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Monday 8 September 2003

0905 Genomes and beyond

GEORGE WEINSTOCK
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With over 100 completed microbial genomes in the public domain, the field of microbial genomics is in full bloom. Although whole genome sequencing of microbes is still evolving, it is now generally considered routine and uncontroversial, a radically different view from less than a decade ago. But while production of genome sequences is routine, the conversion of this sequence data into biologically meaningful information is at an earlier stage of development. Bioinformatics tools for mining the genome and molecular genetic techniques for large-scale analyses are being refined and more widely applied. These issues will be illustrated with the analysis of *Treponema pallidum*, the spirochete that causes syphilis. This bacterium cannot be grown outside of animal hosts, and thus many genetic and biochemical methods are difficult or impossible, making it both a natural target as well as a challenge for genomic analysis. The genome sequence has been determined and analyzed bioinformatically, all the genes have been cloned and expressed in E. coli, attempts at vaccines and immunodiagnostics have shown promise, and whole genome comparisons with related spirochetes have been performed by several methods. These and other results will be reviewed to present a picture of the present and future directions in microbial genomics research.

0945 Comparative genomics of bacterial pathogens

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As a group, the bacterial pathogens of humans are highly diverse, both in terms of their phylogeny, and their strategies for taking advantage of the opportunities offered by the human host. In contrast, many diverse diseases are caused by groups of related pathogens, and often strikingly different pathologies can be caused by very closely related bacteria. Equally, the host ranges available to related pathogens can vary widely. This spread of interactions between genotype and phenotype provides great opportunities for using comparative genomics to investigate the evolution and pathogenic strategies of these organisms. These concepts can be illustrated by comparisons amongst the Enteric pathogens; *Salmonella*, *Escherichia* and *Yersinia*. These and other examples will be discussed.

1100 Molecular profiling for diagnosis of infection

P. GHAZAL
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High throughput technologies, such as microarray-based DNA, RNA and protein detection, have opened new fields in genomics and proteomics. This talk aims to highlight the potential value and limitation of this methodology, to design and extract signature-based markers for infectious disease.

1145 Comparative genomics of alpha-proteobacteria

SIV G.E. ANDERSSON, CAROLIN FRANK, OLOF KARLBERG, BASTIEN BOSSEAU, CHRIS COOPER, MAXIME HUVET & BORIS LEGAULT
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Members of the alpha-proteobacteria display a broad range of interactions with higher eukaryotes. Some are pathogens of humans that cause diseases such as typhus, trench fever and cat scratch disease, while others are animal pathoges, causing for example abortions in cattle. Yet other species have evolved elaborate interactions with plants; in this group we find both plant symbionts and parasites. The recent sequencing of more than 10 alpha-proteobacterial genomes, including our own recently completed genomes form *Bartonella*, provides an excellent opportunity for a global genomic comparison of human, animal and plant- associated bacteria. Here, we present the phylogenetic relationships of the alpha-proteobacteria for which complete genome sequence data is available and discuss genomic features that are shared between human, animal and plant pathogens. We identify differences in genomic contents and architectures that correlate with major lifestyle changes. For example, we show that extreme genome size expansions of a few thousand genes have occurred during the evolution of plant-associated bacteria. In contrast, reductions of a few thousand genes are characteristic of shifts to intracellular animal environments and vector-mediated transmission pathways. The observation is that generation times, population sizes and vulnerability to attack are major determinants for the genomic evolution of bacteria that have developed close interactions with plants and animals.

1330 Alternative splicing in mouse transcription factors – a genomic view

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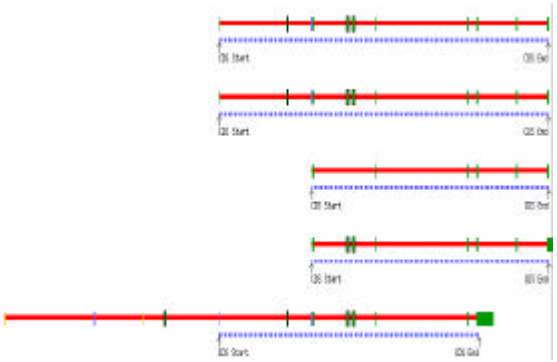
The application of gene identification methods to recently completed genomes from multi-cellular organisms (*C. elegans*, *Arabidopsis*, *Drosophila*, *M. musculus*, *H. sapiens*) has revealed relatively small differences in the number of genes encoded by these genomes. Subsequent EST- and cDNA-based studies indicate that an important factor in the complexity of the eukaryotic proteome is multiplicity of gene structure: a single gene gives rise to developmental stage-, tissue type- and activation state-specific isoforms. Up to 59% (Lander et al 2001) of human and 33% (Brett et al 2002) of mouse genes have alternative splice forms, and a recent analysis of a set of 60,000 full-length mouse cDNAs indicates that the proportion may be as high as 68% (Zavolan et al 2002b). Another substantial finding of the full-length cDNA sequencing projects is the frequent use of alternative promoter and polyadenylation sites (Suzuki et al 2001, Fantom consortium 2002) which is often associated with alternative splicing of initial and terminal exons (Zavolan et al 2002).

Motivated by the Riken set full-length mouse cDNA became available, we developed a systematic approach to characterize splice variation as revealed by cDNA-to-genome mapping. A focus on full-length cDNA sequences reveals the full spectrum of variations generated *in vivo*, rather than having to combine data from multiple EST sequences. We developed computational methods to identify splice variants while eliminating variation that was possibly due to cloning, sequencing and mapping errors. To assess the potential effect on the proteome of alternative splicing events cataloged in the previous section, we aligned the 1667 cDNA sequences in the 581 variant clusters to the protein sequences in the NR section of the Genbank sequence repository. we found that most of the splice variation occurs inside the coding region (68.1%). Only 11.6% and 20.4% alternative splicing events occur in the 5'- and 3'-UTR, respectively. As a case study, we queried the database to retrieve Riken clones annotated with the keyword phrases "transcription factor" or "DNA-binding". 314 genomic clusters were retrieved, of which 221 contained a variant exon, and 93 contained no variant exons. Next, we established whether the alternative splicing event was inside the coding sequence or in 5' or 3' untranslated regions. Checking 10% (30 clusters), 70% of the variants influenced the coding regions, and 25% of those affected the protein in frame within a functional domain, as defined by SMART (Schultz *et al*

1998). The figure below illustrate exons mapped to the mouse genome for one such transcription factor.



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1415 Minimal microbial genome

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Abstract not received

1600 Global views of host/pathogen interactions; transcriptional changes in virally infected cells

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Abstract not received

1645 Herpesvirus genomics

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Members of the family *Herpesviridae* are found in vertebrate animals and bivalve shellfish, and the great majority are highly host specific. They are enveloped, icosahedral viruses that replicate in the cell nucleus and are capable of latently infecting their hosts for life. Their linear, double-stranded DNA genomes are 125-240 kbp in size and contain about 70-170 genes. Over 30 complete genome sequences are available, and understanding of their phylogeny and gene content is in an advanced, though still developing, state.

The family falls into three highly diverged groups, which infect mammalian/avian/reptilian, amphibian/fish and bivalve hosts. The first of these groups comprises three major branches that separated about 200 million years ago, with each lineage inheriting about 40 genes from the common ancestor. In these lineages, herpesviruses have evolved at a rate one or two orders of magnitude faster than their hosts, and have employed all available mechanisms to generate diversity.

As with other organisms, descriptions of gene content have depended on the criteria used. Comparative genomics has proved fundamental, and has been supplemented by other bioinformatics approaches and data from expression studies. It has proved essential to set gene annotation exercises in a strong virological context.

Tuesday 9 September 2003

0900 Prediction of highly expressed genes in sequenced prokaryotic genomes

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The majority of genes in bacterial genomes are expressed at quite low levels (e.g., only a few molecules per cell), whilst only a handful of genes, typically less than 10% of the genome, is expressed at significantly high levels (more than a few hundred molecules per cell). We compare three different computational methods for prediction of gene expression levels in bacterial genomes: codon usage bias, chromatin structure, and a neural network trained on expression data. All three methods give similar performance, although the neural network has the best Pearson's correlation coefficient (a value of 0.83 for Affymetrix *E. coli* experiments). Advantages and disadvantages of each method as well as performance, compared to microarray experiments for several different bacterial genomes will be presented.

0945 Comparative genome analysis of Yersinia

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Yersinia pestis, the etiological agent of plague, and *Y. pseudotuberculosis*, which causes yersiniosis in humans, are thought to have diverged from a common ancestor no more than 20,000 years ago (Achtman *et al.*, 1999). Despite their vastly different etiologies, they are highly similar at the nucleotide level (~97%) and share a 70 kb virulence plasmid as well as a number of chromosomally-encoded virulence-associated factors. In spite of extensive studies, the genetic bases for their dramatic difference in virulence remain unidentified. Comprehensive comparative genomic analyses along with experiments engendered by this data should help elucidate the mechanisms underlying the increased pathogenicity in *Y. pestis*.

We have obtained the entire genome sequence of *Y. pseudotuberculosis* IP32953 (a fully virulent serovar I strain) and carried out a comprehensive analysis of its genome organization and comparisons to the recently published sequence of *Y. pestis* strain CO92 and KIM10+. These studies have revealed a number of striking differences with regards to genome rearrangements, the identification of a large number of frameshift mutations, small insertion/deletions and IS element insertion-mediated gene interruptions that affects as many as 8% of the ORFs in *Y. pestis* when compared with the *Y. pseudotuberculosis* progenitor. Many of these mutations, not found in *Y. pseudotuberculosis*, are consistent with a recent emergence, and may have profound consequences to the biology of *Y. pestis*. The distribution of unique regions and small point mutations characteristic of each species has been investigated across a panel of representative Yersinia members. This aggregate data not only allows us to highlight the general physiological capabilities of these two *Yersinia* species but it points to unique regions in these species

that may account for the observed differences in virulence. Finally, the unique regions may serve in molecular typing, forensic, and other applications. A general genome overview of *Y. pseudotuberculosis* IP32953 along with a detailed comparison with *Y. pestis* will be presented.

The work at Livermore was performed under the auspices of the U.S. Department of Energy by the University of California, Lawrence Livermore National Laboratory under Contract No. W-7405-Eng-48.

1100 Bacterial gene expression and infection: microbes, messengers and microarrays

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Bacterial pathogens response to multiple environments encountered within the host by modulating gene expression patterns, particularly through transcriptional control. This ensures the temporal and spatial production of virulence determinants such as toxins or invasins, the induction of scavenging responses for host-restricted elements (such as Fe or Mn) and the appropriate metabolic adaptations for alternative carbon substrates (such as fatty acids) or electron acceptors encountered in the host during infection. The ability to study transcription regulation at a whole genome level is now possible using DNA microarrays, but requires careful consideration of appropriate infection models and extraction methods for highly labile mRNA. *M.tuberculosis* has a complex pathophysiology, being able to replicate in macrophages and also to remain dormant for many years. Macrophage and experimental murine models of acute, chronic and latent tuberculosis are now amenable to gene expression studies by microarray analysis. Furthermore, profiling host transcriptional responses to defined mutants lacking potential virulence determinants (such as cell wall-associated complex lipids) provides a functional approach to defining their role in host-pathogen interactions. Microarray analysis of gene expression during infection will contribute to elucidating pathogenic mechanisms, to the functional analysis of genes of unknown function and to the identification of gene products with potential to act as targets for new drugs and vaccines.

1145 Proteomics

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Megabase-scale sequencing has already yielded over 100 complete genomes, of which over 90% are of microbial origin. It follows that microbes are at the forefront of the next major wave of biological studies that aims to define the function of every gene product as well as to characterise interactions and patterns of gene expression. Much of this work necessarily has to be carried out at the level of the proteome, as proteins are the ultimate effectors of essentially all cellular processes. Additionally, the abundance of mRNA transcripts is generally poorly predictive of protein levels, not least because <75% of translation initiation events ultimately result in a complete protein. Proteomic analyses have often been hampered by the sheer physico-chemical diversity of proteins and their vast dynamic range of expression. Fortunately, the situation has improved recently with the introduction of new non-gel-based approaches and instrumentation. For example, the use of isotope-coded tags and accurate mass tags has significantly increased the number of proteins that can be quantified and give a precision of better than 10%. Similarly, Fourier transform-ion cyclotron resonance mass spectrometry can measure protein expression over five orders of magnitude and detects proteins down to ~20 zmol (~12,000 molecules). Such advances are generating unprecedented amounts of information about the components involved in specific microbial processes as well as on the control and integration of gene expression. The talk will describe recent advances in microbial proteomics focusing on instances that have yielded fresh biological insights into microbial physiology and/or virulence.

1400 The *Campylobacter jejuni* glycome

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Campylobacter jejuni is the primary cause of bacterial gastroenteritis worldwide and can lead to serious post infection neurological complications. Despite the importance of the organism, little is known about how it causes disease. This was the driving force for the *C. jejuni* NCTC11168 genome sequencing project completed in 2000. This lecture will focus on the discoveries made from this information relating to cell-surface polysaccharide structures.

Prior to the genome sequence *C. jejuni* was known to have LOS and a glycosylated flagellum. Both structures have now been elucidated and shown to be phase variable due to multiple contingency loci. Among the remarkable findings was the identification of a phase variable capsule and a novel N-linked general glycosylation pathway. Proteome analysis revealed that this system is responsible for glycosylating over 40 *C. jejuni* proteins.

In summary, for a genome with a limited genetic repertoire (1.68 Mb), *C. jejuni* goes to great lengths to decorate, disguise and vary its surface structure. It has two glycolipid structures that are phase variable and two glycosylation systems, one variable (flagella modification system) and the other conserved (general glycosylation system). The potential roles of these structures in the life-cycle and evolution of *C. jejuni* will be discussed.

1445 Unravelling the secret life of *Pseudomonas fluorescens*

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Pseudomonas fluorescens SBW25 is a Gram-negative bacterium that grows in close association with plants. In common with a broad range of functionally similar bacteria it plays an important role in the turnover of organic matter and certain isolates can promote plant growth. It is closely related to strains that produce secondary metabolites that have important properties including antibiotic and antifungal activities. Despite its environmental significance, the causes of its ecological success are poorly understood. Here we outlined an integrated approach to study the function of this bacterium in its natural environment. Central to this is the forthcoming genome sequence, a proteomic analysis of cold-induced genes, a suite of promoter trapping strategies and the use of genome-based approaches to the identification of regulatory networks. Together these technologies have facilitated the isolation and characterization of more than 100 loci that are active at the plant-soil interface, but silent in the laboratory environment. The study of these loci has brought many challenges, not least the difficulty of working with genes, that in the laboratory environment confer no phenotype. Nevertheless, progress in understanding the biological function and ecological significance of certain loci has been made and here we report the current state of these studies.

1600 Identification of novel targets for antimalarial drugs

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Identification of the mostvulnerable drug targets in the *Plasmodium* genome database often begins with a search for essential enzymes and host-parasite differences in enzyme active sites. Unfortunately, such concepts may be both old and of limited predictive value in drug development. Amongst other things, too little attention is paid to target levels in cells and responses of cellular machinery to the presence of a drug in a cell. A thorough biochemical characterization of well-established antimetabolites in malaria teaches us general lessons about potency and selectivity of successful drugs. Dihydrofolate reductase-thymidylate synthase (DHFR-TS) offers an important case study: It is the target of clinically useful antifolate drugs, a selectable marker for manipulation of the *Plasmodium* genome, and an important model for understanding gene function in *Plasmodium*. Traditionally, the potent and selective antimalarial activity of DHFR-TS inhibitors has been interpreted in the context of host-parasite differences in drug binding at theDHFR active site. However, some compounds that show little difference in binding between human and malaria DHFR can still be potent and selective inhibitors of *Plasmodium* proliferation. A large portion of this selectivity can now be explained through host-parasite differences in gene expression, both at the translational and transcriptional level. In the immediate future, functional genomics tools are helping us improve and test our understanding of selective chemotherapy. As newer concepts are established and integrated into genome-wide strategies for finding good drug targets, malaria drug discovery should become rapid and cheap.

1645 Vaccine antigen discovery *in silico*

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Abstract not received

POSTERS

MS 01 Identification of protein functions for vaccine antigen candidates selected from *Francisella tularensis*

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Francisella tularensis is one of the most infectious organisms known in man. Currently a live vaccine strain (LVS) of *F. tularensis* is available, however it is unlicensed as the genetic basis of attenuation is unknown.

The aim of our research programme is to develop and validate a recombinant subunit vaccine for protection against challenge with *F. tularensis.* A computer algorithm was developed to exploit differences between known bacterial vaccine antigens and randomly selected proteins and this was utilised to search the predicted *F. tularensis* Schu 4 proteome. From this prediction, the 25 top potential vaccine antigen candidates have been taken forward for further analysis. In an attempt to identify the selected proteins, BLAST searches (Basic Local Alignment Search Tool) were carried out. From the results, putative functions were predicted for some proteins. To gain further insight into the unknown proteins, bioinformatics analyses were extended by using a number of computer programmes other than BLAST. The results showed a significant reduction in the percentage of unknown proteins.

MS 02 *In silico* analysis of known bacterial protein vaccine antigens

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Many vaccines have been developed from live attenuated forms of bacterial pathogens or from killed bacterial cells. However, an increased awareness of the potential for transient side effects following vaccination has prompted an increased emphasis on the use of sub-unit vaccines rather those based on whole bacterial cells. Traditional identification of vaccine sub-units is often a lengthy process. However, the publication of several bacterial genomes has allowed the recent development of bioinformatic approaches to identify candidate protein vaccine antigens. Such methods can screen whole genomes for potential vaccine candidates far more rapidly than empirical approaches.

Comparison of a dataset of known vaccine antigens with that of randomly selected proteins has shown there to be significant differences in the amino acid composition of the two datasets. A novel *in silico* approach to predict vaccine antigen candidates based on these differences has been developed. This algorithm can be applied to the proteome of any pathogenic bacteria to predict potential vaccine candidates.

MS 03 Development of transposon mutagenesis systems for the random disruption of genes in *Streptococcus equi* subsp. *equi*

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Background and objective of investigation: *Streptococcus equi* subsp. *equi* (*S. equi*) is an important equine respiratory pathogen. This project developed Tn*917* and mariner transposon mutagenesis systems in *S. equi*, and subsequently used these to identify genes important in pathogenesis.
Methods: A library of 2000 *S. equi* signature tagged Tn*917* transposon mutants was generated. Two vectors for *in vivo* mariner mutagenesis were also developed, and a library of 200 transposon mutants generated. Transposon insertion sites were determined by sequence comparison (www.sanger.ac.uk). The libraries were screened using a whole blood killing assay.
Results: Analysis of the Tn*917* library by insertion site sequencing revealed that 60% of the insertions had occurred within a 15 kb region of the chromosome. Vectors were generated to allow *in vivo* transposition of a modified mariner transposon in streptococci. This was found to insert randomly into the *S. equi* chromosome. Approximately 1.5% of the library was attenuated for the whole blood killing assay.
Conclusions: Tn*917* preferentially integrated into a 15 kb region of the *S. equi* chromosome. A modified mariner transposon inserted randomly across the chromosome, and was successfully screened for attenuating mutations using appropriate *in vitro* models.
Acknowledgements: Funded by the Wellcome Trust and the Home of Rest for Horses.

MS 04 Extracting phylogenetic information from whole-genome sequencing projects: the lactic acid bacteria as a testcase

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The availability of an ever increasing number of complete genome sequences of diverse prokaryotic taxa has lead to the introduction of novel approaches to infer phylogenetic relationships among bacteria. In the present study we compared the sequences of 16S rDNA and nine housekeeping genes with the fraction of shared orthologous protein-coding genes, conservation of gene order, codon usage and dinucleotide relative abundance among 11 genomes of species belonging to the lactic acid bacteria. In general there is a good correlation between the results obtained with various approaches, although it is clear that

there is a stronger phylogenetic signal in some datasets than in others and that different parameters have different taxonomic resolution. It appears that trees based on different kinds of information derived from whole-genome sequencing projects do not provide much additional information about the phylogenetic relationships among bacterial taxa compared to more-traditional alignment-based methods. Nevertheless we expect that the study of these novel forms of information will have its value in taxonomy, to determine what genes are shared, when genes or sets of genes are lost in evolutionary history, to detect the presence of horizontally transferred genes and/or onfirm or enhance the phylogenetic signal derived from traditional (alignment-based) methods.

MS 05 Bioinformatic identification of putative lipoproteins encoded in the genomes of *Streptococcus agalactiae* strains

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N-terminal lipidation is a major mechanism by which bacteria can tether proteins to membranes. Lipidation is directed by the presence of a cysteine-containing 'lipobox' within specific signal peptides and this feature has greatly facilitated the bioinformatic identification of putative lipoproteins. The appropriate N-terminal sequence features are described by the Prosite pattern entry PS00013. As this is a frequently matched pattern, we have designed previously a taxon-specific pattern (G+LPP) for the identification of Gram-positive bacterial lipoproteins, based on the signal peptides of experimentally verified lipoproteins (Sutcliffe, I.C. and Harrington D.J. Microbiology 148: 2065-2077). Patterns searches with these patterns have been used to identify at least 40 putative lipoproteins in the recently published genomes of *Streptococcus agalactiae* strains 2603/V and NEM316. As in other Gram-positive bacteria, the largest functional category of *S. agalactiae* lipoproteins is that predicted to comprise substrate binding proteins of ABC transport systems. These include a homologue of pneumococcal PsaA and we have experimental data consistent with the lipidation of this protein. Other roles include lipoproteins that appear to participate in protein export, enzymes, adhesins and several species-specific proteins of unknown function. These data suggest lipoproteins may have significant roles that influence the virulence of this important pathogen.

MS 06 Bacterial comparative genomics: gene/operon duplications

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Gene duplications are important antecedents to the acquisition of new genes and evolution of novel gene functions. So far, this has mainly been studied in eukaryotes. However, gene duplications are independent of the physical organisation of the genome and of the replication mechanism, and consequently have occurred both in prokaryotes and eukaryotes. Therefore, a comparative genomics approach to study the contribution of duplication in bacterial genome evolution was set out.

Systematic comparisons of predicted proteomes from 97 organisms with a complete genome sequence have allowed us to quantify the proportion of duplicated genes within each genome. Subsequently, the software tool ADHoRe (Automated Detection of Homologous Regions) was applied to detect regions with statistically significant conserved gene content and order within the genome, i.e. block duplications, and distinguish these from paralogous gene products not in a detectable block duplication (tandem duplications or rearranged duplicates). It was found that 70% of the block duplications have a size of three genes, reflecting the bacterial operon size, whereas only 3% have a size larger than 15 genes. These results contrast sharply with what was found in eukaryotic genomes. A thorough comparative genome analysis will help to build a clearer picture of operon evolution, and consequently of how life adapted to diverse environments, and increased in complexity, events that are important aspects of evolutionary genomics.

MS 07 Application of DNA microarrays to study the evolutionary genomics of *Yersinia pestis* and *Yersinia pseudotuberculosis*

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Yersinia pestis, the causative agent of plague, diverged from *Yersinia pseudotuberculosis*, an enteric pathogen, an estimated 1,500-20,000 years ago. Genetic characterisation of these closely related organisms represents a useful model to study the rapid emergence of bacterial pathogens that threaten mankind. To this end, we undertook whole genome DNA microarray analysis of twenty-two strains of *Y. pestis* and ten strains of *Y. pseudotuberculosis* of diverse origin. Eleven *Y. pestis* DNA loci were deemed absent or highly divergent in all strains of *Y. pseudotuberculosis*. Four were regions of phage origin, whilst the other seven included genes encoding a vitamin B12 receptor and the insect toxin *sepC*. Sixteen differences were identified between *Y. pestis* strains, with biovar Antiqua and Mediaevalis strains showing most divergence from the arrayed CO92 Orientalis strain. Fifty-eight *Y. pestis* regions were specific to a limited number of *Y. pseudotuberculosis* strains, including the high pathogenicity island, three putative autotransporters and several possible insecticidal toxins and hemolysins. The O-antigen gene cluster and one of two possible flagellar operons had high levels of divergence between *Y. pseudotuberculosis* strains. This study reports chromosomal differences between species, biovars, serotypes and strains of *Y. pestis* and *Y. pseudotuberculosis* that may relate to the evolution of these species in their respective niches.

MS 08 A systematic comparison of Pfam and MEROPS: two databases used in genome annotation

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Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge

Pfam is a comprehensive collection of alignments and hidden Markov models representing families of proteins and domains. MEROPS is a catalogue and classification of proteases. These resources are widely used for genome annotation, yet their contents are not peer reviewed. A systematic comparison of their contents could provide a means of independent quality control. An additional reason for carrying out this study was to explore the extent of consensus in the definition of a protein family.

When comparing the numbers of sequences found in the overlap between a MEROPS family and its corresponding Pfam family, in most cases the overlap was substantial but there were some differences in the sets of sequences included in the MEROPS families versus the overlapping Pfam families.

A number of the discrepancies between MEROPS and Pfam families arose from differences in the aims and philosophies of

the two databases. Examination of these discrepancies highlighted additional members of families, which have subsequently been added in both Pfam and MEROPS. This has led to improvements in the quality of both databases. Overall there was a great deal of consensus between the databases in definitions of a protein family.

MS 09 Triplet periodicity is a result of information content maximisation at the extremes of GC% in bacterial genomic DNA

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The cause of triplet periodicity (the propensity for codons to occur in one reading frame) in bacterial open reading frames (ORFs) has remained elusive. We analysed triplet periodicity in coding frames of ORFs from complete bacterial genomes. In high GC% organisms, AT-rich codons are periodic, whereas in low GC% organisms, GC-rich codons are periodic. In non-coding frames, the opposite is found. In organisms with 50% GC, codons have low periodicity in all frames. These data imply that observed triplet periodicity is the result of bacteria maximising information content by using codons that contrast with overall GC% sparingly, and almost exclusively in the coding frame of ORFs. This observation damages the theory that GC% is a result of the usage of particular codons in order to match with tRNA availability, because such selection would not affect the presence of codons in the non-coding frames, and thus, result in coding frame periodicity. These observations also damage the theory that coding periodicity is a mechanism by which ribosomes sense the real coding frame through codon-anticodon interactions with inversely periodic 16S rRNA sequences, since these sequences are absolutely conserved, irrespective of the GC% of a genome, and of which codons are the most periodic.

MS 10 *In vitro* signature tagged transposon mutagenesis of *Campylobacter jejuni*, and the use of these mutant libraries in *in vitro* screens

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Hendrixson *et al*, (2001) *Mol. Micro*. 40, 214-224, previously reported a system for *in vitro* transposon mutagenesis of *Campylobacter jejuni* using a *mariner* based transposon and purified *mariner* transposase. We have modified a derivative of this plasmid by the addition of an OriR6K gamma origin of replication (to facilitate plasmid rescue of mutants), and included signature tags (Holden Supertags) to enable us to follow pools of individually tagged mutants through *in vitro* and *in vivo* assays. We show successful *in vitro* transposition and transformation of four strains of *C. jejuni* - including the sequenced strain (NCTC11168), generating large numbers of signature tagged mutants. We report the distribution of transposon insertion sites within these strains and highlight the propensity for insertion of the transposon into the plasmids of *C. jejuni* 81-176. Initial *in vitro* screens have identified mutants defective in flagellar mediated motility, other screens have concentrated on environmental stresses, such as pH, osmolarity and temperature and we report mutants identified in these screens.

MS 11 Development of an *in vivo* screen to identify *Campylobacter jejuni* genes important in chicken colonisation using a signature-tagged mutant library

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To identify *Campylobacter jejuni* chicken colonisation factors we have generated signature-tagged mutant (STM) libraries in a variety of strains. Mutants were screened in pools through a colonisation model by oral inoculation of two-week old specific pathogen free chickens, sampling caecal contents at up to seven days post-inoculation. We observed random loss of mutants, not correlating with the colonisation potential of mutants when assayed in pure culture. Analysing pools of bacteria recovered from caeca of birds inoculated with individual mutants reduced or eliminated the stochastic loss, enabling large-scale screening of the STM libraries.

We conclude that the population dynamics of *C. jejuni* in the chicken gut leads to random loss of strains from STM mutant pools. Using our modified screening protocol we have identified genes implicated in colonisation of the chicken.

MS 12 Protein-protein interactions of the tetratricopeptide-repeat type-III secretion chaperones CesD and SicA

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Tetratricopeptide repeats (TPRs) are degenerate poorly conserved, 34 amino acid sequences found in a variety of predominantly eukaryotic proteins. They usually occur in tandem arrays of 3-16 motifs which form alpha-helical scaffolds mediating protein-protein interactions. They are commonly involved in the assembly of multi-protein complexes and can mediate molecular recognition.

Three TPRs with variable-length N and C-terminal extensions have been identified in LcrH-like Type III-secretion chaperones through data mining, multiple alignments and homology modelling. Amongst these were SicA from *S. typhimurium* and CesD from EPEC/EHEC.

Studies utilising the yeast-2-hybrid system and mutagenesis have allowed the minimum binding regions of the TPR chaperone substrates to be identified. A deletion mutant in the gene encoding CesD has been created and characterised and paves the way for complementation studies with mutant constructs. Mutagenesis studies utilising QuikChange™ technology are underway on both yeast-two hybrid and complementing plasmids. This will allow us to dissect the determinants required for chaperone-substrate binding and specificity, using our homology models to provide a conceptual framework.

MS 13 *ViruloGenome*: an online facility to help bacteriologists exploit bacterial genome sequences

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We have built an online facility, *ViruloGenome*, which is freely accessible via the web (http://www.vge.ac.uk) and is aimed at providing the academic community with a set of user-friendly tools for analysing and exploiting bacterial genome sequence data, while genome sequencing is in progress, and once it has been completed. *Virulogenome* contains databases of more than two hundred bacterial genomes and provides facilities for BLAST searches against finished and unfinished genomes. A unique feature of the facility is the ability to perform PSI-BLAST searches against proteins from unfinished genomes. From several case studies using *ViruloGenome* PSI-BLAST, it is clear that sequences from unfinished genomes are able to fill gaps in sequence space and reveal otherwise unexpected weak homologies. Improved data visualisation tools and integration with species-specific databases are under development.

MS 14 *coli*BASE, *Campy*DB and *Clostri*DB, online resources for the comparative genomics of *Escherichia coli* and its close relatives, Campylobacters and Clostridia respectively

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We initially developed *coli*BASE as an online resource for *E. coli* comparative genomics. More recently we have adapted the same schema to serve two other resources: *Campy*DB and *Clostri*DB, serving the *Campylobacter* and Clostridial communities respectively. At the heart of all three facilities is a relational database containing data from relevant completely sequenced strains, together with preliminary sequence data from genome projects in progress.

Genes of interest can be retrieved based on their annotation text using a flexible and intuitive Google-like search interface. Alternatively a gene can be selected by chromosomal location or by sequence homology using a customised BLAST search. Once a gene is retrieved a clickable image of the surrounding chromosomal region is generated, allowing easy navigation of the genome. Annotation from GenBank and Swissprot is displayed, including a list of relevant articles. Numerous analytical tools are also provided, including sequence retrieval, BLAST, the applet version of Artemis, a primer design facility based on Primer3 and links to other relevant online resources. Graphical comparisons of the chromosomal region with homologous regions of other strains, as determined using MUMmer, can be displayed using a novel genome alignment viewer.

The databases are available at http://colibase.bham.ac.uk, http://campy.bham.ac.uk and http://clostri.bham.ac.uk, respectively.

MS 15 Identification and comparison of ABC transporters in sequenced bacterial genomes

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The Institute of Genomic Research (TIGR) produces an up-to date list of all completely sequenced and annotated microbial genomes in the form of the complete microbial resource (CMR). This genomic tool allows for a wide variety of data retrieval and was used to identify 76 bacteria with completed genome sequences and annotations. In this study we have identified ATP-binding cassettes in these bacterial genome sequences using the ABC signature sequence prosite pattern PS00211. Such ATP-binding cassettes are specific to ABC transporters and are responsible for the generation of energy for the import and export of a variety of molecules across cellular membranes. Furthermore, we have compared the number of identified ABC transporters, to the genome size and lifestyle of the bacteria.

MS 16 Phenotypic chracterisation of *E. coli* specific genes

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The gamma-proteobacteria are a diverse family that have evolved to occupy a wide variety of ecological niches. The family members include free living soil organisms, pathogenic bacteria and obligate mutualistic bacteria. Diversity such as this results in genomes that vary from a mere 640kb (*Buchnera* sp.) to the largest sequenced bacterial genome of about 6.3 mb (*Pseudomonas aeruginosa*). Differences in the genomes of related members represent sequences/functions which have been acquired, maintained or lost due to evolutionary pressures of niche adaptation. *E. coli* is an important member of the gamma-proteobacterial family and is closely related to pathogenic enteric organisms like Shigella and Salmonella. It occurs naturally in the colon of humans and other vertebrates and certain serotypes are causative agents of gastro-intestinal diseases. The genetic differences between *E. coli* and its relatives, in other words 'Coli specific genes', enable it to occupy the niche of a commensal and an opportunistic pathogen. The basis of my research has been to identify and characterise the function of 'Coli specific' genes.

Full genome sequences of 28 gamma-proteobacteria, including three strains of *E. coli* are now available online. Using comparative BLAST analysis, we identified genes common, yet unique, to all three *E. coli* strains (data available from the MBGD database). Of the 4288 genes known and predicted in *E. coli* K-12 strain MG1655, 102 were found to be unique to the three sequenced *E. coli* genomes. 39 genes had known/annotated functions assigned to them or were found in phage/insertion elements. 63 Genes/annotated ORF's of unknown function which were not present in known phage or insertion elements were then deleted using the modified pKO3 deletion procedure (Merlin et al, 2002). This involves replacing the gene of interest with a deletion construct containing a lacZ reporter cassette. This allows us to study the phenotypic effects of deletion while studying expression of the gene from its native promoter using the reporter (lacZ) molecule. The mutants are being tested for growth in a variety of media/conditions to study the phenotypic effects of gene deletion while measuring changes in gene expression. The growth tests will include alternate carbon, nitrogen and sulphur sources, resistance to metals and antibiotics and ranges of environmental conditions such as salt, pH, temperature and anaerobiosis. Phenotypic characterisation of these mutants may provide new insights into the evolution and metabolism of the *E. coli* family of bacteria.

MS 17 ETT2 and LEE: A tale of two type III secretion systems in *Escherichia coli* and the smile of the Cheshire cat?

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Genomics sequence analysis reveals that there are two gene clusters encoding components of type III secretion systems (TTSS) in enterohemorrhagic *Escherichia coli* O157:H7: the well-characterized LEE region and a gene cluster encoding a second type-III secretion system, ETT2. We have used conventional and long PCR to investigate the distribution and evolution of these clusters among the well-characterized reference collection of 72 natural *E. coli* isolates (ECOR strains), plus several other strains (including non-coli *Escherichia* spp. and some clinical septicemia isolates). In addition, whole-genome scanning by long PCR is underway for some strains.

MS 18 Defining the species-genome of *Streptomyces violaceoruber*

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Background: The whole genome sequence of "*Streptomyces coelicolor*", a member of the *S. violaceoruber* species cluster, provides an unprecedented reference point for the taxonomy and phylogeny of related streptomycetes. Despite the wealth of data from whole genome sequencing studies one genome sequence does not describe the genetic repertoire of any species. And, for bacteria, defining a species is still problematical. The *S. violaceoruber* clade (in the 16S rDNA phylogenetic tree) identifies a well-studied set of closely related streptomycetes within which to identify the species boundary and define the species genome.

Methods: Comparative genomics using "*Streptomyces coelicolor*" micro-arrays and subtractive hybridisation of the streptomycete strains included in the ICSSB† study.

Results: The similarity of all 24 streptomycetes in the ICSSB study was determined by competitive hybridisation against "*Streptomyces coelicolor*" M145 on whole genome ORFmer arrays (BBSRC IGF initiative and Stanford). The results were compared with 16S, 16S spacer region, DNA:DNA similarity and molecular finger-printing. Subtractive hybridisation was used to identify non-"*S. coelicolor*" DNA.
Conclusion: Whole genome comparisons using micro-arrays correlates well with 16S phylogeny, DNA:DNA similarity and other whole genome comparisons such as AFLP and Rep-PCR but offers the promise of a technique which measures similarity from the subgeneric to the subspecific.
†International Collaborative Study of Streptomycete Biology

MS 19 The *Corynebacterium diphtheria* genome project

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Corynebacterium diphtheriae is a Gram-positive, non-spore forming, non-motile, pleomorphic rod belonging to the genus Corynebacterium and the actinomycete group of organisms. This microorganism produces a potent protein exotoxin, diphtheria toxin (DT) that enters the bloodstream and causes the symptoms of diphtheria. It is carried within the genome in an integrated mobile phage, the corynephage β.

This potentially fatal infectious disease is controlled in many developed countries by an effective immunisation programme. However, the disease has made a dramatic return in recent years, in particular, within the Eastern European Region. The largest, and still on-going, outbreak since the advent of mass immunisation occurred within Russia and the Newly Independent States of the former Soviet Union (NIS) in the 1990s.

We have sequenced the genome of a U.K. clinical isolate (biotype *gravis* strain NCTC 13129) representative of the clone responsible for this outbreak. The genome consists of a single circular chromosome of 2,488,635 bp, with no plasmids. It provides evidence that recent acquisition of pathogenicity factors goes beyond the toxin itself, and includes iron-uptake systems, adhesins and fimbrial proteins. This is in contrast to *Corynebacterium*'s nearest sequenced pathogenic relative, *Mycobacterium tuberculosis*, where there is little evidence of recent horizontal transfer. The genome itself shows an unusual large-scale compositional bias, being noticeably higher in G+C near the origin than at the terminus.

MS 20 Proteome analysis of *porphyromonas gingivalis* strains w50 and *rgpB*

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Arg-gingipains are extracellular cysteine proteases of *Porphyromonas gingivalis* encoded by *rgpA* and *rgpB*. Monomeric enzymes $RgpA_{cat}$ and mt-$RgpA_{cat}$ derived from *rgpA* contain 15% - 30% (w/w) of carbohydrate respectively. Inactivation of *rgpB* caused (1) loss of carbohydrate modification to mt-$RgpA_{cat}$, (2) aberrant glycosylation of $RgpA_{cat}$ leading to (3) loss of a glycan epitope which is recognised by monoclonal antibody 1B5 in $RgpA_{cat}$ from W50 and (4) decrease in $RgpA_{cat}$ stability. Inactivation of genes upstream and downstream of *rgpB* had no effect on maturation of *rgpA*-derived enzymes suggesting that there is no polar effect on transcription at this locus. This implied an important role for *rgpB* in glycosylation of *rgpA*-derived enzymes. In order to determine the function of *rgpB* in the glycosylation process, cellular proteins of W50 and *rgpB* were separated by 2D-PAGE. Landmark protein spots and those which differed in the two strains were excised, digested with trypsin and analyzed by MALDI-TOF/MS. *P. gingivalis* W83 genome sequence (http://www.tigr.org) was examined for peptide masses to establish identity of proteins. A GTP-binding protein Type A was down-regulated in *rgpB*. This protein has sequence similarity to bacterial BipA proteins involved in virulence regulation and could be potentially involved in the maturation of *rgpA*-derived enzymes.

MS 21 Characterisation of a genetic locus required for protein glycosylation in *Porphyromonas gingivalis*

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Arg-gingipains of *Porphyromonas gingivalis* are extra-cellular cysteine proteases and are members of a growing family of prokaryotic glycoproteins. They are encoded by *rgpA* and *rgpB* which give rise to five isoforms: non-glycosylated HRgpA, glycosylated $RgpA_{cat}$, mt-$RgpA_{cat}$ derived from *rgpA* and glycosylated RgpB, mt-RgpB derived from *rgpB*. The glycosylated Arg-gingipains contain between 15% and 30% (w/w) of carbohydrate. $RgpA_{cat}$ and mt-enzymes share a glycan epitope with an anionic surface-polysaccharide recognised by mAb1B5. Glycosylation of Arg-gingipains was examined by targeted mutagenesis of *porR*, which has homology to genes involved in protein glycosylation in other organisms. The *porR* mutant has reduced Arg-X and Lys-X activities and release into the culture supernatant is coupled to their synthesis. At early time points in the growth of *porR*, there was an accumulation of a high molecular weight protein which cross-reacted with anti-RgpA suggesting a defect in processing of RgpA polypeptide. Culture supernatant of *porR* contains HRgpA and glycosylated RgpB whereas $RgpA_{cat}$ and mt-enzymes are not present and *porR* does not show cross-reactivity with mAb1B5. Hence, *porR* plays an important role in processing/maturation of the *rgpA* derived enzymes whereas glycosylation of RgpB proceeds via a *porR*-independent pathway.

MS 22 A locus involved in glycosylation of arg-gingipains of *porphyromonas gingivalis*

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Five isoforms of Arg-gingipains are encoded by *rgpA* and *rgpB* in *Porphyromonas gingivalis* W50: non-glycosylated HRgpA, glycosylated $RgpA_{cat}$, mt-$RgpA_{cat}$ from *rgpA*, and glycosylated RgpB and mt-RgpB derived from *rgpB*. The glycosylated enzymes, apart from RgpB, share common structural features with an anionic surface polysaccharide (APS). In order to identify genes required for glycosylation of Arg-gingipains / synthesis of APS, *P. gingivalis* W83 genome (TIGR: http://www.tigr.org/) was examined for genes encoding glycosyl transferases and protein glycosylation systems in other organisms. *porR* / *porS* locus encodes a homologue of a protein required for protein glycosylation in *Campylobacter jejuni* and *Neisseria meningitidis*, several glycosyl transferases and a glucan polymer translocase. Seven genes in this locus were deleted in strain W50 by allelic exchange. The mutant was non-pigmented on blood agar and showed increased haemolytic activity compared to the parent. Arg-gingipain activity reduced to 50% of the wild-type levels was released into culture supernatant within 48h of growth, contained HRgpA and RgpB, whereas $RgpA_{cat}$, mt-enzymes were absent and APS was not synthesised. Therefore, a region of *the P. gingivalis* genome involved in glycosylation of Arg-gingipains / synthesis of APS has been identified and deletion of this locus has multiple pleiotropic effects on its phenotype.

MS 23 EcoK and DNA fifty years on: a 'pas de deux' full of surprises

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1953, the year of the famous DNA helix paper, also saw the first report on restriction by *E. coli* K-12[1]. This paper lacked both the glamour and media coverage of the helix model, yet heralded the era of restriction and modification enzymes with their tremendous impact on biological and medical research in the second part of the 20th century.

Fifty years on, >3000 restriction systems are known. They are classified in three main categories, type I, II and III, and are either chromosomally, plasmid or virus-encoded. The type II enzymes uniquely cut at the recognition sequence and are in world-wide use for DNA cloning, engineering, fingerprinting and genomics. Though of no such commercial value, fundamental research into the above E. coli K-12 type I restriction enzyme, EcoK, led to many serendipitous findings, including the curious ability to track or translocate DNA in a helicase-related way, as well as tight control of nuclease activity against undesired (and potentially lethal) breaks in bacterial chromosomal 'self' DNA[2,3].

While the millennium year still saw only a meagre dozen bacterial species described with type I systems[2], in the wake of the human genome project, today the restriction enzyme database (http://rebase.neb.com) lists over 800 entries of proven, potential or orphan type I systems in Eubacteria and Archaea. This presentation commemorates fifty years research on EcoK and relatives and reflects on the putatively general contribution of these translocating nucleases to bacterial evolution by affecting the influx of genetic information.

References: Bertani G and Weigle JJ. 1953. Host-controlled variation in bacterial viruses. J Bacteriol 65:113-12; Murray NE. 2000. Type I restriction systems: sophisticated molecular machines (a legacy of Bertani and Weigle). Microbiol Mol Biol Rev 64:412-34; Murray NE. 2002. Microbiology 148(Pt 1):3-20. 2001 Fred Griffith review lecture. Immigration control of DNA in bacteria: self versus non-self.

MS 24 The *Bacteroides fragilis* genome project

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Bacteroides fragilis is a Gram negative, strictly anaerobic, bacterium that is part of the normal resident colonic microbiota. It is also the most common cause of anaerobic bacteraemia, with a potential mortality of up to 19%. In order to provide a basis for further investigation into the commensal and pathogenic aspects of the lifestyle of this bacterium, we undertook a project to sequence and analyse the complete genome of the organism. We chose to sequence to completion the type strain NCTC9343, and to generate a draft shotgun sequence of strain AIP638R for comparative purposes. We have finished the sequence of NCTC9343; annotation and analysis will be completed soon. The genome consists of a single circular chromosome of 5,205,140 bp with a G+C content of 43.2%, and a plasmid of 36,360 bp with a G+C content of 32.2%. The sequence was assembled from 107,500 shotgun reads, giving a final coverage of 11-fold. The draft sequence of AIP638R currently contains 39,230 shotgun reads (4-fold coverage) and has been assembled into 733 contigs > 2kb. This project may also be taken to completion at a later stage. All the sequence data for NCTC9343 and AIP638R are available for searching and download from the project website http://www.sanger.ac.uk/Projects/B_fragilis. The latest results from the annotation and analysis of the projects will be presented.

Thursday 11 September 2003

0900 Two component systems

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His-Asp phosphorelay systems regulate many signal transduction pathways in microorganisms and plants. Generally a histidine protein kinase (HPK) whose activity is regulated by environmental signals autophosphorylates one of its own histidine residues using ATP as the phosphodonor. The phosphoryl group is then transferred to a conserved aspartate residue in a response regulator protein. The phosphorylated response regulator is activate to produce an appropriate response. The biochemistry of this common signaling mechanism will be discussed with particular emphasis on lessons that have been learned from studies of the his-asp phosphorelay that mediates chemotaxis responses in bacteria.

0945 Which way to go? bacterial chemotaxis: variations on a theme

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Motile bacteria move towards their optimum environment using a related sensory system, whether swimming or gliding, whether archaea, eubacteria or cyanobacteria. Receptors, usually transmembrane and clustered at the cell poles, sense a change in the local environment and control the activity of a histidine protein kinase, CheA. CheA in turn controls the activity of two response regulators, CheY, which binds the flagellar motor when phosphorylated and causes a switch in rotational direction and a methyl esterase, CheB, which is involved in receptor modification and adaptation.

While this basic scheme is found in all motile species, many genome sequences suggest multiple copies of chemosensory pathways may be common. There is evidence that some pathways may be expressed under different growth conditions, tuning behaviour to specific environments, others may have roles in systems other than flagellar control, such as development and pathogenicity. One of the best studied complex systems is that of the photosynthetic bacterium, *Rhodobacter sphaeroides*. It has genes encoding 4 CheW, 4 CheA, 6 CheY, 3 CheR and 2 CheB proteins on 3 operons plus 12 chemoreceptors, 8 transmembrane and 4 cytoplasmic. The proteins encoded by 2 of these operons are essential for chemosensing. Replacement of the wt chemotaxis genes with *gfp* fusions shows the chemotaxis proteins encoded on one operon localised to the poles of the cell with the transmembrane receptors, while all the components of the other pathway cluster with cytoplasmic chemoreceptors in the centre of the cell. *In vitro* analysis of autophosphorylation and phosphotransfer between the different CheAs and the response regulators has identified patterns of phosphotransfer and suggests that cross talk is prevented by physical separation of the pathways rather than by different specificities. The current data suggest the cytoplasmic chemosensory proteins sense the metabolic state of the cell and can over-ride the signals from the transmembrane receptors.

1030 Sensing the Earth's magnetic field: Magnetosome biomineralization in magnetotactic bacteria

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Magnetotactic bacteria (MTB) are able to orient and migrate along magnetic field lines. This intriguing ability is based on specific intracellular structures, the magnetosomes, which are nanometer-sized, membrane-bound magnetic particles consisting of the iron mineral magnetite (Fe_3O_4). Intracellular magnetosome formation is achieved by an unique biomineralization process, which is poorly understood at the molecular level.

While most MTB species have not been isolated in pure culture, magnetotaxis and magnetosome formation can be studied in *Magnetospirillum gryphiswaldense*, which can be readily grown by microaerobic techniques and is amenable to genetic manipulation. The magnetosome membrane in *M. gryphiswaldense* contains a number of specific proteins that are encoded by the *mam*-genes. Functions of the Mam-proteins involve the compartmentalization of biomineralization, the transport of iron and the control of nucleation and crystal growth. Most of the *mam*-genes that are essential for magnetosome formation could be assigned to different operons clustered within a genomic region, which represents a putative "magnetosome island". Magnetosome assembly is currently being studied by genetic analysis and localization studies involving GFP-fusions.

1330 Circadian oscillatory systems

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Life on earth has evolved under continuos daily fluctuations in both light and temperature. Many organisms have evolved the ability to anticipate these external changes in their environment by using endogenous biological clocks. Molecular components that make up these intracellular clocks display similarities among a wide range of organisms. The clock in a number of eukaryotes, including the filamentous fungus Neurospora contains a negative feedback loop in which two PAS domain-containing proteins heterodimerize and act as positive elements to activate the expression of a negative element, FRQ in Neurospora. One of these PAS-domain proteins in Neurospora, WC-1, acts as the circadian photoreceptor. The negative element(s) in turn feeds back to repress the activity of the positive elements. These transcription/translation-based negative loops generate self-sustaining circadian (circa = about; dies = day) rhythms in the level(s) of one or more of the elements within the loop. Interlocking positive feedback loops and multiple layers of regulation enhances the robustness and stability of these oscillations. The KaiA, KaiB and KaiC proteins in Synechococcus also describe a transcription/translation-based negative feedback loop in the first prokaryotic clock to be described. Transcription in this unicellular cyanobacterium is rhythmic, regulating a wide variety of outputs, unlike in the eukaryotic systems where genes for specific functions are rhythmically regulated.

1415 Fungal-plant signalling with *Ustilago maydis*

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Ustilago maydis is a dimorphic fungus that switches from a yeast-like haploid stage to a filamentous dikaryon after mating. This process is controlled by a pheromone/receptor system and a pair of homeodomain transcription factors. In nature it is the dikaryon that is able to differentiate infection structures and cause disease.

I will review our current understanding of the interplay between cAMP and MAPK signalling in preparation for mating, during mating and during pathogenic development. These gene-driven approaches have recently been combined with random insertional mutagenesis (REMI), the use of regulated promoters in differential screens as well as the exploitation of DNA micro arrays based on the annotation of the *U. maydis* genomic sequence. I will discuss these strategies and highlight their

impact for the identification of novel pathogenicity determinants in this system.

1530 Sensing and responding to redox imbalance in *Streptomyces*

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We are studying the underlying regulatory mechanisms that allow the antibiotic-producing bacterium *Streptomyces coelicolor* to sense and respond to cytoplasmic redox imbalance. Two systems will be described – the SigR-RsrA system that monitors the thiol-disulphide redox status, and the recently discovered Rex regulator that monitors the NADH/NAD^+ redox poise. SigR is a sigma factor that activates expression of more than 40 genes in response to increased disulphide bond formation (disulphide stress) in the cytoplasm. RsrA, a redox-sensitive zinc-containing anti-sigma factor, controls SigR activity. During disulphide stress RsrA forms a disulphide bond that causes it to release SigR, allowing SigR to bind core RNA polymerase and activate transcription of the SigR regulon, which includes several disulphide reductase genes. Rex is a novel repressor, found in most Gram-positive bacteria, that controls the expression of several respiratory components in *S. coelicolor*, including NADH dehydrogenase and cytochrome *bd* terminal oxidase. The DNA binding activity of Rex appears to be directly modulated by changes in the NADH/NAD^+ redox poise, which ultimately allows the organism to adapt its respiratory chain during conditions of oxygen limitation.

1615 Coping with stress; lessons from yeast

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All cells have developed mechanisms to sense and respond to changes in their growth environment. This is particularly important for unicellular organisms which must contend with fluctuations in nutrients, external osmolarity, temperature and pH, as well as exposure to toxic environmental compounds and DNA damaging agents such as UV irradiation. In general, cells respond to such stresses with transient changes in the expression of genes encoding proteins that protect the cell against the encountered stress. Many studies have highlighted the role of the eukaryotic stress-activated protein kinase (SAPK) signalling pathways in regulating stress-induced changes in gene transcription. SAPK pathways are conserved from yeast to man, and I shall discuss some of our recent work which has investigated the role and regulation of the SAPK signalling pathways in stress responses, in the fission yeast *Schizosaccharomyces pombe*, and the medically relevant pathogenic yeast, *Candida albicans*.

POSTERS

CCS 01 Marine algal zoospores exploit bacterial quorum sensing molecules for surface targeting

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Chemical signalling molecules produced by bacterial biofilms are exploited for surface targeting by the planktonic phase of a marine eukaryote. Zoospores of the green algae, *Enteromorpha*, settle preferentially on bacterial cells in biofilms. Using *Vibrio anguillarum* mutants defective in *N*-acyl homoserine lactones (AHL) production, *Escherichia coli* strains harbouring plasmid-bourne AHLs and synthetic AHLs, zoospore settlement was shown to respond to the presence of AHLs (Joint *et al.*, 2002). A better understanding of the mechanisms involved may provide new, novel means of controlling *Enteromorpha* biofouling. To investigate this relationship further, bacteria were isolated from the rocks colonised by *Enteromorpha* and assayed for their affect on zoospore settlement. These studies have shown the relationship between bacterial biofilms and zoospores to be dependant on both the cell density and the age of the biofilm, and how this is related to AHL production by the biofilms. Progress has also been made on elucidating the zoospore AHL-detection method.

CCS 02 Characterization of the *flp* gene of *Rhodobacter sphaeroides*, a gene that is negatively regulated by the Prr (Reg) two-component system

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The globally-acting Prr (also known as Reg) two-component system occurs in several bacterial genera, notably in a number of photosynthetic and nitrogen-fixing species, and senses and responds to changes in redox potential. Under aerobic conditions, phosphorylation is repressed. However, under anaerobic conditions, the histidine protein kinase PrrB becomes autophosphorylated, and the phosphate signal is then transferred to partner response regulator PrrA. PrrA~P is the activated form that can then bind to target promoter regions of many genes including those for photosynthesis, carbon dioxide fixation, nitrogen fixation and electron transport proteins. To date, the majority of the target genes identified have been shown to be *positively* regulated by Prr. Here we report on the identification and characterization of another gene (*flp*) that is shown here to be under *negative* Prr control. We used reporter and 2D gel analyses to identify and confirm that *flp* is under Prr repression; these studies also indicated a role for additional regulators in *flp* regulation. Insertional inactivation of the *flp* gene was been undertaken but the role of Flp remains unclear. Finally, we report on the successful overexpression and purification of a His-tagged version for future structural and functional studies of the Flp protein.

This work is funded by BBSRC (24/P13277)

CCS 03 Investigation of two-component signalling in Myxobacteria

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Two-component systems (TCSs) are signalling systems found in prokaryotes and some eukaryotes. The system involves the phosphorylation of the transmitter domain of a sensor kinase, which then phosphorylates the receiver domain of a response regulator. This causes a response within the cell such as sporulation.

We wish to study protein-protein interactions between two-component proteins in *M. xanthus*. Transmitter-receiver interactions are being studied using a yeast two-hybrid system. One TCS family is the *Pho*-type family, which have a *PhoR, PhoB*-like sequence. Ten of these *Pho*-type systems have been identified and are being investigated experimentally. Two further interesting TCS proteins are the motility response regulator *frzS* which has a coiled coil output domain and *ohyA* sensor kinase which has a coiled coil input domain. These are the only coiled coil two-component proteins in *M. xanthus*.

Pho-type, *frzS* and *ohyA* transmitter and receiver domains will be made as bait and prey fusions. All of these baits and preys will then be tested against each other to ascertain if interactions occur and also if any cross talk between different TCS happens. Those TCSs which interact will be investigated in more detail.

CCS 04 WITHDRAWN - Regulatory mechanisms for hemicellulase gene expression in *Cellvibrio japonicus*

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Celvibrio japonicus is a gram-negative soil born bacterium, capable of degrading the major plant structural polysaccharides. Through northern blots and promoter probes analyses we have investigated the expression pattern of C. *japonicus* hemicellulases. Five of the 6 xylanase genes, an α-glucuronidase, and an arabinofuranosidase in this bacterium are induced by xylan or β-glucan whereas all 5 mannanase genes from the bacterium are induced by galactomannan but not by either xylan or glucan. By construction of transposon libraries and screening of the phenotypes on chromogenic polysaccharides or using methylambelliferyl-based substrates, several mutants were identified that were defecting in degrading plant structural polysaccharides. The mutants were utilised to studying the regulatory factors for expression of hemicellulases. To date, we have characterised genes, which are independent members of two component signal transduction systems. Disruption of these genes has resulted in significant reduction in the level of expression of these enzymes when the bacterium was grown on inducing media. One of the sensor domains has been expressed in *E. coli* and its ligand specificity has been determined.

CCS 05 Regulation of genetic competence in *Streptococcus mutans*: evidence for a novel signalling pathway

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Genetic competence in *Streptococcus mutans* and *Streptococcus pneumoniae* is controlled by a quorum sensing (QS) system consisting of a secreted competence stimulating peptide (CSP), the ComDE two component regulatory system and *ComX*, which is an alternative RNA polymerase sigma factor in *S. pneumoniae*. In the QS system of *S. pneumoniae*, the gene encoding the peptide (*comC*) is co-transcribed with the genes encoding the two-component regulatory system. In *S. mutans*, however, *comC* is transcribed in the opposite direction to *comDE* and has its own putative promoter, suggesting that it can be expressed independently. We have inactivated *comDE* in *S. mutans* UA159 and demonstrated using plasmid DNA that this has no effect on transformation frequency. This contrasts with *S. pneumoniae comDE* mutants which show no detectable transformation. Our data suggest that an alternative pathway responsible for the detection and response to CSP exists in *S. mutans*. We have also constructed a *comX* mutant of *S. mutans* UA159 and shown that this gene is essential for competence development. This work has demonstrated that, as in *S. pneumoniae*, ComX is likely to be the global regulator responsible for the development of genetic competence in *S. mutans*. It has also provided evidence for the existence of a second signal transduction system in *S. mutans* which can substitute for the ComDE two-component regulatory system during the development of genetic competence.

CCS 06 Surface structure analysis of *Salmonella* and *E. coli* by Atomic Force Microscopy (AFM)

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We need to understand the surface properties of microbial cells if we are to determine their function in the natural environment. The surface structure includes many highly species-specific antigenic determinants which are required for bacteria-host interactions, such as flagella, lipopolysaccharide and fimbriae. Atomic Force Microscopy (AFM), a member of the family of so-called scanning probe microscopes, is a powerful tool for mapping the surface morphology of biological specimens and can achieve similar resolution to electron microscopy, without the need for vacuum or staining. We are currently investigating the use of scanning probe microscopy to study the effect of environmental factors on the expression of cell surface structures of *Salmonella* and *Escherichia coli*.

AFM analysis revealed very fine detail of individual *E. coli* cells and showed the surface morphology at high resolution. A modified preparation technique allowed the effect of environmental conditions on surface morphology of *Salmonella* to be observed by AFM. Small differences in experimental conditions proved to be critical for reproducible imaging. The use of LPS mutant strains showed clear differences in the surface structure of *Salmonella*. The pro's and con's of AFM for the visualisation of bacterial cells will be discussed.

CCS 07 The role of motility in the host interactions of *Photorhabdus luminescens*

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Photorhabdus luminescens are bacterial symbionts of the entomopathogenic nematode *Heterorhabditis*, colonising the gut of the infective juvenile stage. The nematodes infect a variety of soil dwelling insects, releasing the bacteria into the insect haemolymph where the bacteria multiply, killing the insect and converting its tissues into a substrate suitable for growth and development of the nematodes. The importance of bacterial motility in symbiotic and pathogenic host interactions has been shown in numerous models. Using transposon mutagenesis we isolated mutants with varying degrees of motility, which were screened for their ability to support nematode growth and for insect virulence. A non-motile, non-flagellate mutant BMM316 was identified and shown to have a transposon insert in the flagella structural gene *flgG*. This mutation was then shown to have no effect on the symbiosis with the nematode or on the pathogenicity of *P. luminescens* towards *Galleria melonella* larvae. However when in direct competition with motile wild type cells BMM316 did show a competitive disadvantage in colonising the nematode host but showed no significant difference in pathogenicity towards *Galleria*. Therefore whilst not essential to symbiosis motility confers a competitive advantage to *P. luminescens* when colonising *Heterorhabditis*.

CCS 08 Two-component regulation in *Myxococcus xanthus*: a genomic approach for defining partnerships and assessing cross-talk through the yeast two-hybrid assay

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Two-component systems (TCSs) are phosphotransfer signalling pathways, common throughout the prokaryotes. Such systems are defined by phosphotransfer between the transmitter domain of a sensor kinase and the receiver domain of a response regulator.

Sequencing the *Myxococcus xanthus* genome has revealed a huge number of two-component system proteins (more than 151 receiver, 7 Hpt and 130 transmitter domains). We have classified the TCS proteins according to operon context, domain structure and phylogenetic relationships. Such an analysis has revealed several interesting features. For instance, classification of response regulators into families has revealed an exceptional over-representation by Che-like and Ntr-like proteins. However, up to half of the TCS genes in the genome are orphaned (not adjacent to potential partner genes) making deduction of entire signalling pathways difficult.

We are using the yeast two-hybrid system as an assay of protein-protein interactions. Using receiver and transmitter domains as bait and prey fusions we are mapping interactions between all receiver and transmitter domains in the *M. xanthus* genome. Preliminary results have identified transmitter-receiver partnerships and provided evidence of inter-system cross-talk. In this context, the two-hybrid assay proves a powerful technique

for studying signalling proteins without needing to appreciate the phenotype of the biological process they regulate.

CCS 09 The role of the KfiB protein in the expression of *Escherichia coli* K5 capsule

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Capsular polysaccharide expression is an important virulence factor for many invasive bacterial pathogens of humans. They play roles in attachment and biofilm development as well as confer resistance to both non-specific and specific host defence mechanisms. The *Escherichia coli* K5 capsule offers a model system to study the mechanisms by which capsular polysaccharides are synthesised and exported onto the cell surface of bacteria. The KfiB protein is essential for the synthesis of the K5 capsule. In order to determine its role the KfiB protein was purified and specific antisera generated. This antisera demonstrated that KfiB was associated with the inner face of the cytoplasmic membrane. The effect of mutations in individual genes encoding proteins involved in synthesis and export of polysaccharide was also studied in relation to the localisation of KfiB protein. Only a strain defective for KpsS, resulted in a loss of membrane targeting of KfiB.

Over-expression of KfiB caused an increase in incorporation of radiolabelled GlcNAc into the lipid fraction, suggesting a possible role for KfiB in the initiation of K5 polysaccharide biosynthesis.

CCS 10 *Escherichia coli* capsule gene expression and its regulation

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E. coli, can express a polysaccharide that is an important virulence factor in extra-intestinal infections. Based on genetic criteria, there are 4 groups of *E. coli* capsules; 1, 2, 3, and 4 with most pathogens expressing a group 2 capsule. The *E. coli* K5 capsule is the group 2 capsules paradigm. K5 capsule gene cluster is composed of three regions, 1,2, and 3. Expression of K5 capsule gene cluster is controlled by two convergent promoters one (P1) 5` to region 1 and the other (P3) is 5` to region 3. Transcription from both promoters is temperature regulated. To study the P1 we constructed a single-copy chromosomal *lacZ* fusion of this promoter using an integrative λ phage. Analysis of *bipA* and *hns* mutants demonstrated that BipA was necessary for maximal transcription from P1 at 37°C and that temperature regulation was controlled by H-NS. Transposon mutagenesis was also applied to P1-*lacZ* fusion strain and 11 mutants with decreased β-Galactosidase activity were isolated and characterized. Out of these there were 2 *bipA* mutants. A plasmid library was constructed to identify genes capable of suppressing the *bipA* mutation. So far two clones suppressing the *bipA* mutation have been isolated and are currently being analyzed.

CCS 11 Enhanced AHL production in *Rhizobium leguminosarum*: identification of novel genes involved in symbiotic nitrogen fixation

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Two quorum sensing regulatory sytems (*cinI, cinR* and *raiI, raiR*), comprising AHL synthases and LuxR type regulators, have been identified in *Rhizobium leguminosarum* lacking a symbiotic plasmid (strain 8401). CinI is responsible for production of *N*-(3-hydroxy-7-*cis* tetradecanoyl) homoserine lactone (3-OH-C_{14}-HSL), wheras RaiI is primaril produces *N*-(3-hydroxyoctanoyl)-L-homoserine lactone (3-OH-C_8-HSL). Expression of *raiI* is autoregulated by RaiR/3-OH-C_8-HSL, and is also influenced by CinI, CinR and iron avilability. Mutation of the *cinI, cinR, raiI* or *raiR* loci, or of genes affecting iron uptake, results in a significant decrease in RaiI made AHLs. A screen was initiated to find mutations that increased AHL production in an iron uptake mutant. From the screen, six mutations in different genes, designated *ina*1-6 (increased AHL), were identified. We have shown that increased AHL production resulting from mutation of the *ina* genes, is not only a result of increased *raiI* expression, but also involves other as yet uncharacterised factors.

Disruption of the *ina2* and *ina3* genes in strain 8401 carrying the symbiotic plasmid, results in the development of Fix^- nodules on pea, this phenotype is suppressed by decreasing AHL production in these strains. The effect of the mutation of the *ina* genes on AHL production and nitrogen fixation will be described.

CCS 12 The quorum sensing-controlled gene *rhiD* is involved in symbiosis in *Rhizobium leguminosarum* biovar *viciae*

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The *rhi* genes, located in the symbiotic plasmid pRL1JI of *Rhizobium leguminosarum* biovar *viciae*, are expressed in the rhizosphere and play a role in the interaction with legumes. A novel ORF termed *rhiD* was found in the *rhi* region encoding rhizosphere-expressed genes. The *rhiD* gene encodes a 215-amino-acid predicted protein that belongs to the OMP25/RopB family of outer membrane proteins. *rhiD* expression is regulated in a cell-density dependent manner, under the control of several quorum sensing regulatory genes. Mutation of the *rhi*-AHL synthase or the regulator reduced *rhiD* expression by about 50%. However mutation of *traR* and *traI* reduced *rhiD* expression to background levels, suggesting that *rhiD* is primarily under the control of plasmid transfer regulator, but mutation of *rhiD* had no effect on plasmid conjugation. Nodulation of the *rhiD* mutant was reduced by 66% on cv. Afghanistan peas and nitrogen fixation was reduced by 96%. Nodule sections showed infected cells, although decreased level of infection was observed compared to the wild type. Taken together, these results suggest a role for RhiD in the infection process for the establishment of symbiosis.

CCS 13 A role for KpsD in export of group 2 capsular polysaccharides in *Escherichia coli*

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Capsular polysaccharide expression is an important virulence factor for many invasive bacterial pathogens of man. The export pathway for group 2 capsular polysaccharides is conserved in *Escherichia coli*, where *E.coli* are capable of polysaccharide export irrespective of the polysaccharide repeat structure. The KpsD protein plays a role in the final steps of the export pathway across the outer membrane. Initially KpsD was thought to be a periplasmic protein but recent evidence from our laboratory would suggest that this is not the case.

- KpsD is detectable in outer membrane fractions, as well as in inner membrane and periplasmic fractions.
- Immuno-fluorescence microscopy has shown KpsD to be localised to the same site as the onset of encapsulation.
- KpsD has been shown to be exposed on the cell surface by FACS analysis. The C-terminus is not exposed.
- Secondary structure analysis predicts up to eight transmembrane β-sheets, potentially forming a β-barrel, and a large periplasmic C-terminal domain.

Work is currently underway to test the predicted topology of outer-membrane insertion of KpsD. A truncated KpsD protein, containing the predicted barrel domain, is localised solely to the

outer-membrane. We are also testing the potential pore-forming ability of full length KpsD and truncates.

Our current model is that KpsD participates in the export pathway by forming pores in the outer membrane, through which group 2 capsular polysaccharides are extruded.

CCS 14 The *dosS/dosR* two component system of *Mycobacterium tuberculosis*: Response to different signals *in vitro*

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The pathogenic bacterium *M. tuberculosis* survives a number of hostile environments during infection and, in addition to causing active disease, is also able to persist within the lung for a number of years. Two component regulatory systems, made up of a sensor histidine kinase and a response regulator, are one of the mechanisms by which the bacterium senses and adapts to the host environment.

M. tuberculosis has 11 probable two component regulator genes. One of these, the *dosRS* (*Rv3133c/Rv3132c)* system has been shown to respond to hypoxia, which is implicated in the switch to a persistent state. We have previously shown that a *dosR* mutant is hypervirulent *in vivo* and in IFNg-activated macrophages (1). We have looked at other signals that may be involved in the up-regulation of this system *in vitro* using quantitative RT -PCR, and have found at least two other conditions that induce this system. We have also investigated the phenotype of the *dosR* mutant under a number of conditions *in vitro*, including a model of persistence. We discuss the relevance of the signals that *dosR* responds to *in vitro*, and relate these to the conditions experienced *in vivo*.

Reference: (1)Parish, T., Smith, D. A., Kendall, S. L., Casali, N., Bancroft, G. J. & Stoker, N. G. Deletion of two-component regulatory systems increases virulence of *Mycobacterium tuberculosis*. Infect Immun 71, 1134-1140 (2003).

CCS 15 Understanding biofilm formation in *Pseudomonas fluorescens* SBW25 – an evolutionary approach

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Pseudomonas fluorescens SBW25 rapidly diversifies when propagated in a spatially structured micrcosm (static broth culture), producing a range of morphologically distinct niche-specialist genotypes. One of these morphs, termed Wrinkly Spreader (WS) has a characteristic wrinkled morphology on agar plates and forms a biofilm at the air-broth interface. There are two operons required for expression of the WS phenotype, a structural locus *wss*, and a chemosensory pathway *wsp*. A primary cause of the morphology on plates and biofilm formation is over-production of an acetylated cellulose polymer (ACP), triggered by a single point mutation in the *wsp* regulatory pathway, which controls ACP production encoded by *wss*. In order to address questions relating to ecological and evolutionary convergence the *wss* operon was deleted from the ancestral (SM) genotype and this ACP-defective mutant was allowed to evolve in a structured microcosm. We were particularly interested to know whether this genotype could, during the course of evolution, generate a genotype that is ecologically equivalent to WS. Within 5 days of evolution (~35 generations) mutants had evolved that were capable of colonising the air-broth interface. The biofilm formed by these genotypes is not made of cellulose. In compensation for the inability to express cellulose, the SM? *wss*-derived biofilm forming genotypes overproduce an attachment factor which requires the *hms* locus and is iron sensitive. The evolved strains isolated appear to be ecologically equivalent to WS, and demonstrate that access and colonisation of a new ecological niche can be achieved by using a number of different genetic and biochemical pathways.

CCS 16 Sensor sensibility: the input domains of mycobacterial two-component system sensor proteins

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The traditional paradigm for two-component systems is of a histidine kinase sensor protein detecting a signal and transmitting a phosphate group to a related response regulator protein. The sensor protein is composed of a signal detecting input domain, and a transmitter domain. The response regulator is composed of a receiver domain, and an output domain, the latter responsible for initialising cellular response to the signal, often by acting as a transcription factor. Herein, the input domains of mycobacterial sensor proteins (particularly those of *M. tuberculosis*), have been analysed using a number of bioinformatic techniques. These investigations shed light on the possible signals detected by the input domains.

Wednesday 10 September 2003

1400 Bioinformatics: how did we get here?

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Bioinformatics is a swiftly growing field centred on the structure of nucleic acid and proteins, and on the genetic code. It aims to yield information about the genome, which can be used for many purposes, from understanding vital processes to drug design. Many techniques are often combined into one complex computer package, so that it may be difficult to appreciate the role of these simpler algorithms.

There are numerous different types of operation involved in bioinformatics. They can be broadly grouped into nine types, which commonly must be done sequentially, although there are some exceptions. These include algorithms (1) to search and sort; (2) to translate; (3) to align sequences; (4) to calculate similarity or distance; (5) to construct groups, including trees and ordination plots; (6) to trace phylogeny, lateral gene transfer and recombination; (7) to identify; (8) to predict function from structure, and (9) to learn from the data and improve the system automatically. This contribution traces the origins of the main concepts. They come from a wide range of disciplines. They include computer science, numerical taxonomy, molecular genetics, medical diagnosis, psychology and statistics. Some outstanding problems remain. These are a challenge if bioinformatics is to fulfil its promise.

1440 How to manage and analyse poxvirus genomes with the Viral Genome Organiser and Poxvirus Orthologous Clusters software and still have time for a beer

C. UPTON
University of Victoria, BC, Canada

More than 30 poxvirus genomes (150-320kb), each encoding more than 150 proteins, have been sequenced. However, despite the availability of this information and significant research efforts, the proteins responsible for smallpox virulence and those important for a protective immune response are not well characterized.

In an effort to simplify characterization of these and other large virus genomes, we have developed a series of computer programs that make use of a single comprehensive SQL database (Virus Orthologous Clusters; VOCs). These programs, Virus Genome Organizer (VGO), JDotter and Base-by-Base (BBB) form a significant portion of the Poxvirus Bioinformatics Resource (www.poxvirus.org) and a new SARS virus Bioinformatics Resource (www.sarsresearch.ca). Our focus has been on providing easy-to-use tools that allow the molecular virologist to make full use of the available sequence data and analyze the data at the level of the genome, ortholog family and single protein/gene. A significant amount of preprocessing is performed on the genomes, genes and proteins to help the users and speed up analyses (BLAST searches, dotplots and the creation of orthologous genes families). Interfaces and menus are intuitive and enable researchers to make complex SQL database queries simply by clicking a series of buttons.

1540 An overview of Artemis

STEPHEN BENTLEY
Pathogen Sequencing Unit, Wellcome Trust Sanger Institute, Wellcome Trust, Genome Campus, Hinxton, Cambs CB10 1SA

During the past decade advances in DNA sequencing technology have lead to an explosion in genomic data generation. In order to handle, analyse and interpret such data many new computer-based methods have been developed. The Artemis software was primarily developed at the Sanger Institute as an annotation tool allowing the user-friendly display of DNA sequences overlaid with relevant features. As well as being able to read and display the output from externally performed analyses, Artemis has a range of built-in functions and the facility to launch database searches. Other features allow the user to easily navigate around a whole genome and to extract and summarise information from the annotation.

The presentation will aim to give an overview of the software as well as highlighting its potential uses both in research and teaching.

1620 MALDI-TOF-MS..A new tool in diagnostic medical microbiology?

VALERIE EDWARDS-JONES
Dept of Biological Sciences, Manchester Metropolitan University, Manchester

Matrix assisted laser desorption ionisation time-of-flight mass spectrometry (MALDI-TOF-MS) is a powerful analytical technique with important applications in microbiology. Sample preparation for MALDI-TOF-MS is relatively simple. Purified or crude sample is co-crystallised with an excess of organic matrix onto a target plate that is placed into the mass spectrometer. In most microbiological applications, a UV laser (337nm) is used to generate ions that are then pulsed into the flight tube. A series of these provide a complex spectrum that is a characteristic fingerprint. The spectra obtained have been shown to be reproducible and sufficiently different to allow bacterial speciation. Versatility and the rapidity of analysis has led to the development of a wide range of possible applications and the subsequent development of sophisticated mathematical algorithms to analyse the complex data output. Examples include analysis of bacterial DNA, detection of recombinant proteins, characterisation of unknown proteins, proteomics, detection of virulence markers, rapid characterisation of bacteria at genus, species, and strain, direct detection of biomarkers from samples, identification of possible vaccine components, detection of adhesion / virulence markers and detection of antibiotic resistance markers.

Studies have shown that differences exist between the major clones of MRSA (EMRSA 15 and EMRSA 16), seen at high frequency in the United Kingdom. Work is currently being undertaken to establish if the technique can be used to detect these strains directly from clinical specimens or short-term cultures. If this proves possible it may be that detection and simultaneous sub-typing directly from specimens in real-time is not too far away. The speed of analysis provided by MALDI-TOF-MS is a very attractive alternative to the development of nucleic acid amplification techniques for rapid pathogen detection and identification.

Thursday 11 September 2003

0900 a bioinformatics teaching programme: do as I say and not as I've done

JAMES O. McINERNEY
Dept of Biology, National University of Ireland Maynooth, Co. Kildare, Ireland

Never under-resource a good idea. This might sound like an obvious statement, however this has been the greatest problem we have faced at NUI Maynooth in our efforts to establish our bioinformatics teaching courses. We teach an undergraduate degree course in computational biology and bioinformatics and we teach an annual international bioinformatics summer school for postgraduates. It is obvious that a biology department would contribute substantially to the teaching of a computational biology degree course, however a biology department probably qualifies as the most ill-equipped place to carry out such an

exercise. The perception is that the only requirement is for a few internet-connected computers and the course will take care of itself. The reality is that the development of an integrated, workable computational biology degree course requires much, much more. In this talk I shall go through the requirements and the desiderata for the development of a teaching programme in computational biology and bioinformatics.

0940 An overview of the pathogen sequencing program at the Sanger Institute

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

The Wellcome Trust Sanger Institute is one of the leading genomics centres in the world, dedicated to analysing and understanding genomes. The Pathogen Sequencing Unit, at the Sanger Institute, focuses on the sequencing and analysis of small Eukaryotic and Prokaryotic microbes, including those that cause some of the most important human diseases, including Malaria, Plague, Tuberculosis and Typhoid.

Presented will be an overview of the process of whole genome sequencing: from DNA to data. Specific examples will be used to show how genomics has increased our understanding of the biology of these organisms and highlighted many interesting aspects of their genomic architecture and evolution.

1100 An overview of EBI

CATH BROOKSBANK
EMBL – European Bioinformatics Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge CB10 1SD

The explosion in genomic sequence and functional genomics information far exceeds the capacity of individual scientists to keep abreast of the data. If we are to fulfil the promise of genomics, we need to collect, store and curate it in ways that allow its rapid retrieval, and we need to build tools that allow us to mine and analyse the data.

The EBI is one of the few places in the world that has the resources and expertise to perform these vital functions. Building on more than 20 years' experience in bioinformatics, the EBI supplies information to biologists across the globe through its databases and other resources. It also performs state-of-the-art research, provides training and supports industry.

The mission of the EBI is to ensure that the growing body of information from molecular biology and genome research is accessible freely to all facets of the scientific community in ways that promote scientific progress.

1140 Horizontal gene transfer accelerates genome innovation and evolution

JAMES A. LAKE[1,4], RAVI JAIN[1,4], JONATHAN E. MOORE[4] & MARIA C. RIVERA[1,2,4]
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Horizontal gene transfer (HGT) spreads genetic diversity by moving genes across species boundaries. By rapidly introducing newly evolved genes into existing genomes, HGT circumvents the slow step of *ab initio* gene creation and accelerates genome innovation. But HGT can only affect organisms that readily exchange genes (exchange communities). In order to define exchange communities and understand the internal and external environmental factors that regulate HGT, we analyzed approximately twenty thousand genes contained in eight free-living prokaryotic genomes. These analyses indicate that HGT occurs among organisms that share similar factors. The most significant are genome size, genome G/C composition, carbon utilization, and oxygen tolerance. Exchange groups are, on average, estimated to contain 10^4 or more prokaryotic species, corresponding to an increase of 10^4 in genome innovation. Hence HGT appears to be responsible for a remarkable increase in genome innovation, greatly exceeding anything that could have been accomplished by clonal evolution alone.

1400 Analysing microarray data

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Biomathematics & Statistics Scotland, BioSS Office at the Rowett Research Institute, Aberdeen AB21 9SB

The introduction of microarray technology has had an huge impact on life sciences in recent years. The possibility to obtain a snapshot of the whole genome of an organism at a certain timepoint by measuring the (relative) expression of all genes simultaneously has opened many new possibilities to gain a deeper understanding of fundamental biological processes in humans, animals, plants but also bacteria and viruses.

However, due to both the enormous volume and the unique structure of microarray data, completely new problems of data handling and statistical analysis have to be tackled.

In this talk we will give an overview over the statistical aspects of microarray experiments. We will particularly focus on the preprocessing steps of image analysis and data normalisation, which play a crucial role in separating the biological effects of interest from experimental noise.

We will illustrate some of the main problems and the remedies that have been proposed to solve these.

1440 A phylogenetic supertree approach to prokaryotic phylogeny reconstruction – evidence for rampant HGT in early prokaryotic evolution

CHRISTOPHER J. CREEVEY
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The search for a phylogenetic tree uniting all life has been underway for more than 150 years. Progress has been hampered for a variety of reasons including difficulty in interpreting morphological characters and often by their scarcity and also by the patchiness of availability of molecular characters. It is still not entirely clear how best to construct a phylogenetic tree, but most would agree that a tree that is strongly supported by the vast majority of available data is a preferred tree. We have developed a meta-analytical supertree method of phylogenetic analysis. This approach makes use of large amounts of data from individual source trees derived from discrete datasets such as individual genes. Using this method we can find a phylogenetic tree that is agrees with the majority of the trees from individual genes. We have developed a randomisation test to detect conflicting signals such as might be caused by horizontal gene transfer. We have also developed methods for evaluating the robustness of phylogenetic hypotheses. We have analysed the prokaryotic phylogeny and demonstrate that strong phylogenetic signal is only located at the tips of the tree, and deeper relationships strongly conflict with one another. This analysis provides strong support for the recently espoused theory of the "Darwinian Threshold" for the formation of species in early cellular life.

1500 Intact cell MALDI fingerprints and biomarkers for the sub-typing of MRSA

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The ability to rapidly differentiate micro-organisms to genus and species level can be a valuable tool. However, in many instances, the pathogenicity and virulence characteristics of a given micro-organism are strain dependent, and it is therefore essential that we can apply these same rapid methods to permit strain level discrimination.

The use of MALDI-TOF mass spectrometry on intact cell micro-organisms; Intact Cell MALDI (ICM), has been shown by numerous workers to be effective for discrimination to species level identification. In order to achieve sub-typing of micro-organisms it has been essential to develop standardised methods. For Methicillin resistant *Staphylococcus aureus*, we have developed such a standardised method.

This was used for the ICM analysis of a collection of isolates, which had been characterised previously using conventional and molecular subtyping methods, namely antibiograms, phagetyping and PFGE. These isolates included diverse sporadic and epidemiologically related isolates. The method developed incorporated standardised conditions for both the culture of the isolates and for preparation of the MALDI slides.

Examination of the data, using software developed "in-house", illustrated that key areas of the spectra allow identification and subtyping of MRSA. Particular strain marker peaks have been identified.

Further studies suggest that these biomarker peaks may be reproducible across many conditions e.g. culture media and incubation conditions. Thus providing a robust tool for the identification and, more importantly, subtyping of MRSA, which could have significant impact on the detection and infection control of nosocomial infections.

1540 Bioinformatics-based strategies for rapid microorganism identification by mass spectrometry

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Approaches for microorganism identification, exploiting the wealth of information contained in prokaryotic genome and proteome databases, will be discussed. They are based on determining the masses of a set of biomarker proteins detected from intact unknown organisms by MS. Matching experimental against sequence-derived masses of proteins (found together with their organismic sources in proteome databases) and ranking the organisms according to matches, results in microorganism identification. Statistical analysis of proteome uniqueness as a function of mass accuracy and proteome size suggests a means to evaluate the rate of false microorganism identifications. Parameters characterizing the overlap of individual proteins within a specified mass window in pair-wise proteome comparisons will be discussed. The identification specificity can be improved by several orders of magnitude with: **a)** imposing rational constraints on the number of potential biomarkers, e.g., including only highly-expressed proteins, and **b)** accounting for the most-common post-translational modification not directly reflected in the proteome database. The use of statistical gene sequence analysis (codon indexes) to create *in silico* a protein biomarker database for microorganism identification by MS will be presented. In that approach, each database entry is selected directly using only microbial genome sequence information.

1620 Bioinformatics: where are we going?

C. MILLER
Paterson Institute for Cancer Research, Manchester

The need to turn raw data into knowledge has led bioinformatics to focus increasingly on the manipulation of complex inoformation. This shift of emphasis parallels similar ones in both cryptography and Artificial Intelligence, and by exploring these we can develop an understanding of the way bioinformatics is changing. With specific examples from the microarray and gene expression field, we'll consider some of the key issues and challenges faced by bioinformatics as it attempts to handle the complexity of modern molecular biology.

Education & Training Group
POSTER

ET 01 Teaching bioinformatics in Thailand, problems and solutions

PANOMWAN UDOMKAN & RUNGRACH WANGSPA
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Bioinformatics has been of interest to undergraduate students of the department of Biology, Faculty of Science, Chiang Mai University ever since it was added to the curriculum 3 years ago. The class began with BLAST searches of a short oligonucleotide sample. Six-frame translation of the BLAST results preceded functional and structural analyses of the translated amino acid sequences. Multiple sequence alignment programs were demonstrated at the end of the course. Several problems were encountered during the years. Limited availability of computers restricted the number of students attending the class. Low bandwidth connection to the internet slowed the course progress. Lacking comprehension of underlying concepts and a good command of English language restrained some students from achieving the course objectives. Some of the problems have been addressed. Larger internet feeds were recently augmented while a new student computer facility will increase the number of students enrolled. Future plans include developing a Thai version of molecular biology tools and locally store nucleotide and protein databases.

Systematics & Evolution Group
POSTERS

SE 01 Use of fluorescent amplified-fragment length polymorphism (FAFLP) analysis to identify polymorphisms in *Mycobacterium tuberculosis* using *Xho*I/*Hha*I: comparison of experimental versus *in silico* data

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The whole-genome fingerprinting technique FAFLP was used to identify polymorphism in different strains of *Mycobacterium tuberculosis*. In our previous study, FAFLP was able to differentiate between clinical isolates of *M. tuberculosis* that possess single copy of IS*6110*. However, this study showed that for this GC rich genome many predicted fragments were not detected experimentally when the *Eco*RI/*Mse*I enzyme combination FAFLP data was compared with the *in silico* data for the sequenced strains of *M. tuberculosis* (H37Rv and CDC1551).

In the present study, we have investigated whether changing the enzyme combination used for digestion of *M. tuberculosis* H37Rv to *Xho*I/*Hha*I would give an exact match between the *in vitro*/*in silico* FAFLP fragment data. The genome sequence of *M. tuberculosis* H37Rv was analysed using TIGR's 'Restriction Digest Tool' (www.tigr.org). Data concerning the size and predicted number of fragments following an *Xho*I+GCT/*Hha*I digest of the genome were imported into a spreadsheet where the fragment size data was adjusted to allow for addition of primer sequences during PCR, before comparing the predicted *in silico* fragments to the *in vitro* results. Only 48% of the predicted fragments were visualised *in vitro*. In conclusion, changing the enzyme combination used in FAFLP analysis of *M. tuberculosis* H37Rv does not allow the *in vitro* and *in silico* FAFLP data for this GC rich genome to exactly match, even when known PCR enhancers such as glycerol and DMSO are added.

SE 02 Molecular evolution of fungi by using of aminoadipate reductase gene

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Aminoadipate reductase(*lys2*) is a fungal-specific gene and generally appears in a single-copy. The *lys2* gene is one of the most useful tools for revealing the phylogenetic relationships among fungi. In this study, we amplified and sequenced the PCR and RT-PCR products using the *lys2*-specific primers from 15 ascomycetes, 4 basidiomycetes, 1 zygomycete, and 4 chytridiomycetes. We analyzed phylogenetic tree based on these above twenty-four of *lys2* gene sequences and the known eight *lys2* sequences from ascomycetes.

The phylogenetic tree based on LYS2 is almost consistent with that based on 18S rRNA. This study reveals that the *lys2* gene was distributed in the course of fungal evolution. In addition, LYS2 would be used for good marker in order to detect fungi in environment.

SE 03 *Zimmermannia helvola* gen. nov., sp. nov., *Zimmermannia alba* sp. nov., *Zimmermannia bifida* sp. nov., *Zimmermannia faecalis* sp. nov. and *Leucobacter albus* sp. nov., a novel member of the family *Microbacteriaceae*

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Seven strains of *Actinobacteria* were characterized by a polyphasic approach to clarify their taxonomic position. On the basis of chemotaxonomic, 16S rDNA analysis and DNA relatedness, strain IAM 14851^T was can be classified into the cluster of the genus *Leucobacter* and was proposed to be a new species, *Leucobacter albus* sp. nov. The other six strains formed a cluster in the family *Microbacteriaceae* with the following characteristics: the major menaquinone is MK-8-10; the DNA G+C content ranges from 62-68 mol%; the diamino acid in the cell wall is diaminobutyric acid (DAB); and the muramic acid in the peptidoglycan is an acetyl type. The major fatty acids are ai-$C_{15:0}$, $C_{16:0}$, i-$C_{16:0}$, and ai-$C_{17:0}$. On the basis of morphological, physiological, and chemotaxonomic characteristics, together with DNA-DNA hybridization and 16S rDNA sequence comparison, the new genus *Zimmermannia* gen. nov. is proposed for these seven strains in the family *Microbacteriaceae*, and four new species are also proposed: *Z. helvola* sp. nov. (IAM 14726^T), *Z. alba* sp. nov. (IAM 14724^T), *Z. bifida* sp. nov. (IAM 14848^T), and *Z. faecalis* sp. nov. (IAM 15030^T). The type species of the genus is *Z. helvola*.

SE 04 *Campylobacter hyointestinalis* and *Campylobacter lanienae*: taxonomic relationships and phylogenetic chronometers

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C. hyointestinalis is found in the intestinal tract of pigs, cattle, deer and hamsters. It may cause sporadic intestinal infections in humans. Two subspecies were defined on the basis of high DNA-DNA hybridisations and phenotypic differences. Comparison of 16S rDNA sequences revealed rather low similarities of 95.7 to 99.0% between both subspecies. *C. lanienae* was recently described from the faeces of healthy abattoir workers. It was initially shown to be distinct from *C. hyointestinalis* subsp. *hyointestinalis* by 16 S rDNA sequence analysis, but subsequently found to be closely related to *C. hyointestinalis* subsp. *lawsonii*. To assess the taxonomic status of *C. lanienae*, we studied 8 strains representing both species. We confirm that 16S rDNA sequences of *C. hyointestinalis* subsp. *lawsonii* are very similar (97.2-98.2%) to those of *C. lanienae*, and less similar to *C. hyointestinalis* subsp. *hyointestinalis* strains (95.4-96.8%). In DNA-DNA hybridisations, we found 84 to 90% hybridisation between both *C. hyointestinalis* subspecies, whereas *C. lanienae* had 31 to 48% hybridisation with *C. hyointestinalis*, clearly demonstrating that *C. lanienae* is a separate species, despite its high 16S rDNA sequence similarity with *C. hyointestinalis*. We are currently assessing the similarity of other molecular chronometers including recA, rpoB and 23S.

SE 05 Genetic instability and agarase production by *Streptomyces coelicolor* A3(2)

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

The linear chromosome of *Streptomyces coelicolor* A3(2) represents one of the most spectacular examples of genetic instability amongst prokaryotes. Sequencing of the genome has revealed 14 regions on the chromosome that are thought to be recently acquired insertions. These insertions are located on the 'arms' of the chromosome, which are considered to be very unstable. Previous studies in this laboratory showed the transfer of the *strA* gene from a putative *S. griseus* to two soil isolates – ASB37 and ASSF15. Phenotypic and phylogenetic analysis showed these to be closely related to *S. coelicolor* A3(2). *S. coelicolor* A3(2) also has the unusual ability to utilise agar as a sole carbon source through the production of an extracellular agarase (*dagA*). Other organisms that have been found to produce agarase are *Cytophaga flevensis* and *Pseudomonas atlantica*, and only one other streptomycete- *S.cyaneus*, therefore it is possible to distinguish *S. coelicolor* A3(2) from other streptomycetes.

The aim of the current investigation is to determine the diversity and structural integrity of the antibiotic gene clusters within *S. coelicolor* populations. Subtractive hybridisation and ARDRA will be used to study the evolution of *S. coelicolor* A3(2) chromosome. The distribution of *dagA* within *S. coelicolor* populations and also within streptomycete type cultures will also be determined as will the origins of *dagA* within *S. coelicolor* A3(2).

SE 06 *Sinorhizobium* and *Ensifer*: taxonomic relationships assessed by sequencing of several housekeeping genes

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Ensifer is a genus of predatory soil bacteria with one species, *E. adhaerens*, that was described in 1982, mainly on the basis of phenotypic characteristics. *Sinorhizobium* was created in 1988 for fast growing rhizobia from soybean and now has at least 10 species. According to 16S rDNA phylogeny and *recA* phylogeny, *Ensifer* forms a single cluster with *Sinorhizobium* and on this basis we have recently requested an Opinion from the Judicial Committee to merge both genera and retain the name *Sinorhizobium*. To collect further evidence in support of this proposal we have now determined the partial sequences of the *gyrB*, *rpoB* and *atpD* genes for *Sinorhizobium* and *Ensifer* strains. Sequence similarities for these genes confirm the close relationship of both genera. Branching patterns of the *gyrB* and *rpoB* neighbour joining trees also support this, but in the *atpD* tree both genera form separate but related clusters. We are currently sequencing additional housekeeping genes, including *rpoD*, *nifH* and *glnA*, to verify if other molecular chronometers will support the proposed merger of both genera.

SE 07 Molecular characterisation of the lower respiratory tract microbiota

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The use of culture to characterise microbial communities has been demonstrated to be inadequate. In some cases less than 1% of the species present can be identified using this approach.

In contrast, molecular-based methods appear to provide a more comprehensive representation of the organisms present. Such methods have been used to characterise the microbiota of many environmental samples but so far have only been applied to a small number of clinical samples.

Many respiratory diseases are believed to have a bacterial component but the microbiota has only been characterised using culture. We aim to use molecular methods to identify uncharacterised bacteria, which may contribute in the pathogenesis of respiratory disease.

Restriction analysis of bacterial 16S rDNA clones have enabled us to characterise the microbiota in lower respiratory tract samples from individuals with no known respiratory condition and those with respiratory diseases (e.g. asthma and COPD). Results have revealed the presence of many different bacterial species in both sample sets, including those which were culture negative. Although this is only preliminary data, we can demonstrate the complexity of bacterial communities in the lower respiratory tract.

SE 08 Identification and discrimination of EMRSA 15 & 16 using a BioNumerics™ Database of Intact Cell MALDI Spectra

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MRSA is major nosocomial pathogen world-wide and is increasingly isolated from systemic infections. Improved infection control and emerging glycopeptide resistance demands timely and accurate surveillance. Recently, the technique of Intact Cell Matrix Assisted Laser Desorption/Ionisation Time Of Flight Mass Spectrometry (ICMS) has been described for the rapid identification of staphylococci including MRSA.

EMRSA isolates from the Northwest of England (previously genotyped and phenotyped) were analysed by ICMS. A subset of isolates was also characterised by Multilocus Sequence Typing (MLST). Data processing scripts were written to allow capture and analysis of ICMS spectra in the software BioNumerics™ and used for combined analysis of ICMS and MLST data.

Previously identified genus and species specific mass components were recognised in all the isolates analysed. The mass differences in the range representing potential strain specific biomarker peaks were reproducibly identified for each of the major MLST types. ICMS data of MRSA reference strains imported into the BioNumerics™ software package was used to create a database that facilitated the rapid identification of unknown isolates and strain specific biomarkers.

ICMS is a novel technique that is low cost, rapid and provides discriminatory and reproducible biological fingerprints. ICMS has the potential to impact significantly on the surveillance and control of major nosocomial pathogens such as MRSA.

SE 09 The use of BioNumerics ä for interpretation MALDI-ToF mass spectrometry data obtained from vancomycin intermediate and resistant *Staphylococcus aureus* isolates

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Matrix-assisted laser desorption ionisation time-of-flight mass spectrometry (MALDI-ToF MS), or intact cell mass spectrometry (ICMS), has been used for the rapid identification and typing of a number of bacterial pathogens of human origin. The technique produces a characterisitc mass/charge spectrum, or fingerprint, from intact bacterial cells in a matter of minutes. Application of the technique to the rapid, routine diagnosis of bacteria from clinical matterials has been hampered by limitations in the software used to examine the complex data generated.

We have used ICMS to examine a collection of vancomycin intermediate and resistant *Staphylococcus aureus* (VISA/VRSA) isolates which were derived from epidemic MRSA strains by serial passage in vancomycin. Data were analysed using BioNumerics™ software which converts the mass/charge ratio peaks into a fingerprint of clear, quantifiable bands. Using BioNumerics™ we were able to readily identify bands specific to VISA/VRSA isolates and group the isolates, using a number of clustering algorithms, into parent and derivative lineages.

Monday 8 September 2003

0900 Characterisation of the human gut microbiota using molecular based methods

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The bacterial community in the human intestinal tract affects gastrointestinal function, health and well-being of the host. To understand antagonistic and synergistic interactions of the intestinal microbiota a more complete coverage of the microbial diversity in this ecosystem is required. So far, 1536 rDNA clones have been retrieved and analysed to improve the 16S rRNA database of human gut bacteria. These analyses indicate that the vast majority of faecal organisms have eluded scientific description. The improved database of ribosomal RNA sequences has greatly facilitated the design diagnostic oligonucleotide probes. So far, more than 50 oligonucleotide probes targeting gut bacteria at different levels of the phylogenetic hierarchy have been designed and validated. Present efforts are directed towards the analysis of high numbers of samples on the basis of 16S rRNA. Flow cytometry has been successfully introduced as a rapid method for the detection of fluorescently labeled gut bacteria. The development of molecular methods enabling the monitoring of functional gene expression is another major challenge.

0945 Stable isotope probing for assessing functional diversity

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Microbial ecologists need to be able to study microorganisms based upon their identity and the environmental processes which they perform. These criteria are key to a full ecological understanding of microbes in their natural environment. However, unlike macro-ecologists, microbiobgists find it difficult to directly observe a single species performing an ecological process *in situ* within a multi-species complex. As such, microbiologists have learnt to apply elegant molecular methodologies to associate microbial identity with environmental functions. One such technique is the stable isotope probing strategy and this lecture summarises the role stable isotopes are playing in addressing issues of phylogeny and its association with function.

1100 Fluorescence *in situ* hybridisation, molecular markers for assessing community structures

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The ribosomal RNA (rRNA) approach to microbial evolution and ecology has become an integral part of environmental microbiology. Large database exist that encompass besides the 16S rRNA sequences of the majority of the validly described bacteria also numerous so-called "environmental" 16S rRNA sequences. rRNA-targeted oligonucleotide probes with defined specificities can now be designed in a directed way. When such probes are labeled with fluorescent dyes or horseraddish peroxidase they can be used to identify single microbial cells by fluorescence *in situ* hybridisation (FISH) directly in complex environmental samples. An update on available methods will be given including possibilities to identify small bacterial cells in oligotrophic environments by catalyzed reporter deposition (CARD)-FISH. With optimized methods and proper controls the FISH technique can yield exact cell numbers and spatial distributions for defined bacterial populations. Furthermore, it will be outlined how FISH together with other methods can be used to study the diversity, structure and function of complex microbial communities.

1145 Enhanced biodegradation of the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) in the rhizosphere of *Trifolium pratense*

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The rhizosphere is defined as the zone of soil directly influenced plant roots. Deposition of plant-derived organic compounds in the rhizosphere results in enhanced microbial activities and numbers. Rhizosphere enhanced biodegradation of organic xenobiotics has been reported, but the mechanisms are not well understood. We have shown that the rhizosphere effect on ^{14}C-2,4-D mineralisation is plant species and plant age specific. For example, the maximum mineralisation rate was significantly ($p<0.05$) enhanced (*Trifolium* = 10.0 d^{-1}, non-planted = 7.6 d^{-1}) for 25- and 60- day old *Trifolium pratense* rhizosphere, but not in 116-day old *Trifolium* rhizosphere or for *Lolium perenne* rhizosphere of any age. Most probable number estimates and single strand conformational polymorphism analysis of *tfdA*α genes revealed that the plant species did not select for or enrich 2,4-D degrading organisms. Based on this evidence, we suggest that specific compound(s) released by the *Trifolium* root stimulate 2,4-D mineralisation by acting as inducers of the pathway. To investigate this possibility, experiments have been initiated to: (a) isolate the active component of the rhizodeposits (exudates or structural root components); (b) mimic the rhizosphere effect by addition of potential inducers; and (c) quantify the effect of rhizodeposit components on *tfdA*α transcription.

1200 Novel molecular methods to study prokaryotic functional diversity in the deep biosphere

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Considerable advances have been made in recent years through the application of molecular biological methods for the analysis of structural and functional genes of prokaryotic communities directly from the environment. Although, these methods have given insights into microbial community structure, succession and allow comparisons of diversity between habitats they are limited since they do not determine which community members are active. This is particularly relevant to environments, which contain high proportions of as yet uncharacterised and uncultured organisms, like the deep sub-seafloor biosphere. Here we report the use of the new technique stable isotope probing with ^{13}C-labelled compounds to study the functional diversity of an acetate-utilising sulphate reducing enrichment from marine sediments. Small concentrations of ^{13}C-labelled DNA were extracted from the enrichment using a refined CsCl-ethidium bromide density centrifugation method and PCR was used to detect 16S rRNA and the functional dissimilatory sulfite reductase (*dsrAB*) genes. Novel bacterial 16S rRNA and *dsrAB* genes were retrieved which led to the design of new 16S rRNA

primers which could specifically target these novel bacterial sequences. Our results suggest the presence of an active uncultured group of bacteria in marine sediment enrichments that is able to utilise acetate for sulphate reduction.

1215 Analysis of the distribution of plasmid and chromosomal genes in strains of enteroaggregative *Escherichia coli* using a DNA microarray

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Enteroaggregative *Escherichia coli* (EAEC) is the most commonly isolated pathogenic *E. coli* group from patients with diarrhoea in England. EAEC are characterised by the way they adhere to HEp2 cells in a stacked-brick formation. Typical EAEC possess a plasmid carrying genes such as *agg*R (fimbrial regulator gene) and *aa*p (antiaggregative protein), whereas as atypical EAEC are less well defined. The aim of this study was to genotypically characterise typical and atypical EAEC in order to design new detection methods targeting genes characteristic of both groups of EAEC. Typical and atypical EAEC were analysed using DNA microarrays to determine whether both groups share certain plasmid and chromosomal genes. Amplified PCR products from fifty-two genes, including adhesins, toxins, and regulatory genes, characteristic of EAEC strain 042, were added to an array previously designed to screen for virulence factors in *E. coli*, making a total of 85 probes altogether. Thirty-six genes were shared by typical and atypical EAEC, thirty-two of which are located on a large pathogenicity island found in EAEC 042. These genes will be targeted for further investigation to develop a novel and rapid detection system for EAEC.

1400 Identification of enterohaemorrhagic *Escherichia coli* genes required for colonisation of cattle

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Enterohaemorrhagic *E. coli* (EHEC) cause severe enterocolitis in humans, which may be complicated by life-threatening systemic sequelae. Ruminants are important reservoirs of EHEC and direct or indirect contact with ruminant faeces is the leading antecedent to human EHEC infection. The O-serotypes predominantly associated with human disease are O157 and O26. Strategies to control EHEC in ruminants are expected to lower the incidence of human infection, however the mechanisms underlying colonisation of the ruminant intestine by EHEC are poorly understood. EHEC O157 and O26 differ in virulence in calves and colonise the bovine intestinal tract by distinct mechanisms. We have used signature-tagged mutagenesis (STM) to identify EHEC O157 and O26 genes required for intestinal colonisation of calves. STM is a DNA hybridisation-based technique to track the fate of uniquely tagged transposon mutants simultaneously during infection. Approximately 1800 mini-Tn*5*Km2 mutants of *E. coli* O157:H7 and 400 mutants of *E. coli* O26:H$^-$ have been screened by oral inoculation of calves. To date 61 *E. coli* O157:H7 genes and 53 *E. coli* O26 H$^-$ genes required for intestinal colonisation have been identified. Our studies have identified new targets for the diagnosis and control of EHEC infections and indicated roles for novel cytotoxins and locus of enterocyte effacement-encoded proteins in EHEC persistence and enteropathogenesis in calves.

1445 Relevance of genome sequencing of campylobacter

J. WELLS
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Abstract not received

1600 Comparative transcriptomics: DNA-array analysis of global iron- and Fur-dependent gene expression in *E. coli* K-12 and *Salmonella*

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Iron is an essential nutrient for virtually every organism, but can be toxic and is often growth limiting due to its poor availability. In Gram-negative bacteria, iron acquisition and iron storage are generally regulated in response to iron availability at the transcriptional level by the ferric uptake regulation protein (Fur). Fur also regulates other aspects of bacterial physiology, such as redox-stress resistance and virulence, and can thus be considered to be a truly 'global' gene regulator. In order to determine the full extent of the Fur 'modulon' and Fe 'stimulon', we have performed a global transcriptomic analysis of Fe- and Fur-dependent gene expression in the gut commensal, *E. coli* K-12, and in the related pathogen, *Salmonella enterica* sv. Typhimurium. These experiments show many similarities regarding the response of these two organisms to iron, as well as some major differences. These similarities and differences seem mainly to reflect the genomic make up of *E. coli* and *Salmonella* – common regulatory responses are seen for 'shared' genes, with differences in expression pattern being mostly restricted to genes unique to either of the two organisms. The differences in expression profile are likely to correlate with the distinct life-styles of *E. coli* and *Salmonella*.

Tuesday 9 September 2003

0900 Hunting for novel genes in the environment

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The direct extraction and analysis of total community DNA from environments such as the open oceans, sediments and soils has resulted in the characterisation of bacterial populations *in situ* without the need for isolation and cultivation. We have used degenerate PCR primers based on conserved regions of genes to detect diversity within adaptive traits such as antibiotic and extracellular, degradative enzyme production. The distribution and diversity of antibiotic gene clusters was studied by targeting key conserved genes/sequences in antibiotic gene clusters and provided evidence of novel sequences within clusters of polyketide biosynthetic genes. A similar approach revealed a cluster of genes involved in streptomycin production, which were present in *S. griseus* strains in place of the well characterised *str/sts* cluster. The data indicates that a further *str* cluster has evolved in *Streptomyces griseus*, perhaps resulting from an extensive duplication, and recombination with existing clusters in closely related strains has resulted in some mosaic structure of the cluster. We have also made lambda and BACS environmental gene libraries to detect by expression novel genes/clusters involved in antibiotic and enzyme production This allowed detection of novel genes without prior knowledge and use of PCR screening. This approach does have disadvantaged being labour intensive and technically difficult especially when dealing with DNA extracted from soil.

0945 MRSA and other Gram positive cocci

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Staphylococci (*S. aureus* and coagulase negative staphylococci, CoNS) are colonisers of human skin and nasal nares, and among the most common opportunistic pathogens. *S. aureus* is a major cause of both community and hospital-acquired infections, including cellulitis, septic arthritis, osteomyelitis wound infection and septicaemia. There has been a dramatic rise in the incidence of methicillin-resistant *S. aureus* (MRSA) bacteraemia throughout the UK. Highly virulent strains of community-acquired MRSA (CMRSA) are also emerging as a significant problem. CMRSA isolates have a type IV *mec* gene that is responsible for the methicillin resistance phenotype. Genome sequencing of a CMRSA isolate in Japan has also revealed the presence of a bacteriocin operon (bsa) that appears to be related to epidermin. We have used a multiplex *mec* typing scheme that identifies the four *mec* types found in MRSA and have used this to type MRSA isolates collected from this hospital and elsewhere. We have also developed a PCR method to screen MRSA isolates for the presence of the bsa operon. We are investigating whether the production of a bacteriocin gives CMRSA strains a colonization advantage and could explain their rapidly increasing incidence of isolation.

1130 Clostridia, other Gram positive rods

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The Health Protection Agency Foodborne Pathogen Reference Unit provides reference services for the detection, identification and characterisation of Gram-positive food-borne pathogens including *Clostridium botulinum*, *Clostridium perfringens*, *Listeria monocytogenes*, *Staphylococcus aureus*, and *Bacillus* species. Traditional microbiological methods used in the Unit have included phenotypic and immunological assays for identification, typing and toxin detection which are expensive, slow, labour intensive, lacking in discrimination and require the use of specific reagents. As alternatives to these traditional approaches we have developed and implemented a range of generic molecular techniques for the investigation of food poisoning. These techniques have included: DNA extraction protocols applicable to cultures, enrichment broths and clinical specimens; high throughput real time PCR assays; and generic amplified fragment length polymorphism (AFLP) analysis for fingerprinting of isolates. This talk will illustrate some of the application of these molecular strategies, including: real time PCR assays for detection of *C. botulinum* neurotoxins, *C. perfringens* enterotoxin and *L. monocytogenes* haemolysin gene fragments; and the AFLP analysis of *C. perfringens* and *L. monocytogenes*. These approaches have provided previously unavailable diagnostic information to other public health professionals allowing a better understanding of foodborne infections, in a more timely manner.

1215 Validation and standardization of diagnostic pcr for *Listeria monocytogenes*

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Conventional methods for the detection of *Listeria* in foodstuffs are generally cumbersome and time-consuming. The use of primary enrichment in Fraser broth and the use of Oxford and Rapid'Lmono (RLM) agar were assessed in comparison with polymerase chain reaction (PCR) for their ability to accurately detect and confirm the presence of *L. monocytogenes* in food products. Of the 27 food samples tested, 74 % were presumptively positive for *Listeria* on Oxford agar, while 44% were presumptively positive for *L. monocytogenes* on RLM. Samples confirmed to be positive for *L. monocytogenes* by the amplification of the *hly* gene (732 bp) were only 37%. The PCR was able to eliminate the false positives and detect all positive *L. monocytogenes* in the food products, unlike the conventional methods used in the industry. In addition to the incidence of *Listeria* species being higher than *L. monocytogenes* on selective media, there was also the occurrence of *Listeria*-like organisms. These organisms had typical appearance on selective media, but were non-*Listeria* species as confirmed by the PCR and API *Listeria*. The PCR proved to be a highly sensitive and rapid technique for the detection of *L. monocytogenes*.

1400 Dual reporters in Gram-positive bacteria

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The bacterial luciferase reporter (*luxCDABE*) originates from a Gram-negative bacterium. Difficulties expressing the genes in Gram-positive genera originally precluded this technology from being used in bacterial pathogens such as the *Staphylococcus* and *Listeria*. We have re-engineered the *lux* operon to allow expression in these organisms and have used it to study the behaviour of these bacteria under a wide range of environmental conditions. However problems have been found when studying phenomena such as bacterial motility and chemotaxis, when the physiological condition of the cell is altered in response to changes in the environment. We also evaluated the use of green fluorescent protein (GFP) as a marker and transcriptional reporter in these bacteria. Although GFP is less sensitive than *lux* it can be used in fixed samples for fluorescence microscopy for both tracking bacteria and following patterns of gene expression in particular environmental niches.

Lux and GFP have their own particular advantages and disadvantages as reporter/marker genes. To capitalise on the complementary strengths of each we have constructed dual reporter operons (*gfp-luxABCDE*) for expression in Gram-positive organisms. These have been evaluated as reporters in both *Listeria* and *Staphylococcus* and, as anticipated, allow gene expression to be assessed both in "real-time" *in vivo*, and also "retrospectively" on fixed samples by fluorescence microscopy/fluorometry.

1445 The rise and spread of catabolic genes for the degradation of *s*-triazine herbicides: from the field to the lab and back again

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Atrazine is one of the most widely used herbicides in the United States for control of broadleaf weeds in corn, sorghum, and sugarcane. Most of our knowledge concerning catabolic enzymes involved in the degradation of *s*-triazines and atrazine come from studies done using *Pseudomonas* sp. strain ADP. This bacterium uses atrazine as a sole source of N for growth and initiates catabolism via three enzymatic steps encoded by the *atzA*, *B* and *C* genes. These proteins are members of the amidohydrolase superfamily. Growth and culture-independent PCR methods have shown that genes essentially identical to *atzA*, *B* and *C* are present in many different genera of gram negative and gram positive bacteria isolated from geographically diverse locations in the world, suggesting their rapid global spread. The atrazine degradation genes have been localized to a 108 kb, self-transmissible plasmid in *Pseudomonas* ADP, which has been completely sequenced. Plasmid pADP-1 contains 104 putative ORFs and regions encoding transfer and replication functions were highly related to those present in pR751, an IncP plasmid. Complete copies of transposases flank each of the atzA, atzB and atzC genes, forming structures resembling nested catabolic transposons. This suggests that the pathway assembled via gene transfer and transposition events.. Functional analyses identified three new co-transcribed atrazine catabolic genes, atzD, atzE, and atzF, which encode cyanuric acid hydrolase, biuret hydrolase and allophanate hydrolase, respectively. The structure, function, and use of these catabolic genes to bioremediate atrazine-contaminated soils will be discussed. Taken together, our data reveal the complete structure of a catabolic plasmid and provide insight into how plasmids can rapidly evolve to encode the catabolism of compounds recently added to the biosphere.

1600 Whole cell microbial biosensors – applications in environment and health

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Biosensors have been applied very successfully to determine glucose concentrations from blood samples and a range of specific analytes for example in pregnancy testing. In general, these types of biosensor rely upon immobilised enzymes or antibody-antigen binding and do not report on cell physiology. In contrast, whole cell biosensors can help assess the impact of a variety of compounds on general cell health which is particularly valuable when trying to assess the impact of mixtures.

This presentation will describe how whole cell microbial biosensors have been developed to report upon cellular toxicity. Bacteria, algae, yeast and nematodes have been engineered to constitutively express either prokaryotic or eukaryotic luciferase and assays systems have been optimised to allow the determination of both acute and chronic toxicity from a variety of sample types including soil, water, food and heterogeneous waste material. When these luminescent biosensors are exposed to acutely toxic samples, they respond with a rapid decrease of light output. Similarly, dividing cell assays can be used to determine chronic toxicity. Of particular value is the ability of whole cell biosensors to report upon bioavailability of toxicity in environmental samples thus allowing a risk assessment to be carried out.

More recently, these biosensors have been used in combination with analyte-specific biosensors in both aqueous and immobilised formats to build up a picture of both bioavailable as well as potential pools of toxicity in a variety of sample types.

Environmental Microbiology Group
POSTERS

EM 01 Detection of sulphate-reducing bacteria in estuarine sediments by competitive PCR

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We developed PCR primers and a new quantitative method using PCR for the detection and the enumeration of SRB in natural environments. A PCR primer set selective for dissimilatory sulphite reductase gene (*dsr*) of SRB was designed. PCR amplification using the single set of *dsr*-specific primers resulted in PCR products of the expected size from 27 SRB strains tested, indicating that PCR using the newly designed primer set are useful for the selective detection of SRB from a natural sample. This primer set was used to estimate cell numbers by *dsr* selective competitive PCR using a competitor DNA. This procedure was applied to sediment samples from the River Colne estuary, Essex together with simultaneous measurement of *in situ* rates of sulphate reduction. High densities of SRB ranging from 0.2 - 5.7 x 10^8 cells ml^{-1} sediment were estimated by the competitive PCR assuming that all SRB have a single copy of *dsr*. Using these estimates cell specific sulphate reduction rates of 10^{-17} to 10^{-15} mol of SO_4^{2-} $cell^{-1}$ day^{-1} were calculated, which is within the range of, or lower than, those previously reported for pure culture of SRB. Our results show that the newly developed competitive PCR technique targeted to *dsr* is a powerful tool for rapid estimation of SRB numbers *in situ* and is superior to the use of culture-dependent techniques.

EM 02 Withdrawn

EM 03 Isolation and characterization of aerobic denitrifiers from agricultural soil

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Denitrificiation is generally accepted as an anaerobic process. However, in recent years it has been shown that bacteria can also reduce nitrate to nitrite under aerobic conditions. Characterization of biologically available nitrogen forms and their biological interconversion mechanisms is important for ecological and agricultural implications. In this study, aerobic nitrate reducers were isolated from greenhouse soil. Using nitrate reduction assay, it was found that 39 out of 60 isolates can reduce nitrate to nitrite under aerobic conditions. Five of those 39 isolates were selected for further characterization of their aerobic nitrate reduction activity. Four of those five isolates were found to have periplasmic nitrate reductase activity using MV^+ as a non-biological electron donor. Nitrite production of these isolates under aerobic conditions was determined using different nitrogen forms as sole nitrogen sources. It was found that nitrite accumulation in all isolates was higher when nitrate was the sole nitrogen source than when nitrate + ammonium was the sole nitrogen source. No nitrite accumulation was observed when ammonium was the sole nitrogen source. This study suggests that when ammonium exists together with nitrate, aerobic nitrate reduction does not lead to high nitrite accumulation.

EM 04 The use of PCR-TGGE and fluorescence microscopy in the study of a lab-scale contaminant plume

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A flow cell with internal dimensions 200x100x5mm, containing quartz sand (212-300µm) covered with a biofilm was set up to allow a degrading plume of potassium acetate to be formed. Mineral media was used as the flow through solution and 10mM potassium acetate added to mineral media was injected from a point source within the back wall of the flow cell. The biofilm was produced in a glass column by dripping an environmental culture through the sand. The culture used was grown from a contaminated water sample from borehole MW7 at a contaminated site in St Albans using 1mM potassium acetate as the carbon source.

Plume formation was monitored by measuring oxygen or nitrate distribution using UV excitation of the tracer dyes $Ru(phen)_3Cl_2$ and RBOE respectively. Images were taken using a CCD camera. Changes in the microbial population across transects of the plume and over time will be monitored with PCR-TGGE and fluorescence microscopy including total cell counts with DAPI, and active counts with the tetrazolium salt, CTC. The microbial populations formed on the quartz sand in the flow cell will also be compared to the original inoculum for the environmental culture and the culture used for producing the biofilm.

EM 05 Molecular detection of colonic microbial involvement in ulcerative colitis

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Ulcerative colitis is a severe chronic relapsing disease characterised by inflammation of the mucosa and submucosa of the human large intestine. There is compelling evidence that the colonic microflora plays an important role in both initiation and perpetuation of the disease - either through an improper host immune response to commensal flora or via the interaction of pathogenic bacteria with colonocytes. Faecal samples were obtained from healthy subjects and ulcerative colitis patients in

active and quiescent disease states. Fluorescence *in situ* Hybridisation (FISH) was used to detect differences in the colonic bacterial populations - principally *Bacteroides*, *Lactobacillus*, *Bifidobacterium*, *Clostridium* and sulphate-reducing species. Those patients with active inflammation of the colon had less total bacteria and lower numbers of lactobacilli when compared with those in remission. Colonic biopsies from colitic and asymptomatic patients were analysed by Denaturing Gradient Gel Electophoresis (DGGE). Profiles of bifidobacteria adherent to the colonic mucosa were similar in the two study groups. However, *Clostridium nexile*, *Lactobacillus salivarius*, *Bacteroides vulgatus*, *Bacteroides fragilis* and *Lactobacillus gasseri* were found exclusively in the diseased colon. These investigations are combined with a human dietary intervention study to examine the ability of a novel prebiotic combination to alter the gut microflora in colitis patients towards that of a healthy individual.

EM 06 The use of denaturing gradient gel electrophoresis as a tool for determining the bacterial species present in biopsy samples

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Until recently it has not been possible to accurately determine the exact bacterial species present in biopsy samples. The aim of this research was to design a method to more accurately identify bacterial species in biopsy samples. Human biopsy samples from the sigmoid rectal region were obtained from patients suffering from ulcerative colitis. Total DNA was extracted and PCR was carried out initially using previously described Ribosomal 16s gene primers pA (bps 9-28) to pH (bps1 540-1521). A second round of PCR (optimised annealing temperature of 50^0C) was then carried out using pA and gamma-GCclamp (bps 361-342), this last being a universal primer designed using INGENYMELT software. (This two stage approach was necessary because when PCR was carried out using a GCclamp primer on total DNA the GC rich regions in human DNA interfered with PCR.) The resulting fragment was 333bps long with a diagnostic hypervariable region flanked by conserved regions. The resulting PCR products were then separated by DGGE with a gradient running from 40% to 80% urea/formamide denaturants. Previously identified PCR products were run along side the samples as controls. The above improves on current diagnostic methods by employing a new universal PCR primer, significantly extending the length of the PCR fragment produced for sequencing, therefore enhancing diagnostic capabilities when analysing mixed bacterial populations in samples from ulcerative colitis.

EM 07 Analysis of the microbial composition of macroscopic "streamer" growths in acid mine drainage using a combination of terminal restriction enzyme fragment length polymorphism and fluorescent *in situ* hybridisation

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Acid streamers found in acidic and metal-rich waters in North Wales (Parys copper mine and Trefriw Iron Spa) were studied using both cultivation-based and molecular techniques. The most abundant microbial isolates in Trefriw Spa were acidophilic iron-oxidisers, while heterotrophs and iron-oxidisers were present in similar numbers in the Parys mine streamer. Total bacterial counts using 4',6-diamidino-2-phenylindole (DAPI) showed that cultivated bacterial numbers accounted for 1-2 % of the total microbial population from either site.

Fluorescent *in situ* hybridisation (FISH) was also used to study the streamer communities with RNA-targeting oligonucleotide probes. This revealed that 80-90% of microbes in both streamers were unknown β-*Proteobacteria*. Terminal restriction fragment length polymorphism (T-RFLP) of 16S rRNA genes amplified from total community DNA suggested that there was one dominant microbe within the Parys mine streamer, while two microbes dominated the Trefriw spa streamer. The identities of these microbes were determined by construction of 16S rRNA gene clone-libraries and sequencing of selected clones. Using these sequence data, probes were designed and used in further FISH analysis to confirm that these two β-*Proteobacteria* dominated both streamers as suggested by T-RFLP. These data demonstrate the effectiveness of the combined molecular approach to the elucidation of unknown microbial communities.

EM 08 Unravelling the contribution of individual *Pseudomonas fluorescens* genes to ecological success in the plant rhizosphere

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Pseudomonas fluorescens can successfully colonise the rhizosphere, a highly complex and dynamic environment whose composition varies both spatially and temporally. To determine the contribution of specific rhizosphere-induced genes to fitness in the rhizosphere, knowledge of the spatial and temporal patterns of gene expression are required so that competition assays can be performed in precisely the niche where expression of that gene occurs. To this end, we have developed a recombinase-based *in vivo* expression technology (RIVET) procedure to monitor gene expression in the rhizosphere, although have incorporated modifications to enable more efficient selection of induced cells. Here, the promoter of the gene of interest drives a reporter gene, *tnpR*, that encodes a site-specific resolvase. When *tnpR* is expressed in a genome containing the artificial substrate cassette (*res1-tet-lacI*^{q}*-res1*) it catalyses the excision (resolution) of the *tet* and *lacI*^{q} genes, rendering the cell and its progeny tetracycline sensitive. To the genome, we have further introduced a gene encoding green fluorescent protein (GFP) under the control of a pLac promoter, thus GFP is also expressed after an induction event. Resolution is both stable and heritable, and this modified RIVET procedure allows gene expression to be assessed directly in the rhizosphere, as well as *in vitro*.

EM 09 Interactions between bacteria via metabolic intermediates within artificially constructed environmental biofilms

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One of the ways in which bacterial interactions in biofilms occur is via metabolic intermediates. Such metabolic interactions can have a profound effect on biofilm structure, and in turn the biofilm architecture can profoundly affect metabolic interactions between the different microorganisms within the biofilm. To study such interactions we have developed novel techniques for rapidly constructing biofilms with defined internal architecture based on electrokinetics. Bacterial cells are guided to specific areas of a microelectrode array by dielectrophoresis, and immobilised on the electrodes. This allows the rapid formation of biofilms that have a specific and defined structure, microcolonies of different bacterial species and strains in different locations. We have made artificial, structured biofilms of *P. putida* and *Acinetobacter* sp., in which *P. putida* had the TOL plasmid *Pm::gfp* cassette inserted into its chromosome. When a consortium of *Acinetobacter* sp. and the *P. putida Pm::gfp* is grown on benzyl alcohol, interaction occurs between the different bacteria via the metabolic intermediate of benzyl alcohol, benzoate. Interaction is observed via the production of GFP by *P. putida Pm::gfp* in response to the benzoate produced by *Acinetobacter* sp. in the biofilm. This novel technique provides a new tool for the study of interactions between bacteria and will

enable the relationship between the structure and function of environmentally and industrially significant biofilm forming species to be elucidated.

EM 10 Interactions via inter-cell signalling molecules within artificially constructed bacterial biofilms

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Bacterial interactions in biofilms often occur via inter-cell signalling molecules such as the homoserine lactones (HSLs), in a phenomenon known as quorum sensing. Quorum sensing is known to play an important part in the formation and structure of biofilms. We have developed novel techniques for rapidly constructing biofilms with defined internal architecture based on electrokinetics. Bacterial cells are guided to specific areas of a microelectrode array by dielectrophoresis, and immobilised on the electrodes. We have used these techniques to make biofilms in which interactions via the inter-cell signalling molecule AHL could be observed between genetically modified *Agrobacterium tumefaciens*, which over expresses AHL, and *E. coli*. The *E. coli* strain was genetically modified to produce GFP in response to the presence of AHL. This novel technique provides a new tool for the rapid construction of biofilms that have a defined internal structure and will greatly assist in the study of how cell-cell signalling occurs within biofilms.

EM 11 Conjugation of mobile genetic elements between bacteria within artificially constructed bacterial biofilms

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The movement of DNA between bacteria of different species can lead to a change in evolutionary dynamics within an ecosystem and has become a major concern in the use of genetically modified organisms. The causes and mechanisms of how the transfer of DNA between bacteria occurs in natural environments is little studied due to the lack of techniques that can mimic the natural environment, which for most bacteria is the biofilm structure. We have developed novel techniques for rapidly constructing biofilms with a defined internal architecture based on electrokinetics. Bacterial cells are guided to specific areas of a microelectrode array by dielectrophoresis, and immobilised on the electrodes. We have made biofilms of two different strains of *P. putida* R1, one of which contained a *lac* operon repressor on its chromosome as well the well-characterised TOL plasmid which had been tagged with the genes for GFP production. GFP is expressed when the plasmid is transferred to a *P. putida* without the *lac* operon repressor. This novel technique of coupling well-known molecular biology methods with the construction of biofilms using dielectrophoresis provides a new tool for the investigation of DNA transfer between bacteria within the microenvironment of a biofilm.

EM 12 Regulation of phenazine antibiotics in relation to the autoinducer N-hexanoyl-L-homoserine lactone (HHL) production

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Regulation of bacterial gene expression can be coordinated in response to a variety of external environmental stimuli, including self produced diffusible signal molecules that allow cell-to-cell communication within a population. The most common signalling mechanism in Gm-ve bacteria uses acyl homoserine lactones. Phenazine antibiotics produced by the biocontrol agent *Pseudomonas aureofaciens* PGS12 are regulated by homoserine lactones utilizing DNA *lux* box homologues *phzI* and *phzR*, and by another level of regulation via the global regulatory genes *gacA* and *gacS*.

Our study is aimed to investigate the rate of HHL production under different cell densities and to determine the extent nutrient flow has affected production of phenazines, and to identify any another regulatory genes involved in phenazine production. We used a *lux* marked strain to report on the transcriptional activity of the phenazine operon. We were able to demonstrate that the rate of HHL production per cell was greater under nutrient deprived conditions rather than related to cell density. In a further study we have developed a mutant strain of PGS12 that acted as a biosensor based on OD measurements of PCA, specific for HHL. New regulatory genes for phenazine antibiotics were discovered.

EM 13 Use of a *fis:gfp* reporter to assess intracellular growth of *Salmonella typhimurium* in *Acanthamoeba polyphaga*

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Acanthamoeba polyphaga feeding on *Salmonella typhimurium* in a simple model biofilm were observed by light microscopy and a detailed record of interactions kept by digital image capture and image analysis. A strain of *S. typhimurium* SL1344 carrying a *fis:gfp* reporter construct (pPDT105) was used to assess intracellular growth in *A. polyphaga* on non-nutrient agar (NNA) plates. Invasion of the contractile vacuole (CV) was observed at a frequency of 1:100-1000 acanthamoebae at 35ºC. The salmonellae contained in CVs illustrated significant up-regulation of *fis* relative to extracellular bacteria indicating that they were in the early stages of logarithmic growth. Up-regulation of *fis* was also observed in a proportion of *S. typhimurium* cells contained within food vacuoles. Filamentation of *S. typhimurium* and *E. coli* cells was frequently observed in co-culture with *A. polyphaga* on NNA plates, with bacterial cells reaching lengths of up to 500µm after 10 days incubation at 35ºC. *A. polyphaga* was also seen to mediate bacterial translocation over the agar surface, egested salmonellae subsequently formed micro-colonies along amoebal tracks. This illustrated intracellular survival of a fraction of the *S. typhimurium* population. These phenomena suggest that protozoa such as *A. polyhaga* may play an important role in the ecology of *S. typhimurium* in soil and aquatic environments.

EM 14 Horizontal gene transfer of streptomycin gene cluster

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HGT plays an important role in the evolution of antibiotic biosynthesis in streptomycetes. Streptomycin resistant streptomycetes were isolated from diverse soil sites in the UK and Germany. A polyphasic study of the prevalence of streptomycin resistant streptomycetes was done, including type strains and synonyms isolated from the natural environment. Streptomycetes were identified by partial sequencing of the 16S rDNA including the hypervariable γ region. And the identification is confirmed by partial sequencing of *gyrB*. Isolates were screened for the streptomycin resistance gene, *strA*, and flanking biosynthesis gene, *strB*1. Streptomycin genes were detected in all the *S. griseus* isolates, and also in some of the *Streptomyces* species identified as other species. The latter indicated the dissemination of partial gene clusters or individual resistance genes. The expression of these genes was studied utilising RT PCR. Evidence of horizontal gene transfer of the whole streptomycin gene cluster from *S. griseus* was recorded in isolates identified as *S. platensis* recovered from two different sites which had acquired the whole streptomycin gene cluster from *S. griseus*, and was found to be streptomycin producers.

EM 15 Development of PCR methods to monitor gene bioaugmentation in soil contaminated with 2,4-D

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Bioaugmentation of soil has received a great deal of interest over the last decade. This is because the introduction of degradative genes, particularly on conjugative plasmids, has great potential for the cleanup of contaminated sites. The process involves horizontal plasmid transfer from the inoculant population to the indigenous microflora. We have assessed this approach using the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) as the target pollutant. Soil microcosms were bioaugmented with either pJP4, a plasmid carrying genes (*tfdA-F*) for the partial degradation of 2,4-D, or pEMT8k, which carries only *tfdA*. The plasmid carrying host *Ralstonia eutropha* JMP222 was chromosomally tagged with a constitutively expressing green fluorescent protein (gfp) cassette using the highly site specific Tn7 delivery system. PCR primers specific to the *gfp* gene were used as the basis for quantitative PCR. Competitive DNA was constructed, differing from the primary sequence by 4 base pairs, and resulting in a unique restriction site. The sample and competitor DNA, both of same size, can then be quantified following restriction enzyme digestion. The approach will determine the gene copy number for a chromosomal gene (*gfp*) and allow donor strain survival to be monitored in a culture independent manner. It is hoped to apply the technique to plasmid encoded genes, allowing monitoring of the relationship between donor strain survival and plasmid transfer.

EM 16 The microbial and metabolic mysteries of enhanced biological phosphorus removal in activated sludge

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Activated sludge wastewater treatment processes with alternating anaerobic and aerobic phases are successfully operated for enhanced biological phosphorus removal (EBPR). Within these systems microorganisms are conditioned to accumulate large amounts of phosphorus (P) in the form of intracellular polyphosphate resulting in the desired P removal. While EBPR processes have been optimised empirically, details of the microbiology and biochemistry of EBPR elude researchers. Such details are essential for improving EBPR performance, in particular its stability. A sequencing batch reactor has been set up for EBPR in our laboratory. High P removal performance has been obtained. Protein extracts from the anaerobic and aerobic phases have been analysed using one dimension polyacrylamide gel electrophoresis (1D-PAGE) and 2D-PAGE. Also, a metagenomic library has been constructed in a broad host range cosmid (pLAFR3) using DNA extracted from the EBPR reactor. The library comprises of ~2000 unique clones, each containing 25-30kb of metagenomic DNA. Fluorescence *in situ* hybridisation suggests that the microbial community of the reactor is diverse although comprises mainly of β-proteobacteria. Correlation of the reactor P removal performance with protein profiles, microbial community structure and metagenomic investigations will provide new insights into the microbial and metabolic details of EBPR.

EM 17 The distribution of the gentamicin gene cluster in nature

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Gentamicin (GM) (an aminoglycoside antibiotic) is produced by *Micromonospora echinospora* ATCC 15835. Self-resistance in aminoglycoside producers is achieved by ribosomal methylation of the A-site residue. Previous work revealed a *grmA* (methyl-transferase encoding) gene from *M. echinospora* DNA when cloned and expressed into a sensitive host *Streptomyces lividans* conferred resistance to gentamicin. *M. echinospora* chromosomal DNA was shown to not only contain *grmA* but possibility a second gene when probed with *fmrO*, a methyl-transferase resistance gene from *M. olivasterospora*. More recently, a cosmid library was constructed from *M. echinospora* DNA and a positive clone, pSPWAR001, was identified using *grmA* gene as a target. The 39.5 kb cosmid insert was characterised and sequence analysis identified a number of genes implicated in the biosynthesis, regulation and resistance of gentamicin. Functional assignment of *gntA* (a presumed L-glutamine:2-deooxy-*scyllo*-inosose aminotransfersase) was confirmed by in vitro bioassay of recombinant protein after heterologous expression in *S. lividans*. It is the aim of this study to increase the current knowledge of gentamicin producing and non-producing *Micromonospora* spp in nature. In this study selective isolation studies for gentamicin producing and non-producing *Micromonospora* spp have yielded a large number of isolates from Mediterranean soil communities providing the opportunity to study these natural populations. Resistant quotients revealed population sensitive and resistant to gentamicin at high and low levels. Soil isolates were screened using key target genes, *grmA* and *gntA*, known to be involved in gentamicin biosynthesis and resistance. Sequences were analysed for the diversity and dissemination of these genes in natural populations.

EM 18 Gene flow in Streptomycetes

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Analysis of the *S. coelicolor* genome has identified 14 regions, which may have recently acquired lateral insertions. This was based on unusual codon use-age and DNA content. Under natural conditions in soil, conjugation is thought to be the primary mechanism of gene transfer, but adaptive mechanisms such as antibiotic resistance can be acquired through plasmids, pathogenicity islands, insertion elements and integrons. The aim of this study is to determine the extent of horizontal gene transfer within populations of soil bacteria. The *str/sts* gene cluster is responsible for streptomycin production and resistance in *S. griseus*, and contains about 40 genes. Most of these have been characterised. The *str* gene cluster is being used as a model to study horizontal gene transfer of individual genes and clusters which can provide an adaptive advantage. Previous work in our laboratory has demonstrated possible *strA* and *strB1* transfer between isolates, which were phenotypically and genetically diverse. The distribution and diversity of other genes in the cluster (e.g. *strR*, *strS* and *strF*), was investigated in distinct soil populations and several cases of horizontal gene transfer were found. One involved the entire cluster and the other just the resistance gene *strA* and some flanking genes. Determination of the location of the *str* genes and possible ISL elements was investigated by utilising ligation mediated PCR, which failed to generate a PCR product for the flanking region. Universal fast chromosome walking which allows direct and versatile determination of flanking sequence is currently being used to locate possible ISL elements.

EM 19 Development of a molecular toolkit to detect *Actinobacteria* in environmental samples: PCR detection of key genes in antibiotic biosynthesis pathways using CODEHOP degenerate primers

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One approach to maximise the probability of finding novel antibiotics is to focus on groups of bacteria that are known producers of bioactive compounds but have not been intensively screened in the past due to difficulties in isolation and cultivation rather than their scarcity in the environment. Many members of the class *Actinobacteria* fulfil both of these criteria. Some streptomycetes are known to devote a large part of their genomes

to the synthesis of secondary metabolites and are historically very important producers of medicinally important compounds.

Our aim is to select key biosynthetic genes and design degenerate primers to detect environmental samples that are rich in actinobacteria. We have tested primers to Isopenicillin N-synthases (β-lactams), Type II polyketide synthases (polyketides), Non-Ribosomal Peptide Synthases and L-glutamine:N-scylloinosose aminotransferases (aminoglycosides) against a reference set of 127 actinomycetes. Our results confirm the specificy of the primers and support earlier suggestions that genes encoding polyketide synthesases and non-ribosomal peptide synthases are common in actinomycetes. Further, we are in the process of testing the utility of these targets in terminal restriction fragment length polymorphism (T-RFLP) based community analysis studies.

EM 20 Impact of pollution on the dissemination of bacterial genes encoding resistance to quaternary ammonium compounds and evidence for co-selection of drug resistance genes in environmental bacteria

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Quaternary ammonium compounds (QACs) are used as antiseptics, biocides and fabric softeners; they are relatively non-biodegradable and are detectable in waste water and sewage sludge. QACs are membrane active agents and are highly toxic. Resistance mechanisms include multi-drug efflux systems encoded by a group of genes known as *qac* resistance genes, these are commonly situated on integrons, which may also carry antibiotic resistance genes. This may produce co-selection of antibiotic resistance by exposure to QACs.

Samples were taken from a reed bed system used to treat effluent from a textile mill where QACs (DTDMAC) were used during wool finishing. Plate counts showed high levels of resistance to DTDMAC at 50µg/ml, and lower levels of resistance to CTAB. Isolates from river sediment downstream of the sewage plant used to treat the reed bed effluent showed no resistance to CTAB. Isolates were screened for Class1 integrons, 19 gave bands with specific primers. *Vibrio parahaemolyticus, Aeromonas salmonicida, Pseudomonas* sp., *Bacillus megaterium* and *Enterobacteriaceae* sp. were identfied as carrying integrons. The variable region of Class1 integrons was characterised using PCR to identify the size and likely number of gene cassettes carried by each integron. Sequencing of these bands will give the identity of inserted cassettes and the QAC and/or antibiotic resistance that they encode.

EM 21 Spatial and temporal heterogeneity of bacterial biofilm populations along a river ecocline

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The Cloghoge River is an episodically acidic, pristine upland river in Co. Wicklow, Ireland. It is surrounded by bogland in its upper reaches, is largely devoid of human disturbance, and provides an ideal site for baseline studies of riverine biofilms in their pristine state. In flowing systems, microbial communities are concentrated within biofilms, which typically develop on surfaces associated with the streambed, with the water flow carrying transient planktonic communities.

Biofilms were sampled along a 15km stretch of the Cloghoge River between July 2001 and September 2002. Terminal Restriction Fragment Length Polymorphism was used to profile the bacterial biofilm communities along the river. TRFLP is a rapid and sensitive molecular technique based on the variability of the 16S rRNA gene as determined by restriction endonucleases and detection of the terminal fluorescent-labelled fragment by a fragment analysis system. The biofilm communities varied down stream, mainly in relation to minor members of the community. Number of species remained relatively constant with an average of 22 per sample. Dominant species were observed at all the sites and accounted for 50- 70% of the community.

In order to investigate the phylogenetic diversity of this system, a clonal library was constructed from one site. RFLP analysis of the library showed 19 OTUs, with 5 dominant OTUs accounting for 69.5% of the total clones analysed. 16S rDNA sequencing revealed that a- Proteobacteria and Cyanobacteria dominate these biofilms but also include members of the CFB and ß- Proteobacteria groups.

EM 22 Molecular methods for oilfield microbes at Saudi Aramco

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Microbial consortia remain poorly characterized despite their importance to the oil industry. Attempts to describe microbial oilfield communities by molecular biotechnology methods have been limited. The availability of gene probe technologies offers an opportunity to conduct more comprehensive characterization of oilfield microbes.

In this study, molecular diagnostic methods based on application of gene probes were used in the fingerprinting of oil field microbial consortia. These microbes including sulfate reducing bacteria are major causative agents in microbially influenced corrosion (MIC), biofouling, souring and plugging. Samples were collected from various sites in Saudi Aramco Operations. These samples were enriched in liquid media and colony purified. Bacterial DNA were isolated and amplified by 16S rRNA followed by DNA sequencing.

Twenty five unique bacterial genomes have been identified and they are referred to as standards. These standards are being utilized as tools to identify important bacteria within microbial consortia, and study consortium population dynamics. Further, these genome probes will be used to study the effects of bactericides and other stressors on microbial oilfield communities.

Food & Beverages Group
POSTERS

FdBev 01 Immunomagnetic-flow cytometric detection of staphylococcal enterotoxin B in milk

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A rapid and sensitive method for detection of staphylococcal enterotoxin B (SEB) in milk samples with the use of antibody-based immunomagnetic separation (IMS) in conjunction with flow cytometry (FCM) was developed. The sheep anti-SEB IgG was immobilized onto Dynabeads M-280. The SEB binds initially to the capturing antibody which is bound on the magnetic beads. The rabbit anti-SEB IgG binds to the captured toxin and is further labeled with a Cy5-labeled goat anti-rabbit IgG antibody. The percentage of fluorescent beads in total beads was measured by FCM. Measurement was done for 1 min and the FCM data were viewed as both histograms showing particle size (forward light scatter) and fluorescence intensity. The number of the fluorescent beads and the fluorescence intensity increased with increase in the SEB concentration in samples. In this IMS-FCM assay, the lower limit of detection of SEB was estimated to be 0.01 and 0.25 ng/ml for buffer and milk samples, respectively.

FdBev 02 Effect of chitosan and its oligosaccharide on gut microflora populations using classical microbiological and molecular methods

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Chitosan and its oligosaccharides are used as preservatives by the food industry and have well-documented antimicrobial properties. Chitosan is also used in many brands of slimming aids with the claim that it blocks the absorption of dietary fat. The effects of these polymers on the gut flora and its fermentation are currently unknown. To determine these effects pH controlled anaerobic batch cultures were performed. Three different molecular weights of chitosan and chitosan oligosaccharide lactate were added to the medium as the sole carbohydrate source and fructooligosaccharides and glucose were used controls. Bacterial groups were enumerated using classical microbiology techniques and fluorescent in situ hybridisation (FISH). Species-specific denaturing gradient gel electrophoresis (DGGE) was used to check for any changes to the flora at species level. Short chain fatty acids were also analysed. Chitosan and its oligosaccharide lactate used as the sole carbon source caused changes in most bacterial groups measured indicating a non-selective fermentation. Differences were also seen between the classical and molecular methods of analysis with the former not being entirely reliable for recovering target genera.

FdBev 03 Monitoring the *in vitro* effect of prebiotics on the faecal microflora of infants using Fluorescence *in situ* Hybridization (FISH)

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A stimulation of bifidobacteria and lactobacilli in formula-fed infants may provide protection against pathogens similar to that observed in breast-fed infants. The addition of prebiotics to existing formula feeds may contribute towards the development of these beneficial populations of bacteria. Several oligosaccharides have bifidogenic effects but their fermentative activities have not been widely tested using infant microflora. Mixtures of fructo-oligosaccharides (FOS) and either galacto-oligosaccharides (GOS) or isomalto-oligosaccharides (IMO) were tested in the following proportions 100:0, 25:75, 50:50, 75:25, 0:100 in a series of single-stage chemostats inoculated with the faecal flora of formula-fed infants (n=4). Fluorescence *In Situ* Hybridization was used to measure changes in the microbial populations developing in the fermenters at time 0, 5, 10 and 24 hours. Irrespective of treatments, a significant increase of the total number of bacteria was observed between T0 ($1.2*10^9 \pm 1.35*10^1$ cells/g) and T24 ($5.9*10^9 \pm 1.64*10^1$ cells/g). *Bifidobacterium* spp. predominated at T0 ($9.1*10^7 \pm 1.52*10^1$) with lower counts of *Bacteroides* spp. ($9.9*10^5 \pm 2.28*10^1$ cells/g) and *Clostridium* spp. ($8.1*10^5 \pm 7.32*10^1$). All prebiotic treatments induced a bifidogenic effect. In the presence of FOS, however, populations of potentially harmful bacteria such as clostridia and bacteroides were maintained at low levels suggesting that the addition of FOS in formula feed would achieve a better microbial selectivity than GOS or IMO.

FdBev 04 Identification of factors involved in the recovery of heat-injured *Salmonella enteritidis*

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Heating is one of the most common sterilization methods, but insufficient heating generates injured bacteria. For detection of viable bacteria, it is important to recover injured bacteria during enrichment. We investigated the injury and recovery of heat-treated *Salmonella enteritidis* (SE). Overnight-cultured SE was diluted with 50 mM citric acid – phosphate buffer (pH6.0) and cell suspension with 10^7 CFU/ml was prepared. After heat treatment at 55°C for 15 min, 99% of SE detected on TSA were injured. During incubation of heat-treated SE in Tryptic Soy Broth, viable cell numbers on TSA did not increase for 2 hr, whereas those on DHL agar increased after 30 min of incubation and reached same level with that on TSA after 2 hr. The results indicate that SE has recovered. To investigate the mechanism for the recovery, total cellular proteins have been analyzed by 2D-PAGE. For this purpose, both genes expressed after heat treatment and during recovery have been identified by differential screening.

FdBev 05 Anti-pathogenic activity of probiotic strains against *Helicobacter pylori*

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Helicobacter pylorus (*H. pylorus*) presents itself as an enigma to researchers as it is uniquely adapted to the colonisation of the human gastrointestinal tract. However this prevalent human pathogen is implicated in the aetiopathogenesis of a variety of disease states such as Type B Chronic Active Gastritis, Non-Ulcer Dyspepsia, Duodenal Ulcer Disease and perhaps ultimately Gastric Cancer. The determinants of these clinical outcomes are multifactorial, complex and not well understood, as is the nature of *Helicobacter pylori* (*H. pylori*) virulence. Current research focuses on the use of an in vitro assay to screen the ability of a range of lactobacilli and bifidobacteria to inhibit 5 selected *H. pylori* strains, common probiotic traits of the inhibitory bacteria are also being defined. The assays are also evaluating the inherent characteristics of any inhibitory substances. The application of prophylaxis within this field is relatively novel and allows us to study *Helicobacter* inhibition with the intention of developing an effective synbiotic towards the reduction of colonic and possibly gastric carriage of this gram negative curved bacillus. The emergence of antibiotic resistance strains and the concurrent failure of first line eradication therapies and gastric carriage clearance have elevated concerns on the direction of *Helicobacter* eradication. The sheer diversity of *Helicobacter* strains and the emerging likelihood of faeco-oral transmission and re-infection have stimulated important research into the use of dietary intervention procedures towards the reduction or indeed eradication of this prevalent human pathogen.

FdBev 06 Specific bacterial groups as potential milking practice hygiene indicators

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Total bacterial counts (TBCs) are universally used the dairy industry as indicators of raw milk quality. In Europe, one of the reasons for the adoption of TBCs as the hygiene indicators was a European Union directive (92/46) for milk quality that is based on this test and requires milk to contain less than 10^5 CFU ml^{-1}. Similarly, in the United States milk with a TBC greater than 10^5 CFU ml may not be sold as Grade-A milk. Milk produced from alveoli in healthy bovine udders is sterile. Microbial contaminants are introduced into milk from the farm environment, external or internal surfaces of udders and poorly cleaned milking equipment and storage tanks. Although TBCs are useful as gross indicators of milk quality, when high counts are obtained, a TBC does not give further specific information about which part of the production process introduced bacteria into the milk. A result of a study looking at potential bacterial indicators of sources of breakdown of on-farm hygiene is discussed.

FdBev 07 The effect of heat stress on *Escherichia coli* and a comparison of its recovery by the plate count method with flow cytometry

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Owing to the importance of microbiology for human health, methods have been developed to enumerate viable bacteria. Traditional methods such as dilution plating are considered to be the 'gold standard' for proof of cell viability. These methods which rely on post sampling growth are limited by our ability to grow cells in an artificial environment. Additionally they are unreliable for the detection of stressed or sub-lethally cells which are known through the phenomena of viable but non-culturable (VBNC) micro-organisms. These results have significance in food industry, where VBNC cells are of particular concern. For example determination of microbiological quality of food may be required for estimation of shelf life or its suitability for human consumption of ready-to-eat foods, which undergo no further processing. If these VBNC cells are left undetected by conventional techniques they may be pathogenic bacteria and still be able to cause foodborne illness. Today single cell measurements by flow cytometric methods can rapidly provide information about growth, and also allow assessment of population heterogeneity. This research was therefore used to compare the recoveries of heat stressed *E. coli* obtained by the 'traditional' colony count method of detection and the 'rapid' multi-parameter flow cytometric analysis. Preliminary experiments were carried out using the colony count method to determine the effect of heat stress on the cells, which is a stress type typical to heat preservation in the food industry. The results from flow cytometry were used to compare those obtained using existing cultural techniques in order to provide information on reproductive viability as well as the existence and nature of the viable but non-culturable state.

The results showed that the percentage viabilities obtained by flow cytometry were significantly higher than that of the percentage viabilities using the colony forming unit method.

FdBev 08 Development of a synbiotic for the treatment of irritable bowel syndrome

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Irritable bowel syndrome (IBS) is a gastrointestinal disorder that effects approximately 22% of the population and is characterised by diarrhoea and/or constipation and severe abdominal bloating. The causes vary between patients and include the use of antibiotics, ovarian hormones, food intolerances, stress and depletion of beneficial gut bacteria. It is also believed that the yeast *Candida albicans* may be implicated in the onset and maintenance of IBS. There is evidence that the ingestion of probiotics mixed with prebiotics (a synbiotic) is likely to restore numbers of beneficial bacteria and alleviate the symptoms of IBS. Therefore a novel probiotic *Lactobacillus plantarum* strain with anti-candida activity was assessed for its probiotic traits including a unique molecular DNA fingerprint. Industrial scale development of *L. plantarum* was conducted ready for incorporation into a synbiotic product. The prebiotic galactooligosaccharide (GOS) was selected for the synbiotic product based on its ability to optimally support the growth of *L. plantarum*. The synbiotic product is to be fed to thirty IBS patients in a randomised, double-blinded, placebo controlled multi-centre clinical trial and the microflora assessed by FISH, SCFA analysis and DGGE.

FdBev 09 Species-specific PCR identification of *Zygosaccharomyces* yeasts *sensu stricto*

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The trend towards reduced preservative concentrations in the food and beverage industries is causing the incidence of yeast spoilage to increase. Rapid and reliable yeast species identification is economically advantageous because it can dictate the most efficient course of remedial action. Existing molecular identification techniques can provide species-specific fingerprints but they are only suitable for the research laboratory and poorly reproducible between different laboratories, require interpretation, are relatively slow and are labour intensive. Our aim has been to develop a simple, rapid and robust method that is also capable of automation. Species-specific PCR primers have been designed to distinguish between the closely related members of the monophyletic *Zygosaccharomyces* genus. The PCR primers capitalise on nucleotide sequence variation that occurs between the species *Z. rouxii, Z. mellis, Z. lentus, Z. kombuchaensis, Z. bailii, Z. bisporus* and two recently identified *Zygosaccharomyces* species. The method is also capable of dealing with natural polymorphisms that occur between different isolates of each species. This proof of principle suggests that the approach can be widely used for other fungal genera.

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FdBev 10 Actinomycete diversity on the surface of European smear-ripened cheeses

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Background: A part of European culture which is jealously guarded is regional food and European smear ripened cheeses are a distinctive element. The propagation of a cheese surface flora on soft and hard cheeses with a salt-wash generates diverse regional cheeses manufactured in small to large-scale cheese-making operations in many parts of Europe to produce such well-known cheeses as Livarot, Limburger, Tilsit, Reblochon and Gubbeen. Both food-safety and quality are influenced by the smear cheese flora which includes cheese starter cultures, yeast, staphylococci and many coryneform-related bacteria and other actinomycetes. The objective is to characterise the smear cheese flora and determine its influence on food safety and quality.
Methods: Isolation studies and molecular ecological techniques have been applied to characterise the complex actinomycete flora and their population dynamics.
Results: New actinobacteria-specific primers have detected both novel and previously characterised cheese micro-organisms from the actinomycete flora of Tilsit cheese. The molecular composition is compared with the results of isolation, dereplication and identification studies.
Conclusion: Additional actinomycete diversity was detected using new primers. The actinomycete diversity present on the Tilsit cheese surface was complex and variable and contained previously described novel species detected on other European smear ripened cheeses.

FdBev 11 Spore heat resistance of *Bacillus* food spoilage isolates

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Bacterial endospores are extremely resistant to heat, desiccation, radiation and chemicals. Due to the stress-resistance of spores, spore-forming microorganisms are an important source for food spoilage. We have studied the heat resistance properties of a number of *Bacillus* food spoilage isolates. Our goal is to identify molecular targets for the development of innovative antibacterial strategies and to identify molecular markers that can be used for tracing and tracking Bacilli in the food chain. We have studied

endospore formation of a number of *B. subtilis* spoilage isolates by using genetic and biochemical methods. In addition, we have studied the effects of food ingredients on spore heat resistance. Importantly, we have detected increased expression levels of a number of spore-specific proteins in the food spoilage isolates in comparison to *B. subtilis* laboratory strains. The increased expression levels of these spore proteins may contribute to an increased spore heat resistance of the food isolates. Increased spore heat resistance was also observed in the presence of a number of food ingredients (spices and milk powder) in the sporulation medium. Spore mineralisation appears to be an important contributor to spore heat resistance.

FdBev 12 Detection and genotyping by conventional and real time PCR/RFLP analyses of *Cryptosporidium* species and *Giardia intestinalis* from human faeces

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We report a new nested-PCR/RFLP assay (PCOWP-PCR) based on the amplification of a fragment of the cryptosporidial COWP gene. This highly sensitive technique uses primers made of 5to 6 different oligonucleotides and enables the detection of a wide range of *Cryptosporidium* species using DNA directly extracted from faeces. The assay also distinguishes between *Cryptosporidium parvum* genotype 1 and genotype 2. We also report a nested-PCR (TPI4-PCR) amplifying a fragment of the triose phosphate isomerase gene for detecting *Giardia intestinalis* and distinguish between assemblages A and B. the COWP-PCR and TPI4-PCR have been evaluated using DNA extracted from purified cysts or oocysts, whole faeces, faecal smears and bacterial suspensions. Both techniques were highly specific, sensitive and reproducible. The assays were adapted to real time PCR format using a LightCycler and the dsDNA-binding dye SYBR Green I. Amplicon characterisation was made by melting point analysis and non-specific amplification products could be easily distinguished from true positives. The LightCycler assays were as sensitive and reproducible as their conventional counterparts but were quicker to perform. We believe that these protocols, since they are performed from DNA recovered directly from faeces, can be used for routine diagnosis of human cryptosporidiosis and giardiasis.

FdBev 13 Detection of *Cryptosporidium* and *Giardia* in samples of shellfish by PCR

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Seventy-nine samples of clams, cockles, mussels and oysters were collected in Spain and England that originated from Spain, Italy, England, Ireland or New Zealand. In some of the samples from Spain, Italy, England or Ireland, *Cryptosporidium* oocysts were detected by immunofluorescence microscopy with a monoclonal antibody in sediments purified from diphasic PBS/diethyl-ether extracts from either gills, digestive tract or a shellfish homogenate. DNA was extracted from these sediments using a protocol developed for the examination of faeces using mechanical disruption with a Beadbeater in the presence of guanidinium thiocyanate and using activated silica. The presence of *Cryptosporidium* spp. or *Giardia duodenalis* DNA (COWP or *tpi* gene fragments respectively) was detected using a novel nested multiplex PCR assay. Amplicon characterisation was performed by restriction fragment length polymorphism analysis and gene sequencing. No inhibition of the PCR reactions was detected using DNA extracted from shellfish that had been spiked with either cryptosporidial or giardial DNA. *Cryptosporidium* species were detected in 26 samples. *C. parvum* genotype 1 in 1 sample, genotype 2 in 22, and 3 containing mixtures of genotypes and those requiring further characterisation) and *Giardia duodenalis* Assemblage B in one of the samples. The development of such methods will greatly enhance the ability to detect *Cryptosporidium* and *Giardia* in these foods and will facilitate formulation of risk assessments for prevention of infection from these parasites from the consumption of shellfish will be discussed.

FdBev 14 Molecular diagnosis of *Clostridium perfringens* diarrhoea

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Clostridium perfringens is a significant cause of diarrhoea in the UK, and thus *C. perfringens* food poisoning is one the five major foodborne diseases targeted by the Food Standards Agency to be reduced by 20% before 2006. All *C. perfringens* contain an alpha-toxin, but only those that cause classical diarrhoea produce an enterotoxin. This study investigates the usefulness of molecular biological techniques for characterising *C. perfringens* directly in faeces as well as grown *in vitro*.

Conventional confirmation of identify of *C. perfringens* is performed by: characteristic colonial morphology, nitrate reduction positive, motility test negative, lactose fermented, and gelatine hydrolysed. *C. perfringens* enterotoxin is detected in samples of faeces by reverse passive latex agglutination (RPLA).

Block based and real time PCR methods have been developed for amplification of fragments of the alpha- and enterotoxin genes, and these have been applied to DNA extracted directly from faecal samples, colonies on agar plates and cooked meat broth cultures.

Molecular methods were shown to be sensitive, specific and cost effective for the diagnosis of *C. perfringens* diarrhoea. The detection of enterotoxin gene fragments directly in faeces was of similar sensitivity to the RPLA. The optimal strategy for diagnosis of *C. perfringens* diarrhoea will be discussed.

FdBev 15 Rapid detection of *Clostridium botulinum* by real time multiplex PCR for neurotoxins A and B

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Botulism is a disease caused by the neurotoxins of *Clostidium botulinum* of which there are seven types designated A-G: in humans most cases are associated with types A, B, E and F. Current laboratory diagnosis relies on the detection of toxin in paient's body fluids and the growth, isolation and toxin production of *C. botulinum* in vitro. Isolation techniques are slow, labour intensive, and non-specific, and the detection of toxin principally relies on the use of a bioassay. The aim of this project was to improve the diagnostic procedures for human botulism by developing rapid multiplexed PCR assays to detect neurotoxin gene fragments.

Primers and fluorescently-labelled probes were designed to amplify and hybridise to fragments of toxin types A, B and a multiplex PCR assay was developed based on ABI Taqman chemistry. This assay was evaluated using an ABI 7700 and SmartCycler platforms with DNA from, pure cultures and inoculated cooked meat broths as well as crude cell lysates from colonies. The assays were found to be sensitive, specific and produced results within 3 hours. These assays have the potential to improve the diagnosis of botulism and the detection of *C. botulinum* in clinical and environmental samples.

FdBev 16 Species diversity analysis of the elderly gut microflora through cloning of whole community 16S rRNA

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The human gut microflora changes with age. Coupled with changes in gut physiology (reduced gastric acid) and sub-optimal immune function, these changes may account for the increased incidence and severity of gastrointestinal infections seen in the elderly. We measured the diversity of the elderly gut microflora through whole community 16S rRNA sequencing. Briefly, 16S rRNA genes amplified from elderly faecal samples (n=10) were cloned into *Escherichia coli* and sequenced, giving a comparative phylogenetic framework of bacterial diversity within the elderly gut microflora. As in previous studies with younger individuals, the majority of sequences belonged to the *Clostridium leptum* and *C. coccoides* groups. Other dominant groups included the *Bacteroides* spp., *Lactobacillus* spp. and *Bifidobacterium* spp. *Enterobacteriaceae, Sporomusa* spp., *Acholeplasma-Anaeroplasma* spp. and *Atopobium* spp. appeared unusually prevalent in comparison to younger adults. On average about 30% of sequences were novels. The phylogenetic frameworks constructed for the elderly gut microflora showed a much higher degree of species diversity than those of younger individuals. This increase in gut microflora diversity within the aged may be symptomatic of a reduction in gut colonization resistance. On going work, within the EU funded project CROWNALIFE (www.crownalife.be), aims to fortify the elderly gut microflora and possibly colonization resistance, through the use of probiotics, prebiotics and synbiotics.

Wednesday 10 September 2003

0910 Understanding acclimation in green algae: physiology to genomics

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For the past 3 years we have been developing genomic tools for the unicellular green alga *Chlamydomonas reinhardtii.* The development of these tools, funded by NSF, has involved 1) sequencing cDNAs isolated from cells exposed to various environmental conditions, 2) construction of a high density DNA microarray, 3) construction of genomic contigs that nucleate around specific physical and genetic markers, 4) generation of a complete chloroplast genome sequence and analyses of chloroplast gene expression, 5) placement of genomic information into searchable databases that can be readily accessed on the internet by the general public, and 6) the creation of links between the information generated in the NSF funded project with the complete genome sequence information that is being generated by the Joint Genome Institute (Department of Energy). I will briefly describe the Chlamydomonas genome project and then emphasize the use of genomic tools to generate information on gene expression under different environmental conditions. The primary focus of the talk will be on sulfur deprivation and the use of microarray analysis to explore the ways in which deprivation influences gene expression, how changes in gene expression may alter the metabolism of the cell and the ability of the cell to survive sulfur deprivation conditions. We have also isolated and characterized expression profiles in mutants that are unable to acclimate to sulfur limitation. These studies will be put into the general context of the strategies that organisms use to withstand nutrient deprivation.

0950 Genetic analysis of drug resistance in African trypanosomes

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Genetic mapping and positional cloning of genes of interest has become a potentially tractable approach to analysis of traits of interest in *Trypanosoma brucei* with the rapid development of the genome sequencing project and our creation of a genetic map. We have used this approach to investigate resistance to arsenical drugs which are first choice for treatment of sleeping sickness and with which drug resistance is a clinical problem. We first developed a simple, verified screening assay for assessing drug sensitivity based on the use of AlamarBlue. Three stocks, used as parents in genetic crosses, differed in drug sensitivity: STIB247 - sensitive, STIB386 - resistant and TREU927 – resistant. Genetic linkage analysis using 101 polymorphic markers was used to examine the co-segregation into F1 progeny of markers and the resistance phenotype using crosses of 247 x 386 and 247 x 927. Inheritance of resistance in both crosses was compatible with a simple single locus genetic model with one dominant allele determining resistance. Linkage analysis indicated that the gene conferring resistance lies within a ~ 25 kilobase region on Chromosome II, which contains 6 open reading frames (ORFs), none of which have been linked previously to drug resistance. A combined 'knock out' and 'knock in' analysis identified one gene 'ORF4' as the determinant.

1100 Comparative genomics of the apicomplexans

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Comparing the genomes of multiple species (comparative genomics) provides a means to study the biology and evolutionary history of those species to a greater extent than analysis of individual genomes allows. Anumber of parasite species belonging to the phylum Apicomplexa, a complex taxon consisting of nearly 5,000 species all of which are parasites and characterized by the presence of an 'apical complex', are in the closing stages of whole genome sequencing, and the era of comparative genomics of apicomplexan species has arrived. This presentation will outline the initial comparative analysis of the genomes of several species of malaria parasite, among them the human malaria parasites *Plasmodium falciparum* and *Plasmodium vivax*, and a rodent malaria model *Plasmodium yoelii*, which has resulted in the generation of genome-wide synteny maps of the genus and identification of genes under different evolutionary pressures. Comparative analyses of the *Plasmodium* genome with other apicomplexan parasite genomes will also be shown. This is a stimulating time for comparative genomics of apicomplexan parasites, and it promises to produce useful results for the development of better methods of disease control.

1140 The exploitation of the comparative genomics of *Plasmodium*: the role of rodent models in the search for malaria vaccines

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There is an abundance of malaria parasite genome sequence from both human-infectious and model parasites. It is very clear that the similarity of the biobgy of different Plasmodium species is reflected in the largely conserved organisation of their genomes. The differences between the parasite genomes are most obviously confined to the sub-telomeric regions where the multigene families that are involved in antigenic variation are located. Comparitive genomics may help improve the annotation of the conserved non sub-telomeric regions of the prototype Plasmodium genome. The significant conservation of gene content including genes that encode surface proteins involved in cell-cell interactions means that the greater flexibility of model parasites can be exploited to provide biological insights that remain relevant to drug and vaccine research. This will be illustrated by data drawn from the research activities at Leiden.

1400 Gene order and MAT locus evolution in yeasts

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The genetics of the mating-type (MAT) locus in *Saccharomyces cerevisiae* have been studied in great detail, but relatively little is known about how this complex system evolved. We compared the organization of MAT and MTL (mating-type-like) loci in nine species spanning the hemiascomycete phylogenetic tree. We infer that an *S. cerevisiae*-type system with Ho endonuclease-mediated switching between an active MAT locus and silent HML/HMR cassettes exists only in the *Saccharomcyes sensu stricto* group and their closest relatives: *Candida glabrata*, *Kluyveromyces delphensis* and *S. castellii.* The more distantly related species *K. lactis* has silent cassettes but appears to undergo mating type switching without the aid of an Ho

endonuclease. Very distantly related species such as *C. albicans*, *Pichia angusta* and *Yarrowia lipolytica* do not have silent cassettes. The MAT loci of species that have Ho differ in two significant ways from their counterparts in other species. First, conservation of the gene order on one side of MAT became disrupted when the Ho nuclease was adopted. Second, a gene for an HMG-domain protein is present in the MAT**a** idiomorph of all the species from *Y. lipolytica* to *S. kluyveri* but was lost relatively recently, in the ancestor of the Ho-containing species.

1440 The process and biological implications of mating in Candida

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Candida albicans is the major fungal pathogen of humans, causing both systemic and localized disease. It is diploid as usually isolated, so thatresearch into the genetic basis of its virulence has been restricted to reverse genetics. Recently, due to the availability of the complete genomic sequence (available at the Stanford Genome Technology Center, http://www-sequence.stanford.edu/group/candida/), genes orthologous to the *MAT* genes in *Saccharomyces cerevisiae* have been identified. Using gene disruption or induced chromosome loss to induce homozygosity at the Mating-Type Like (*MTL*) locus, two laboratories have induced mating. We have focused on two aspects of mating: the occurrence and properties of homozygous strains among clinical isolates and their mating ability, and the genes regulated by the *MTL* locus. Among 120 clinical strains, 12 were found to be homozygous at the *MTL* locus. All but one could mate, although phenotypic switching to the opaque state was not observable in 7 of the 12. The strain that failed to mate was shown to have a frame-shift in the *MTL*α_1 gene. One set of serial isolates showed a transition to homozygosity which was followed by chromosome rearrangements, including fragmentation of chromosome 5, the site of the *MTL* locus, and the emergence of fluconazole resistance. Scanning the genome for potential pheromone genes has led to the identification of a putative alpha pheromone gene. *MTL**a*** cells with this gene deleted fail to mate, while *MTL***a** cells mate normally. Opaque *MTL***a** cells when treated with the peptide encoded by this gene undergo shape changes similar to those in mating mixtures. We conclude that this gene encodes the *Candida a**l**bicans* equivalent of the *Mfa* gene in *S. cerevisiae*. This work was supported by grant AI 16567 from the National Institute of Allergy and Infectious Disease, USA.

1550 The sex chromosome of *Chlamydomonas*: structure and evolution

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Mating type in the green alga *Chlamydomonas reinhardtii* is determined by the mating-type (MT) locus on chromosome VI, a 1 Mb region in which recombination is suppressed. Within this 1 Mb is a 200 kb domain (the R domain) in which four homologous DNA blocks are in rearranged order in the two mating types, interspersed with sequences unique to one mating type or the other. Thus chromosome 6 is dimorphic in the two mating types. The unique sequences include several genes that determine mating type or perform mating functions. Newly available genome sequencing has identified about 15 "housekeeping" genes within the four homologous domains, the presence of which may prevent these blocks from degenerating. To assess whether the recombination suppression at MT seen in the laboratory translates into actual linkage disequilibrium in nature, we sequenced several genes both within and just outside the R domain from 13 natural isolates. Two R domain genes that have alleles in both mating types showed numerous polymorphisms restricted to one mating type or the other, consistent with near total suppression of recombination within the R domain. There is, however, evidence of rare gene conversion events within the R domain which may limit how diverged the alleles of these genes can become.

1630 Genomics reveals sexual secrets of *Aspergillus*

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Fungi from the genus *Aspergillus* exhibit a range of reproductive strategies, including homothallism (selfing), heterothallism (obligate outbreeding) and asexuality. We are investigating the molecular basis for differences between these modes of reproduction. In the past year, genome sequences of the homothallic species *A. nidulans* (http://www-genome.wi.mit.edu) and the asexual species *A. fumigatus* (http://www.tigr.org) have been released. Sequencing of genomes of other aspergilli is currently in progress. Sequence analysis of available genomes has allowed us to identify mating type genes (*MAT*) in both homothallic and asexual species, with complimentary *MAT-1* alpha domain and *MAT-2* high-mobility group genes detected. *MAT* specific primers have been designed and are being used in PCR analysis to investigate the distribution of mating-type genes in a range of *Aspergillus* species to assess their sexual potential.

In parallel studies, genes from *Saccharomyces cerevisiae* with known roles in sexual reproduction are being used as probes to BLAST search the genomes of different aspergilli. Several matching genes have already been identified in *A. nidulans* and *A. fumigatus*, and we are now conducting a detailed comparative and functional analysis of these genes. This work illustrates how genomics can provide new insights into the reproductive lifestyles of filamentous fungi.

1650 Identification and characterisation of components of cyclic nucleotide signalling pathways from the malaria parasite *Plasmodium falciparum*; possible regulators of differentiation

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Cyclic nucleotides (cAMP and cGMP) are ubiquitous signalling molecules which mediate a whole range of physiological processes. These range from complex functions such as colour vision and nerve cell communication in higher eukaryotes to locomotion and growth responses in some protozoans. Cyclic nucleotides have been implicated in sexual differentiation of the human malaria parasite *Plasmodium falciparum*. We therefore sought to isolate genes (using a PCR-based approach) encoding cyclase enzymes which are responsible for the synthesis of these signalling molecules. We identified two unusual, bifunctional guanylyl cyclases with an N-terminal P-type ATPase-like domain and a C-terminal domain with an overall structure that resembles G protein-dependent adenylyl cyclases. Residues diagnostic of purine-binding specificity, however, were characteristic of guanylyl cyclases consistent with the biochemical activity of recombinant proteins. More recently we have used unfinished *P. falciparum* genome project data to identify many other unusual components of the cyclic nucleotide signalling pathways including functional adenylyl cyclases and cyclic nucleotide-dependent protein kinases. Genetic and biochemical characterisation of these molecules is underway and has provided insight at the molecular level into key differentiation events in the parasite life cycle.

1710 Growth-rate-dependent modulation of gene expression and metabolic profiles in yeast

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The influence of growth rate on the pattern of gene expression (transcriptome) and metabolic profiles (metabolome) in yeast was studied in aerobic chemostat cultures under nutrient-limiting conditions (carbon, nitrogen, sulphur and phosphorus limitation, growth on ethanol, and growth on glucose under oxygen limitation). Transcriptome and metabolome studies were performed using an Affymetrix GeneChip system and by Electrospray Mass Spectrometry (ES-MS), respectively. The results show a clear influence of growth rate on the patterns of gene expression and metabolic profiles. Marked differences were observed in the numbers of up- and down-regulated genes throughout the range of dilution rates. Also, gene expression patterns and metabolic profiles were seen to be characteristic of specific growth rates and cultivation conditions. These results have direct implications both for the systems biology of *S. cerevisiae* and for the design of experiments in functional genomics studies.

Thursday 11 September 2003

0900 Lessons learned from the genome of the filamentous ascomycete Ashbya gossypii

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S.cerevisiae grows as a single cell and is found in nature on surfaces of grapes and fruits. The filamentous fungus *A.gossypii* grows as multinucleated hyphae and is a pathogen for cotton and citrus. In order to understand the similarities and differences between these two ascomycetes at the genome and at the cellular level, we initiated in 1996 structural and functional genomics with *A.gossypii*. Sequencing of the 7 chromosomes (9MB) revealed extensive synteny with the *S.cerevisiae* genome. Our annotation of the 4720 protein coding genes uses three categories: SH for syntenic homologue (90 %) , NSH for non-syntenic homologue (5 %) and NOHBY for no homologue in bakers yeast (5 %). Comparative genomics revealed 26 novel ORFs and 90 ORF extensions or fusions for *S.cerevisiae*. In addition, 1090 ORFs presently annotated as hypothetical at SGD have a syntenic homologue in *A.gossypii*. The synteny map based on the *A.gossypii* gene order consists of many clusters composed of groups of up to 50 *A.gossypii* ORFs which, with few exceptions, always align with *S.cerevisiae* ORFs form two genomic regions each displaying incomplete (relaxed) synteny. Implications of this finding with respect to evolution and biology will be discussed.

Functional genomics with *A.gossypii* dwells on an annotated genome and efficient PCR-based gene targeting like in *S.cerevisiae*. Examples showing similarities and differences in cellular roles of syntenic homologous will be presented.

0940 Functional genomics of the rice blast fungus *Magnaporthe grisea*

N. TALBOT
University of Exeter

Abstract not received

1120 Proteomic approaches to investigate the infection process of *Phytophthora infestans*

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A thorough understanding of the molecular events taking place during early interactions between *P. infestans* and host and non-host plants is crucial for developing new control measures. The appressorial stage of the interaction is the first point in which direct contact between the pathogen and the plant occurs via the formation of highly specialised infection structures, such as the appressorium, penetration peg, and the infection vesicle. Moreover, it is during this phase that plant defence responses are initiated. Resistance is based, principally, on recognition of a particular elicitor component from the pathogen. Therefore, we anticipate that secreted and cell wall proteins from the appressorial infection stage of *P. infestans* are likely to be rich in important signalling molecules involved in disease resistance or establishing a successful infection process. A proteomic approach is employed to accelerate the discovery of novel extra-cellular and appressorial stage-specific proteins. Here we present our latest results.

1140 Functional analysis of the *Candida glabrata ACE2* gene and its role in virulence

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Inactivation of the *Candida glabrata ACE2* transcription factor gene results in an increased virulence phenotype. Inoculation of 10^6 *ace2* blastospores into immunosuppressed CD1 mice results in 90-100% mortality within six days. A dose of 2 x 10^8 wild type fungi is required to yield similar mortality levels. Mice infected with *C. glabrata ace2* blastospores develop systemic infection with all major organs involved. Fungi can be recovered from brain, heart, kidney, liver, lungs and spleen. Histological analysis of these organs shows a very high tissue burden compared with wild type infection and invasion of the tissue parenchyma was seen in all organs examined.

To investigate this increased virulence phenotype we have utilized the COGEME proteomics facility at the University of Aberdeen to identify differences in the proteomes of wild type and *ace2 C. glabrata* cells. Using 2D-gel electrophoresis, a total of 1,839 spots in the *C. glabrata* proteome have been resolved. Of these 55 proteins were reduced at least 2-fold in *ace2* mutants, 138 were absent in the mutant, and 15 were increased at least 2-fold. Additionally 8 spots were absent in wild type strains. We are currently attempting to identify these proteins by mass spectrometry of tryptic digests and interrogation of the *C. glabrata* genome sequence.

1200 Genome-wide expression profiling in fission yeast

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In every cell, hundreds of genes and their products function together in complex and orchestrated networks that regulate biological processes. DNA microarrays are now used to monitor expression levels of all genes on a global scale, and such genome-wide approaches will help to fully understand life and disease.

We are pursuing functional genomics research using the fission yeast *Schizosaccharomyces pombe* as a model organism. We are keen to gain an overview of gene expression programs and their regulation, both during normal cell growth and in response to unfavourable conditions. To this end, we have developed DNA microarrays containing all known and predicted genes of fission yeast. We have reported global gene expression programs during sexual differentiation and in response to various environmental stresses (Mata et al., 2002, Nat Genet 32:143; Chen et al., 2003, Mol Biol Cell, 14:214). Another project addresses periodic transcriptional regulation during the fission yeast cell cycle.

Integration of large datasets from different experiments performed under standardized conditions creates additional insights and hypotheses to follow up. These comprehensive analyses help to dissect transcriptional networks and regulatory circuits and will provide a framework for understanding gene function and gene expression programs in more complex organisms.

1400 Candida morphogenesis and the regulation of host-pathogen interactions

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Candida albicans is an opportunistic human pathogen. A variety of lines of evidence establish that the ability to undergo a morphological switch from yeast growth to hyphal growth is important for the virulence of this organism. We have been investigating the molecular events associated with this switch and the consequences to the host-pathogen interaction. Several signalling pathways are involved in the switch. These pathways transmit information from the environment to the intracellular machinery controlling growth and morphogenesis. We haveused transcriptional profiling to identify the genes whose expression is regulated during the switch from yeast growth to hyphal growth. We have also disrupted a number of genes in key signalling pathways to determine the consequences both to the controlof morphology and to the control of gene expression. In particular, adenylyl cyclase plays a key role in controlling the morphological shift. Cyclase is required for all the transcriptional regulation associated with the yeast to hyphal transition. In addition, deletion of the gene for adenylyl cyclase disrupts the expression of hundreds of other genes distinct from those involved in the yeast-to-hyphal shift in *Candida albicans*. We are currently investigating the transcriptional profiles of wild-type and mutant versions of the pathogen when they are interacting with mouse macrophage.

In addition we are measuring the transcriptional profiles of the mouse macrophages during these interactions. The response of the macrophage appears to depend on the genotype of the fungal pathogen; cyclase defective mutants are readily engulfed relative to wild type cells. We have done extensive analysis of the response of *C. albicans* to various stresses, and have used this information to determine that the engulfed fungal cells activate a number of stress related genes. These results establish that the interaction between the host and the pathogen involve complex and interactive transcriptional responses.

1440 The genomics of host-pathogen interactions: How *Candida albicans* survives phagocytosis

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Phagocytes of the innate immune system are a front-line defense against bloodstream fungal infections, such as those from the yeast *Candida albicans*. Patients with defects in innate immunity are thus at increased risk for fungal infections. Morbidity is as high as 40% for systemic candidiasis and approaches 100% for invasive aspergillosis. The critical interaction between phagocytes, such as macrophages, and *C. albicans* is dynamic, with vigorous responses from both cell types. To understand these complex responses at a transcriptional level, we are taking advantage of genomic technologies by using a complete DNA microarray for *C. albicans*. When inside the phagolysosome, the yeast turns off much of the machinery for transcription and translation including translation factors, RNA polymerase subunits and most ribosomal proteins. Accompanying this shift is the upregulation of a variety of genes involved in nutrient import and utilization – the glyoxylate cycle, peptide and sugar permeases, and some aspects of amino acid metabolism. Further, genes to repair DNA damage, strengthen the cell wall, and mediate changes in cell morphology are also activated. We now have a picture of how this fungal pathogen responds to in vivo stresses and have identified a wealth of candidates for further study that may mediate this interaction.

1550 Pharmacogenomics in *Dictyostelium*

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The simple eukaryote *Dictyostelium* has the complexity of metazoa, but the molecular genetic advantages of a microorganism. It is amenable to molecular genetic, biochemical and cell biological techniques, making it ideal for a multidisciplinary approach to study of chemotaxis and signal transduction.

Dictyostelium has a 34 Mbp, haploid genome of approximately 12,000 genes. The genome and 6,000 non-redundant cDNAs have been sequenced (http://dictybase.org/dictyostelium_genomics.htm). Homologous recombination is used for targeted gene disruption, and random insertion mutations made by Restriction Enzyme Mediated Integration (REMI).

Dictyostelium has a unique development strategy where cells grow as unicells, but when starved arrest growth and undergo multicellular development to form a fruiting body. The demarcation between growth and development allows us to raise mutants in signal transduction pathways that, although not essential for growth, are required for development.

We have generated a bank of approximately 25,000 REMI mutants. This can be used to isolate mutants with defective development, or those with altered resistance to teratogenic drugs. Such drugs include the mood stabilizers lithium and valproic acid, which are treatments of bipolar depression. Analysis of lithium mutants has revealed a new pathway for the regulation of inositol phosphate metabolism and suggests a molecular mechanism for mood disorders.

1630 *Candida* biofilms: genetic and genomic approaches to a poorly understood mode of growth

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Biofilms are three-dimensional associations of microorganisms that develop on various surfaces. In the case of *Candida albicans*, the main fungal pathogen of humans, biofilms have been found in association with catheters and prosthetic devices. Because they have a reduced susceptibility to antifungal treatments, these biofilms constitute reservoirs for subsequent reinfestation. In order to understand the molecular basis of biofilm formation in *Candida* species and the physiology of *Candida* biofilms, we have initiated two parallel approaches. First, we have taken advantage of several models of biofilms formation and of *C. albicans* whole–genome DNA-arrays to investigate the transcriptome of *C. albicans* biofilms. Using this approach, we have observed that diverse *C. albicans* biofilms have homogeneous transcript profiles that differ significantly from those of planktonic cultures. Furthermore, we have identified a set of genes that are significantly over-expressed in mature biofilms. The role of several of these genes in the formation of *C. albicans* biofilms will be reported. A second approach is based on the characterization of a collection of insertional mutants of the haploid yeast *Candida glabrata* and has resulted in the identification of several strains impaired for biofilm formation. The nature of the genes whose inactivation results in this phenotype is being investigated.

1650 The genomics of cell cycle regulation in *Candida albicans*

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The ability of *Candida albicans* to form yeast, pseudohyphal and true hyphal morphologies is important for virulence. As in many other fungi, *C. albicans* morphogenesis is regulated, at least in part, by the cell cycle machinery. Cells depleted for either *C. albicans* B-type cyclin (Cyb1p or Cyb4p) are unable to switch between cell types and form either highly elongated hyphal-like projections that rarely displayed septa and eventually stopped growing (Cyb1-depleted) or form pseudohyphal cells (Cyb4p-depleted) under all conditions tested. Thus, Cyb1p and Cyb4p display distinct roles in morphogenesis. Fkh2p, a homolog of the *S. cerevisiae* transcriptional regulator of B-cyclin expression is required for morphogenesis in *C. albicans*: Fkh2p-depleted cells, like Cyb4p-depleted cells, form pseudohyphae. Furthermore, small-scale cDNA microarray studies indicated that *CYB4* transcript levels were altered in asynchronous *fkh2?* cells. We report here the use of *C. albicans* full genome cDNA microarrays to identify genome-wide transcriptional changes in cells depleted of Cyb1p, Cyb4p or Fkh2p. Transcripts whose levels were altered in one or more of these strains encode a G1 cyclin, factors involved in cell wall biosynthesis and metabolic proteins. These data will help us unravel the molecular connections between the cell cycle machinery and morphogenesis in *C. albicans*.

POSTERS

EUK 01 Detection of the protozoan parasite *Cryptosporidium parvum* by Nucleic Acid Sequence Based Amplification (NASBA) of the DNA replication gene *Cp-RPA1*

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Background: The protozoan parasite *Cryptosporidium parvum* is the causative agent of cryptosporidiosis in several mammals, including humans, causing severe gastrointestinal distress. While usually self-limiting, the disease can become chronic in the immunocompromised, contributing to increased morbidity and mortality in these populations. The emergence of *C. parvum* as a globally significant human pathogen has led to a recognition of the need for sensitive and reliable methods of detection for the transmissable oocysts of this pathogen.
Methods: Nucleic acid sequence based amplification (NASBA) is an isothermal, RNA-based rapid amplification method, which utilises electrochemiluminescent probe techniques to detect the amplified target sequence. The gene *Cp-RPA1* encodes the large sub-unit of the *C. parvum* replication protein A (RPA1), a single-stranded-DNA binding protein essential for DNA replication.
Results: An optimal set of NASBA primers for a sequence within the *Cp-RPA1* gene was identified, with a detection limit of 50 oocysts. The ruthenium-tagged NASBA amplicon was subsequently directly sequenced to confirm the target.
Conclusion: This report presents the first application of NASBA to a DNA replication gene in any organism. NASBA of the *Cp-RPA1* gene is proposed as a sensitive molecular detection system for the presence of *Cryptosporidium* oocysts, with potential as a measure of viability.

EUK 02 Tracking new pathogen species by using a macro-array-based technique

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Many vector-borne species are infecting different host species and can be found simultaneously in blood or vector samples. Therefore, there is a need for an integrated method able to identify known species and new species that cannot be directly detected with the published probes. Our molecular method is based of the use of PCR and reverse line blot hybridisation, a macro-array-based method. Known probes are used to target at the same time different pathogen species that could be present in host blood samples or tick samples. However, field collections made in different countries and with different hosts showed that new species could be identified even if species-specific probes are not yet available. Using a 16S and 18S rRNA-based PCR for Anaplasmataceae and Piroplasmidae, respectively, we were able to identify new species in small ruminants, horses and cattle, which have never been identified by molecular techniques before. The reverse line blot hybridisation is based on amino-linked probes specific for species or families, the latter being able to catch new species in the targeted pathogen families. A chemiluminescent reaction allows a greater sensitivity compared with PCR alone demonstrating the usefulness of a method able to target mixed infections at an early stage of infection. This paper shows the technicality of the method and its advantage/disadvantage aspects.

EUK 03 Genotypic microarray analysis can provide significant phylogenetic information

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Phylogenetic trees reveal evolutionary relationships between organisms. With sequence homology studies of the rDNA, phylogenetic trees have been constructed for the *Saccharomyces sensu stricto* species. However, depending upon the region chosen (18S or the ITS), the position of the species varies (Naumov *et al.*, 2000). In the post-genomic era, phylogenetic trees can be constructed on the basis of whole genome information. We have used comparative genomic hybridisations (CGH) with *S. cerevisiae* spotted microarrays to reassess the phylogenetic tree of the *S. sensu stricto* species. The normalised data produced from the CGH of each species has been used to identify species-specific gene differences in comparison to *S. cerevisiae*. These genes are currently being investigated in an attempt to explain the species characteristics and possibly reveal more unique traits.
Reference: Naumov, GI., James, SA., Naumova, ES., Louis, EJ., Roberts, IN. (2000) *Int J Syst Evol Microbiol.* **50**: 1931-1942

EUK 04 Proteomic analysis of Nrg1 targets in *Candida albicans*

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The human pathogen *Candida albicans* is able to undergo reversible morphogenetic transitions between budding yeast and

hyphal growth forms, which are thought to be important for virulence. In *C. albicans*, Nrg1 is required for the repression of hypha-specific genes under yeast-inducing conditions, and Δ*nrg1* cells are constitutively filamentous. Partial transcript profiling of the *C. albicans Dnrg1* mutant was carried out previously using membrane arrays spotted with 2,002 ORFs, corresponding to about one-third of the *C. albicans* genome (Murad *et al.*, 2001). This transcript profiling has now been extended using comprehensive arrays carrying 6039 ORFs representing about 98% of the *C. albicans* genome. Also, in order to examine the global roles of Nrg1 in *C. albicans* at the protein level, proteomic analysis has been undertaken by COGEME Proteome Service Facility I, based in *Aberdeen Fungal Group*. We are comparing the Nrg1 targets identified by this proteome analysis and our transcript profiling. Also, we are examining the cellular roles of these Nrg1 targets.

EUK 05 Antisense *frequency* RNA and its role in the circadian clock of *Neurospora crassa*

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Circadian clocks regulate a host of biochemical and physiological processes that occur rhythmically over the course of each day. Cycles of temperature and light are detected by the clock and can reset it, effectively synchronizing internal and external time. Another feature of circadian clocks, that allows them to keep accurate time, is that their period length is nearly constant over a range of temperatures.

In the model eukaryote, *Neurospora crassa*, the *frequency* (*frq*) gene encodes central components of the circadian clock. Apart from mRNA transcripts encoding the FRQ protein, antisense *frq* transcripts are also transcribed and these have been shown to affect the response of the clock to light. To investigate further the link between antisense *frq* RNA and the clock, other clock characteristics were investigated in antisense-defective strains.

Altering the expression of antisense *frq* does not affect the clocks ability to maintain its period between 25-30°C nor its ability to entrain to temperature cycles. However, preliminary data indicate that when temperature pulses are administered at different times of the circadian day the response is markedly different compared to a wildtype strain. In conclusion, antisense *frq* appears to regulate the response of the Neurospora clock to light and temperature.

EUK 06 The effects of temperature on the circadian clock of *Neurospora crassa*

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In the filamentous fungus *Neurospora crassa* a circadian clock drives rhythms in biochemistry, gene expression and development (conidiation). Light and temperature changes can reset the time of the circadian clock. Light exerts this control by feeding through the WHITE COLLAR / FREQUENCY oscillator. To determine how temperature affects the Neurospora circadian system, we have investigated the effect of temperature pulses on the clock and on gene expression.

The effects of 1 hour temperature pulses of 30, 35, 45, 48 or 50°C were assayed by studying the timing of circadian clock-controlled conidiation. Interestingly, after a pulse of 48 or 50 °C growth was suspended for 1-2 days. Although initiation of mycelial growth between replicate cultures differed, the circadian clock in these cultures was reset to the same time. Moreover, two-pulse experiments indicate the clock is responsive during this halt in growth.

Treatment of cultures with 5-azacytidine suggest that RNA or newly made protein is required for temperature sensing and/or resetting of the clock. Microarray expression profiling of 800 genes was carried out. After a 45°C temperature pulse 22 genes were up-regulated (including hsp70 and hsp78) and 55 down-regulated. One of these is known to encode a protein important for circadian rhythmicity.

EUK 07 Global roles of repressors involved in *Candida albicans* morphogenesis

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The Tup1-Ssn6 protein complex is a conserved eukaryotic global repressor, which has been shown to repress many diverse groups of genes in *Saccharomyces cerevisiae*. The ability of the human pathogen *Candida albicans* to undergo reversible morphogenetic transitions between budding yeast form and hyphal form is essential for virulence. In *C. albicans*, Nrg1 is required for the repression of hypha-specific genes under yeast-inducing conditions, and *Dnrg1* cells are constitutively filamentous. The *Dtup1* cells display similar morphological defects to *Dnrg1*, indicating a similar function (Braun and Johnson, 1997). However, *Dssn6* cells display a different morphological phenotype, indicating that Ssn6 does not simply act as a co-repressor with Tup1.

Transcript profiling of the *C. albicans Dnrg1* and *Dtup1* mutants has been carried out previously using membrane arrays spotted with 2002 *C. albicans* ORFs (Murad *et al*., 2001). More recently the European Galar Fungail Consortium's database (http://genolist.pasteur.fr/CandidaDB/) has been used to generate glass slide arrays carrying 6039 ORFs representing 98% of the *C. albicans* genome.

These new, more comprehensive, arrays have been used to carry out transcript profiling of *Dnrg1*, *Dtup1* and *Dssn6* mutants to expand on the previous work and to determine if the three proteins function together in the regulation of morphogenetic genes.

EUK 08 *Aspergillus nidulans* NpgA is a 4'-phosphopantetheinyl transferase involved in primary and secondary metabolism

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The genomes of filamentous fungi contain a large number of phosphopantetheine (ppant)-dependent pathways of primary and secondary metabolism, all requiring the post-translational addition of ppant into the carrier protein domains by 4'-phosphopantetheinyltransferases (PPTases). While many PPTases of varying specificity have now been identified from a number of bacteria, a filamentous fungal PPTase had yet to be characterised. Through database searching of the *Aspergillus fumigatus* genome sequence against Sfp from *Bacillus subtilis*, we identified a unique sequence which appeared to encode a PPTase, as deduced from conserved residues considered important in PPTases. The PPTase candidate was used to search the NCBI database and an unexpected homologue in *A. nidulans* was identified as *npgA*. To examine the PPTase activity of NpgA, the full length NpgA and *Candida albicans* Lys2 were expressed in *Escherichia coli* as hexahistidine fusions and purified by nickel affinity chromatography. The inactive apo *C. albicans* Lys2 was activated *in vitro* to holo Lys2 in the presence of coenzyme A as the source of the ppant group and NpgA as the PPTase; the activated holo Lys2 had a-aminoreductase activity. These experiments demonstrate that NpgA is a functional PPTase that efficiently recognises and modifi-ed a carrier protein involved in primary metabolism. A strain carrying *cwfA2*, an allele of *npgA* is defective in ppant-related pathways such as spore pigmentation and penicillin biosynthesis. NpgA, as the prototypic *B. subtilis* Sfp, is able to transfer the ppant cofactor to a broad range of enzymes with carrier protein domains; it is the first example of a eukaryotic PPTase that serves primary and

secondary metabolism. Alignment of NpgA homologues detected in filamentous fungal databases shows that most of the conservation among the NpgA homologues is around the three motifs previously described (1). We have also identified in 6 filamentous fungi a distinct PPTase of the AcpS type, involved in ppant modification of acyl carrier proteins (ACPs) of the primary metabolism. We suggest that the target for this PPTase is the mitochondrial Acp1.

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EUK 09 Screening *Saccharomyces cerevisiae* gene knockouts for stationary phase survival

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The entire set of gene knockouts of the yeast *S. cerevisiae* has been screened for ability to survive in stationary phase (SP). From 4831 viable haploid strains, 92 were found unable to survive 3 months in stationary phase at 28ºC after growth in a nutrient rich medium (YPD). A further 13 could survive stationary phase in YPD but not in minimal medium. Interrogation of various databases enabled assignment of potential functions to most of the genes identified. Eleven ORFs were similar to other genes but had no assigned function and 3 ORFs had no assigned function and were not similar to any known gene. Some of these ORFs overlap other known genes but the identified overlapping genes were not essential for stationary phase. The remaining genes all have an assigned function, or are similar to genes of known function. There is a strong mitochondrial and/or respiratory bias to this group. A Calorie Restriction (CR) effect has been discovered to extend genealogical lifespan by increasing catabolite flow via respiration but does not extend SP survival for many of these strains and many are respiratory incompetent. Addition of reduced glutathione that protects against oxidative damage rescued many knockouts.

MRH thanks the BBSRC for a studentship.

EUK 10 Identification of a prokaryotic ATPase in the eukaryotic Trichomonads

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Trichomonads are parasites commonly associated with vaginitis and may be a cause for infertility. *Tritrichomonas foetus* is the bovine parasite and *Trichomonas vaginalis* is the human parasite. Trichomonads are lower eukaryotes and it would not be too surprising to find that their genomes share some similarities with prokaryotes. The use of Random Amplified Polymorphic DNA (RAPD) primers has allowed us to isolate a product of a size of 690bp that shows similarity to a prokaryotic gene encoding an ATPase transporter. Bioinformatic tools were used to identify the sequenced PCR product and to derive specific primers to further isolate the trichomonad gene. Primers were derived to target (1) a region within the original product obtained, and (2) to protein motifs conserved between cadmium ATPases of prokaryotic and lower eukaryotic species. PCR (Polymerase Chain Reaction) products were obtained with the former but not the latter primers. However, the latter did produce products from *Escherichia coli* DNA. This implied that trichomonads possess an ATPase transporter similar to that found in prokaryotes but do not possess the genes for the cadmium ATPase. However, the absence of PCR products could be a function of the primer design.

Euk 11 Genomic and transcriptomic analyses of *Aspergillus* species

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The filamentous fungi *Aspergillus sp.* are an important group of organisms both commercially and medically. They have a high capacity for producing large amounts of extra-cellular proteins, which has been exploited to produce both homologous and heterologous enzymes, antibiotics and specialty chemicals. As a result, several species have been awarded 'generally accepted as safe' (GRAS) status which allows them to be used as hosts for the synthesis of novel products.

The use of microarrays in the analysis of gene expression is becoming widespread for many organisms. However, although the genomes of a number of filamentous fungi have been fully or partially sequenced now, microarray analysis is still in its infancy in *Aspergillus nidulans*. A description of the fabrication of microarrays from PCR products of ESTs from cDNA libraries of *Aspergillus nidulans* will be given.

A glucose up-shift experiment (ethanol to glucose) has been chosen to validate the microarrays as it results in relatively well-characterised physiological changes due to the expression of the negatively acting global repressor creA. The available literature provides a set of expected results against which array data could be compared which will be presented.

A genome-wide phylogenetic study of the similarities between 5 species of *Aspergillus* has been undertaken using *A.nidulans* microarray slides. Genomic DNA from *A.nidulans, A.niger, A.fumigatus, A.parasiticus* and *A.terreus* was digested with *Taq* 1 restriction endonuclease and labelled with either cy3 or cy5 fluorescent dyes and then hybridised to the arrays. An analysis of the results will be presented showing the relatedness of these species to *A.nidulans*.

EUK 12 A global approach to the study of protein turnover in yeast

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Cellular protein levels change in response to physiological and pathological states. The level of individual proteins in a cell is determined by turnover dynamics, the balance between rates of synthesis and degradation. We have developed a method to determine turnover rates of individual proteins in the yeast proteome. This method has been applied to steady-state growth in aerobic continuous culture. We have turnover rates for proteins associated with over 300 spots from a 2-D gel. The turnover rates for some proteins is very close to the doubling time for cell growth; these proteins are therefore lost from the cells by dilution into daughter cells and their intracellular degradation is near zero. Other proteins show turnover rates that exceed 9% per hour. We are developing mass spectrometric approaches that obviate gel-based separations to analyse low-abundance proteins and proteins that are not amenable to 2-D gel based separation.

EUK 13 Post genomic analysis of energy metabolism in trypanosomes: the role of adenylate and arginine kinases

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Maintenance of energy homeostasis is a crucial cellular process. Two enzymes with core roles in maintaining energy balance are adenylate kinase and arginine kinase. Arginine kinase activity provides a spatial and temporal energy buffer that can be readily mobilised during cellular stress, whilst in mammalian cells the former enzyme is suggested to communicate energetic signals between different cellular compartments.

In Africa, the uniflagellated protozoan *Trypanosoma brucei* is a serious pathogen of people and livestock; it also has a complex developmental life cycle that requires replication and cell differentiation in the strikingly contrasting environments of both host and insect vector. Here we report that *T. brucei* expresses not only arginine kinase, but also at least seven distinct adenylate kinases; this is more than any other organism for which significant genome sequence is available (including mammals and several other eukaryotic microbes). The expansion of this gene family and biochemistry of the gene products reflect the highly compartmentalised metabolism of the organism and atypical strategies utilised for regulate gene expression. Intriguingly three of the seven trypanosome adenylate kinases contain unusual N-terminal extensions. The extensions are required on two isoforms for incorporation into a flagellar structure, establishing this organelle as an intracellular site of likely dynamic metabolism. However, whilst silencing of each adenylate kinase isoform by inducible RNA interference gives growth phenotypes for two enzymes, an absence of phenotype for the remaining family members indicates that, as in some other organisms, there is likely to be considerable complexity within the relationship between adenylate kinase activity and cellular energetics. A realistic goal is therefore to uncover the metabolic changes that accompany the loss of adenylate or arginine kinase activity and relate these to the metabolic profiles that are necessary for the successful completion of the developmental cycle through host and vector.

EUK 14 Genomic approaches to assess contribution to fitness of individual genes in yeast

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The contribution to fitness of individual genes of the yeast *Saccharomyces cerevisiae* was assessed via competitive growth experiments coupled with high-throughput analysis. We intend to compete ca. 6000 bar-coded heterozygous deletant strains in chemostats under different nutrient limitations. The barcodes serve as strain identifiers and allow large number of deletion strains to be pooled and analysed in parallel. As a preliminary experiment, we grew 1500 strains, carrying the deletion of all the essential genes, in carbon-limited and nitrogen-limited medium with a dilution rate of 0.1 h^{-1}. The percentage of each strain in the mixed population after nine and eleven days was detected via hybridisation of all the bar-codes to a high density array, and this genome profile was compared to that at time zero (beginning of the steady state). The use of heterozygous deletant diploids allowed us to identify genes that exert a high level of control over the pathways in which they are involved, and those which show a haploinsufficiency phenotype.

Wednesday 10 September 2003

0900 DNA shuffling and the design of vaccines and biopharmaceuticals

R.G. WHALEN
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Abstract not received

0940 Toward the development of potent DNA vaccines for humans

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DNA vaccines have been extensively studied in animal models of infectious and non-infectious disease. One of the potential advantages of the DNA vaccine technology, compared to certain other types of vaccines, is the ability to induce broad-based immunity, including both humoral and cellular responses. Therefore, DNA vaccines may be generally applicable for immunization against diseases caused by both viral and bacterial pathogens. So far, several "naked" DNA vaccines have been tested in human clinical trials. The vaccines have been generally well tolerated, but induction of immune responses has been limited. Therefore, one key to the ultimate success of DNA vaccines will be maximizing their potency in humans. Means by which this could be accomplished include increasing antigen expression through manipulation of the DNA plasmid, facilitating transfection of cells in situ via DNA delivery systems, and coadministration of adjuvants to modulate or enhance the immune responses induced. Such technologies added to "naked" DNA vaccines will have an impact on their complexity and may raise regulatory and safety issues that will need to be addressed.

1050 Development of a DNA prime-MVA boost prophylactic vaccine for HIV

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We have found that a successive immunization with DNA- and modified vaccinia virus Ankara (MVA)-based vaccines expressing a common immunogen is a potent way of inducing CD8+ CTL. Encouraged by the immunogenicity of this approach in primates, we have designed and constructed a DNA-MVA-based HIV vaccine candidate for clinical trials in humans. With a view to proceed to efficacy trials into high-risk cohorts in central and eastern Africa, where 70% of infections are currently caused by HIV clade A, the immunogen HIVA was derived from consensus HIV clade A gag p24/p17 sequences and a string of clade A CTL epitopes. This vaccine is the first HIV-1 clade A-derived vaccine tested in humans, it does not contain the envelope gene and focuses solely on the induction of cell-mediated immune responses. In pre-clinical studies, both the pTHr.HIVA DNA and MVA.HIVA vaccines were highly immunogenic in mice, and induced multi-specific T cell responses in rhesus macaques. Small phase I clinical trials in healthy uninfected human volunteers run under the auspices of Medical Research Council, UK and the International AIDS Vaccine Initiative (IAVI) showed that the vaccine was safe and immunogenic. These results are being confirmed and extended in trials in UK, Kenya and Uganda. We have also constructed and tested in pre-clinical studies a second immunogen RENTA, which complements the HIVA immunogen and will join the present vaccine in a four-component DNA/HIVA-RENTA prime-MVA/HIVA-RENTA boost formulation early in year 2004. RENTA is a fusion protein derived from consensus HIV-1 clade A sequences of tat, reverse transcriptase, nef and gp41. We inactivated the natural biological activities of the HIV components and demonstrated the immunogenicities of the pTHr.RENTA and MVA.RENTA vaccines in mice. Furthermore, we demonstrated broadening of HIVA-specific T cell responses by a parallel induction of those recognizing RENTA.

1400 Building better bacteria for biotherapeutic production

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Genetically modified *Escherichia coli* are responsible for a significant proportion of the world's recombinant biopharmaceutical products. Strain optimisation is critical for improving the yield, purity and safety of the product, particularly through the elimination of antibiotics and removal of host-derived contaminants such as nucleic acids.

Antibiotics are used for plasmid selection and maintenance but they have a number of disadvantages, including a loss of selection pressure due to degradation, a significant metabolic burden and a risk of antibiotic gene transfer to pathogens. Our Operator Repressor Titration (ORT) system for antibiotic-free plasmid selection and maintenance relies on modified *E. coli* strains containing an essential gene under the control of the *lac* operator/promoter. When these strains are transformed with plasmids possessing the *lac* operator, the repressor protein is titrated away from the single chromosomal copy by the multicopy plasmid, allowing gene expression and cell growth.

Our approach to avoid product contamination with host nucleic acids is to integrate the bovine RNaseA and Staphlococcal nuclease genes into the *E. coli* chromosome, tagged with a periplasmic targeting sequence. These nucleases are translocated to the periplasm, and are released upon cell lysis to degrade RNA and chromosomal DNA prior to purification.

1440 The quiescent cell factory: exploiting the synthetic capacity of non-growing *E. coli*

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Escherichia coli continues to occupy centre stage as a host for large-scale recombinant protein production, especially for simple proteins where post-translational modifications are not essential for biological activity. We have been considering ways to solve the conflict between the biotechnologist's desire for more and better product, and the bacterium's inclination to funnel resources into biomass production. In an attempt to shift the balance in favour of the biotechnologist we have developed the Quiescent Cell (Q-Cells) expression system in which a plasmid-encoded protein is expressed in non-growing cells. We have explored the capacity of Q-Cells to express a biologically-active single chain antibody fragment (scFv) in shake flask and fermenter culture. We first monitored scFv 3PF12 expression in high-density shake-flask culture and showed that the protein is exported efficiently into the culture supernatant. The system was then scaled-up in a fermenter where fed-batch techniques allowed us to hold the cells in quiescence at densities up to OD_{600}=50. Under these conditions, the rate of synthesis of biologically-active scFv in fed-batch Q-Cell culture was more than ten-fold greater than in a conventional culture control.

1550 Manufacture and characterisation of DNA Vaccines and the use of particle mediated delivery

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Currently at GSK, a number of DNA vaccines are being developed for use with PowderJect 'needle-free' particle mediated delivery technology. Following large-scale fermentation, the purified plasmid drug substance is precipitated on to gold particles and then loaded onto the pressurised delivery devices. These devices are then used to deliver the vaccine-coated particles to outer skin layers naturally rich in professional antigen presenting cells. By such targeted delivery, suitable and robust immune responses can be generated from very low doses of the DNA vaccine.

An overview of the process as well as an outline of the issues concerning the manufacture and characterisation of the drug substance (plasmid DNA) and drug product (DNA-loaded device) will now be presented.

1630 Genetically Modified Organisms: legislation, risk assessment and control measures

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This presentation provides an overview of the health and safety legislation that applies to genetically modified organisms, and focuses on the Genetically Modified Organisms (Contained Use) Regulations 2000. Topics include:

- The overlap between CoSHH, MHSW, and GMO(CU) regulations - risk assessment, information instruction and training, containment measures, and competent advice.
- What is and is not genetic modification for the purposes of the regulations - DNA vaccines, mutagenesis, cell fusion and self-cloning.
- Derogations in activity notifications.
- A good risk assessment - human health, the environment and detail.
- Classification of activities and when control measures do not affect the risk class.
- Waste inactivation and what is meant by 'a validated means'.
- Inspection - what do inspectors look for?

POSTERS

FB 01 Multi-parameter flow cytometric analysis of plasmid producing *E. coli* cultures in batch and fed batch fermentations

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Over-expression of recombinant products such as plasmid DNA in *Escherichia coli* can significantly increase metabolic loading and affect cell growth. Continual monitoring and control of bioprocesses are vital for obtaining optimal quality and quantities of biological products, usually relying on standard microbiological techniques. Multi-parameter flow cytometry facilitates direct optical analysis of culture physiology on a single cell basis. In this study, fermentations in complex media of an untransformed *E. coli* DH1 (host) and transformed *E. coli* DH1 strain carrying plasmid pTX0161, were profiled using flow cytometry and fluorescent probes alongside traditional techniques.

During batch culture of both strains traditional methods revealed a decline in recoverability after 8 hours' cultivation. Flow cytometry and fluorescent staining of the untransformed host demonstrated no significant energy limitation affecting membrane polarisation throughout fermentation. Increased fluorescent staining of the transformed host revealed progressively increasing cell stress due to depolarisation of cytoplasmic cell membranes.

Fed-batch fermentations demonstrated a longer lag phase for the transformed strain but similar final optical densities and dry cell weights. Cell recoverability peaked on c.f.u. plates between 6-7 hours. For the untransformed host, flow cytometry revealed no significant cell stress over an extended period of 18 hours, though there was a significantly higher percentage of BOX-stained (stressed) transformants.

These data would indicate that flow cytometry successfully profiled the heterogeneous physiology of cells due to plasmid biosynthesis.

FB 02 Mutagenesis of a complex stereoselective alkene monooxygenase

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Alkene monooxygenase (AMO) of *Rhodococcus rhodochrous* B-276 is a three-component binuclear iron active-site oxygenase that catalyses NAD(P)H-dependent oxygenation of propene and other aliphatic alkenes to give chiral epoxides with high enantiomeric excesses. In order to facilitate protein engineering of AMO, we previously developed a system for heterologous expression of the four-gene operon that encodes AMO in *Streptomyces lividans*, which overcame problems of very low or zero levels of activity when the operon was expressed under the control of a strong promoter in *Escherichia coli*. Here we describe a development of this system to facilitate site-directed mutagenesis of AMO and preliminary investigation of the functions of key active-site residues.

FB 03 Studies related to the scale-up of high cell density recombinant *E. coli* fed-batch fermentations using multi-parameter flow cytometry: effect of a changing micro-environment with respect to ph. and glucose

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Genetic engineering has allowed the manufacture of high value therapeutic and other heterologous proteins in large quantities, using suitable hosts such as yeast and bacteria. Fed-batch fermentation is commonly employed in industrial processes, but on scale up additional problems arises such as concentration gradients between the feed zone and the bulk of the reactor. These gradients are due to non–ideal mixing and as such mass transfer limitations will cause the micro-organism to be exposed to a rapidly changing microenvironment. Scale down simulation studies, which mimic the operating conditions at large scale, have been successfully used by our group to examine the effect of glucose gradients on the physiology of *E. coli cell* during high density fed batch fermentation. This study has extended this work by assessing the effect of a changing microenvironment with respect to pH variation, oxygen limitation and glucose gradients on the physiology, and biomass yield of a recombinant *E. coli* strain. Flow cytometric analysis of samples taken during the course of high density *E. coli* fermentation showed a progressive change in the physiological state of *E. coli* cells, with respect to propidium iodide and bis-oxonol staining. This technique has revealed that cells respond quite quickly to small changes in their micrenvionment. The overall reduction in productivity can be explained as a switch of resources from product synthesis to cell maintenance.

Wednesday 10 September 2003

0900 Communication and group activity in bacteria

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Quorum sensing often controls the expression of virulence genes in pathogenic bacteria, and in some bacteria under some conditions it can control the process of biofilm development. Biofilms are groups of bacteria organized in a self-produced extracellular matrix. Quorum sensing in Pseudomonas aeruginosa has attracted considerable attention because this pathogen causes many hard to treat infections including chronic biofilm infections in the lungs of people with cystic fibrosis. In this bacterium, quorum sensing is a key virulence determinant, mutants that cannot communicate are seriously impaired in their ability to cause infections in any number of laboratory models. This is because P. aeruginosa uses quorum sensing to control hundreds of genes, many of which code for the production of extracellular virulence factors, secreted factors that are toxic in one way or another to the host. This presentation will center on how the expression of the hundreds of genes in the quorum regulon of P. aeruginosa is coordinated. The expression of one particular quorum-controlled operon in biofilms, the rhlABC operon, will be described, and correlated to specific biofilm behaviors.

0945 Microarray analysis of *Campylobacter jejuni* gene expression *in vivo*

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In the environment *C. jejuni* has a wide host range and is habitually found as a commensal in chickens. One of the main sources of *Campylobacter* infection in humans is consumption of under-cooked poultry products. The pathophysiology of *C. jejuni* in the chicken host is poorly understood and research in this area may lead to the reduction of *Campylobacter* in the food chain. In experimental trials it is known that some *C. jejuni* strains colonise through out the chicken intestinal tract, with high bacterial numbers being consistently found in the caecum. The genes required for *C. jejuni* to colonise and survive the chicken caeca are unknown. We have used microarray technology to investigate changes in the expression of *C. jejuni* genes during colonisation of the caeca compared with in vitro grown broth cultures. An adapted hyper-motile *C. jejuni* NCTC 11168 strain (109 cfu/ml) was used to orally inoculate 1 day old chicks. After 18 hours the ceacal contents were harvested and total!

RNA extracted, from which fluorecescently labelled complementary DNA was generated. The sample and control cDNA was mixed and hybridised to a second generation oligonucleotide microarray based on *C. jejuni* NCTC 11168. The most notable differences were up-regulation of several genes involved in energy metabolism, chemotaxis, flagellar biosynthesis and putative sensory histidine kinase proteins. Therefore, this initial data indicates that *C. jejuni* undergoes physiological changes in response to environmental conditions in the chick *caecum*.

1005 Expression patterns of *M. tuberculosis:* the intracellular life phase and beyond

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Pathogenic mycobacteria are able to survive and proliferate inside host macrophages and inhibit maturation of their phagosome. Therefore, intracellularly expressed mycobacterial genes/proteins represent putative virulence factors as well as antigens detected by T cells on infected cells in vivo. At different time points after infection, phagosomes were purified and mycobacterial mRNA as well as proteins were analysed using filter based DNA microarrays/radioactive cDNA hybridisation and 2DE/MALDI-MS, respectively. In resting macrophages, mycobacterial gene expression is characterized by an increase in mRNA of genes for oxidoreductases, oxygenases, and those involved in cell wall and fatty acid synthesis. In interferon γ activated macrophages the expression pattern changed significantly showing increased expression of the isocitrate lyase gene (isl) and genes involved in siderophore synthesis and amino acid metabolism. Proteome analysis revealed a number of proteins being upregulated inside phagosomes including a long chain fatty acid CoA ligase (fadD2), Ag85 A and B precursors, fabG4 (fatty acid synthesis), cysQ and PrpD homologue putatively involved in propionate catabolism. It should be noted that a substantial number of intraphagosomally expressed genes/proteins are involved in lipid/cell wall biosynthesis. In this context it is interesting that parallel analysis of the "lipidome" of intracellular mycobacteria revealed a significant increase of the cell wall lipid trehalose dimycolate during intracellular growth. This lipid has been shown to inhibit fusion of vesicles. In summary, complete analysis of mycobacterial gene/protein expression inside host cells will lead to the identification of virulence/persistence associated genes, prerequisits for the development of rational anti-mycobacterial preventives and therapeutics.

1400 Salmonella in the chicken intestine behave differently to those grown *in vitro* and show stationary-phase characteristics

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Poultry are a major source of food-poisoning serotypes of *Salmonella enterica* for man. Most serotypes rarely produce clinical disease but colonise the alimentary tract and thus enter the human food chain from carcass contamination. Understanding the characteristics of intestinal colonisation are therefore crucial to rational methods for control of this infection. *Salmonella* ser. *Typhimurium* organisms were harvested from the ceca of newly-hatched chickens 24 hours after being inoculated. PAGE analysis showed increased levels of expression of outer membrane proteins, compared with bacteria grown *in vitro*. This was emphasized by western blotting using sera from immune birds. The bacterial cells, harvested directly from the gut, also showed increased resistance to heat shock and hydrogen peroxide which was comparable to stationary-phase, *in vitro*-cultured bacteria. Analysis of gene expression at the genomic level using a *S. Typhimurium* microarray indicated extensive down-regulation of large numbers of genes with increased anaerobic metabolism and reduced expression of SPI1 genes.

1445 Identification of *Mycobacterium tuberculosis* proteases as novel therapeutic targets by microarray expression profiling

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The emergence of multi-drug resistant *Mycobacterium tuberculosis* strains is a source of major concern in the clinical management of tuberculosis. The prevention and control of tuberculosis urgently requires the development of novel therapeutic approaches to redress the failure in the treatment of tuberculosis by currently available drugs. Little is understood about the intracellular or extracellular pathogenicity factors of *M. tuberculosis*. The approach we have selected is to look at the total gene expression profile using the hypoxic shift-down (Wayne) model. This model mimics the *in vivo* extracellular latent state of the bacilli in the human host. We have prepared RNA from bacteria grown in this model and hybridised them to whole genome microarrays in order to look at gene expression in physiological states that are relevant to infection. The results reveal a number of differentially regulated genes including several proteases. The expression patterns of these proteases have been analysed in order to select candidates for mutagenesis and further biological studies. Such an approach will allow us to determine the role of proteases in *M. tuberculosis* virulence.

1525 Characterisation of the *S. typhimurium rpoE* regulon

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The extracytoplasmic stress response (ESR) is controlled by at least two partially overlapping pathways in Salmonella, the alternative sigma factor σ^E (*rpoE*) and the two-component regulator, *cpxAR*. These pathways regulate the expression of various genes in response to different environmental stimuli. We are currently investigating the potential roles of ESR regulated genes in the Salmonella stress response and virulence, and have previously demonstrated that *rpoE* is critically important for virulence of *S. typhimurium* in a mouse model. In order to establish putative members of the Salmonella *rpoE* regulon we have utilised a variety of techniques. These include microarray analysis, a 2 plasmid system, S1 mapping, RT-PCR and reporter studies. Each of these methods has their own limitations, but through using them in combination we aim to draw a definitive consensus of *rpoE* regulated genes. From these preliminary investigations, as well as identifying genes reported to be *rpoE* regulated in other organisms, we have also discovered a number of other genes that thus far have not been reported as *rpoE* regulated. For some of the putatively *rpoE* regulated genes identified we have tried to ascertain their roles in the ESR and virulence, using a variety of *in vitro* assays as well as the well recognised competition index *in vivo*.

1545 The regulation of type III secretion in *Escherichia coli* O157:H7

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Enterohaemorrhagic *Escherichia coli* O157:H7 is a cause of gastrointestinal disease worldwide and is of particular concern in Europe, Japan, Canada and the USA. While the number of infections identified is relatively low in comparison to other bacterial food-associated zoonoses such as *Camplylobacter* and *Salmonella*, the consequences of infection can be severe, especially to young children. The majority of infections arise by direct or indirect contact with ruminant faeces, with cattle considered the main host. Cattle are colonised by *E. coli* O157:H7 asymptomatically, with the majority of the bacteria present at the terminal rectum of the animal. This region is characterised by a high density of lymphoid follicles and ongoing work is trying to elucidate the bacterial and host factors that drive this tropism. Once at this site the bacteria form attaching and effacing (A/E) lesions that promote their persistence in the animal. A/E lesions require the expression of a type III secretion system encoded on the locus of enterocyte effacement (LEE) pathogenicity island of EHEC O157. A/E lesions result from the injection into the epithelial cell of the translocated intimin receptor (Tir) which then inserts into the host cell membrane and can bind to the bacterial surface protein intimin. This interaction leads to cytoskeletal rearrangement in the host cell with pedestal formation and the tight association of bacterium and host cell. The injection of Tir and other proteins into the host cell requires a multi-protein basal unit spanning the two bacterial membranes and an injection needle, which in EHECO157 is extended into a filament structure composed of EspA. This allows the delivery of EspB and EspD which form a pore into the host cell. Proteins can then be injected through the basal and translocon apparatus into the host cell.

Our research has focused on the regulation of the translocon apparatus (EspADB) as well as that of intimin and Tir. The work demonstrates that the translocon apparatus is only expressed by a subpopulation of bacteria when cultured under conditions that favour type III secretion. The same subset of bacteria that are expressing EspA filaments also have intimin on their surface and are expressing Tir for delivery. The remainder of the population have not switched on transcription of intimin and Tir. This negative population have produced an *espADB* mRNA transcript but it appears not to be translated. The current working model is that in the bovine host quorum and key environmental signals up-regulate the *LEE* locus and ensure expression of the basal apparatus. The *espADB* mRNA is also produced ready for rapid production of translocon filaments. On contact with the epithelial surface (or using a signal that indicates presence at the epithelium) translation is coupled to the secretion of EspADB. The same regulatory pathway switches on intimin and *tir* transcription. The reason for this regulation is that intimin and EspA, in particular, are key antigens and the bacteria are trying to restrict their exposure to the immune system. The primed expression of the translocon apparatus maybe necessary inorder to rapidly deliver effector proteins into the first cells it encounters at the terminal rectum. The FAE at this site means that these are likely to include antigen sampling, M-like cells. As with Yersinia's 'deadly kiss' encounter with the macrophage, it maybe necessary for *E. coli* O157:H7 to fire the first round immediately upon contact. Experiments are ongoing to investigate this regulation both *in vitro* and *in vivo*.

Thursday 11 September 2003

0900 Regulatory networks during host pathogen interactions

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Pseudomonas aeruginosa is an important opportunistic pathogen of immunocompromised individuals and those with cystic fibrosis. The ability of this organism to cause life-threatening infections depends on the expression of certain virulence factors in response to the host environment. In particular, the genes encoding the *P. aeruginosa* Type III secretion system (TTSS) are coordinately activated in response to contact with host cells. Using whole-genome DNA microarrays we have discovered that expression of the TTSS is mediated by a receptor-like adenylate cyclase designated CyaB. When the appropriate environmental signals are detected CyaB synthesizes cAMP, which acts as a cofactor for the transcriptional regulator Vfr. The cAMP-Vfr complex functions as a positive regulator of TTSS gene expression. Transcriptional profiling of a mutants lacking either adenylate cyclase activity or Vfr indicates that this regulatory network is also involved in coordinating the expression of additional virulence determinants including type IV pili and

flagella. Mutants defective for components of this regulatory network are unable to kill culture human epithelial cells following contact and are attenuated for virulence in an adult mouse model of acute pneumonia. These results define a global regulatory network that controls and coordinates the expression of host-directed virulence determinants in *P. aeruginosa*.

0945 Transcriptional analysis of the effect of inactivation of the response regulator PhoP in *Yersinia pestis*

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Yersinia pestis is the aetiological agent of bubonic plague and is transmitted between susceptible hosts by flea vectors. Although primarily an extracellular pathogen, the organism undergoes an intracellular phase within macrophages *in vivo*. As a result of this complex life-style, the bacterium is exposed to diverse and stressful environments and must modify expression of its genes to optimise survival. The PhoPQ regulon is important in controlling the expression of genes in several pathogens. When compared to the wild type strain, an isogenic *Y. pestis phoP* mutant has previously been shown to be partially attenuated in the mouse, more susceptible to a range of stresses *in vitro* and showed a different lipo-oligosaccharide composition. Preliminary protein analysis showed PhoP to have pleiotropic effects on gene expression, with over twenty differences being observed on 2D gels. In order to characterise the effect of inactivation of PhoP more fully, we have analysed gene expression using a microarray based on the annotation of the *Y. pestis* CO92 genome. Many genes showed altered expression in the *phoP* mutant when compared to the parent strain. The differential expression of these genes may explain the increased sensitivity of the *phoP* mutant and may contribute to the attenuation observed *in vivo*.

1100 *Salmonella* gene expression during infection of mammalian cells

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The success of *Salmonella enterica* as a pathogen is partly explained by its ability to adapt to the intracellular environment of the phagocytic cell. However, we have understood little about this process of adaptation because of the difficulty in measuring bacterial gene expression during infection. In the past, reporter gene fusions were used to monitor changes in expression of certain genes during the transition from the extracellular to the intravacuolar environment, but until now it has not been possible to visualise gene expression at the RNA level. We have now developed a method for stabilising and purifying *Salmonella* RNA from infected mammalian cells, and have used DNA microarrays to determine the complete transcriptional profile of intracellular *S. enterica* sv. Typhimurium following infection of macrophage and other cell lines.

During replication in murine macrophage-like J774-A.1 cells, 919 of 4451 *S.* Typhimurium genes showed significant changes in transcription. The expression profile identified alterations in numerous virulence genes, and revealed unexpected findings concerning the biology of the *Salmonella*/macrophage interaction. Our latest experiments have revealed that *Salmonella* has a distinct expression profile during infection of human epithelial cells. Comparison with the data from macrophages showed differential expression of many *Salmonella* gene clusters including SPI1. This approach promises to be a useful tool for understanding how bacteria cause disease.

1145 GFP and DNA microarrays offer an integrative approach for the monitoring of Salmonella Virulence gene expression during infection

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Salmonella enterica sv. Typhimurium has developed a sophisticated way of communicating with its mammalian host to ensure its survival and multiplication throughout the process of infection. Although many genes have been shown to be required to cause disease, little is known about the organ and host cell-type specificity of *Salmonella* gene expression. In order to address these questions we have developed an improved system to assess the transcriptional response of *Salmonella* virulence genes. Based on the bright GFP^+ variant of the Green Fluorescent Protein reporter, this system allows the visualisation and the quantification of gene expression in individual bacteria using flow cytometric and fluorescence microscopic techniques. Combined with our microarray-based technology for identifying *Salmonella* genes that are regulated during infection (Eriksson *et al*., 2003, *Mol. Microbiol*., 47:103-118), this GFP system offers an integrative approach for understanding the expression of particular promoters at different stages of infection. We will describe the expression of two *Salmonella* genes in cultured mammalian cells and within specific organs of the BALB/c mouse model.

1205 *Campylobacter jejuni* gene expression in contact with intestinal epithelial cells

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Campylobacter jejuni invasion and interaction with tissue culture cells generally correlates with virulence. Using an ORF-specific microarray, we identified a set of *C. jejuni* genes that were immediately up-regulated in the presence of INT407 cells, including several on the pVIR plasmid, an uncharacterized two-component system, and *spoT*. *spoT* and its homolog *relA* have been shown in other bacteria to mediate production of "magic spots" (p)ppGpp which in turn activate the stringent response, a general stress response also important for virulence in intracellular pathogens. We found that *C. jejuni* mounts a strong stringent response that requires *spoT*. A *spoT* mutant failed to produce (p)ppGpp during nutrient deprivation, was defective for stationary phase survival, exhibited a marked decrease in rifampicin resistance, and could not survive under high O_2/low CO_2 conditions. The *spoT* mutant was also attenuated for invasion and intracellular survival in two tissue culture models. Microarray experiments comparing mutant, wild type, and a spontaneous suppressor revealed several marked transcription differences, including oxidoreductase, stress response, phosphate utilization, stationary phase, and pVIR genes. The heretofore undescribed *C. jejuni* stringent response thus affects multiple aspects of *C. jejuni* biology, including host cell interaction, and may lend insight into stringent response characteristics in other bacteria.

1415 Cross-talk between respiratory pathogens and mucosal epithelium

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Studies in our laboratories have shown that bacteria belonging to at least three distinct genera (*Neisseria*, *Haemophilus* and *Moraxella*) that reside in the human nasopharynx bind to members of the human immunoglobulin superfamily, the CEA-related cell adhesion molecules (CEACAMs). Several species within these genera have the propensity to cause localized or disseminated infections in susceptible hosts. Interactions of bacteria with cell-expressed CEACAMs have been shown to result in transmigration across polarised epithelial monolayers. In

order to analyse the molecular determinants of host cell targeting and tissue penetration, we have undertaken to identify the ligands of these bacteria that interact with CEACAMs and the binding sites of the ligands on the receptors. In addition, we have studied bacterial manipulation of host cell signalling via CEACAMs and the manner of crossing epithelial barriers. These studies have identified the CEACAM-binding ligands of *Neisseria*, *Haemophilus* and *Moraxella*, which are structurally distinct. Despite this, from studies using site-directed mutants of the receptor, they appear to bind to the same site on the receptor. They also compete for the receptor on target cells. Using well characterised *Neisseria* and *Haemophilus* isolates, we have investigated microbe-host crosstalk by inhibiting specific post-transcriptional/translational events in target cells and by transcription profiling using DNA microarrays. Our studies have shown that although primarily targeting the same receptor, *Neisseria* and *Haemophilus* clearly send distinct signals to target cells and appear to transmigrate across polarised monolayers by different mechanisms.

1500 The challenges of microarray transcription profiling *Neisseria meningitidis* during human endothelial interactions using a novel Pan-Neisseria microarray

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Background: *Neisseria meningitidis* (Nm) interaction with human brain endothelial cells is central to the pathogenesis of meningococcal meningitis. Microarray technology provides the opportunity for global gene expression profiling of the Nm gene expression events that occur during endothelial interaction. However, to avoid a distorted view of the transcription profile, there are significant challenges in experimental design and implementation that have to be overcome.

Aims: 1) To isolate sufficient undegraded meningococcal RNA(i.e. >5 µg), free of human RNA after human brain endothelial cell (HBEC) infection ; 2) To assess the utility of this RNA when applied to a novel pan-Neisseria microarray.

Methods: HBECs were infected with Nm MC58 derivatives for 30 and 60 minutes. Cellular transcriptional profiles were preserved with RNALater and the RNA extracted. Microbial RNA was separated from contaminating HBEC RNA using an oligo-dT/oligo-sandwich capture system. RNA quantity and quality was assessed using an Agilent 2100 bioanalyzer. Alexa-fluor labelled cDNA was generated and then hybridised onto the Pan-Neisseria array.

Results: Bioanalysis of isolated bacterial RNA revealed no eukaryotic RNA contamination after microbial enrichment. Hybridisations with >99.9% reproducibility at all microarray elements were achieved for both time points. Major changes in capsule biosynthetic machinery, opacity proteins and a significant ribosomal protein recruitment at both time points were observed.

Conclusion: Addressing the issues of RNA stabilisation and RNA isolation/enrichment allows generation of truly representative transcriptional profiles for infecting bacteria and a unique insight into the mechanisms of bacterial adaptation to different host cell environments on a global scale.

1540 Global gene expression analysis of intracellular *Francisella tularensis*

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Francisella tularensis, the causative agent of tularaemia, is a highly infectious facultative intracellular pathogen. The aim of this study was to identify *F. tularensis* genes that are up- and down-regulated during infection of macrophages. The on-going *F. tularensis* Schu S4 genome sequencing project was used to design oligos for the creation of a microarray for the study of global gene expression patterns. Mouse macrophage-like-cells (J774.A) and human monocyte-derived macrophages (U937) were infected with *F. tularensis* and the kinetics of uptake and growth were investigated. *F. tularensis* subspecies *novicida* was transformed with a plasmid encoding green fluorescence protein (GFP), and was used to further investigate infection of macrophages. Protocols for the isolation of RNA from intracellular bacteria have been investigated and compared in order to find a suitable method that does not induce changes in gene expression profile. At various time points post-infection, bacterial RNA will be isolated, using the most appropriate method, and hybridized to the *F. tularensis* microarray. It will be compared with RNA harvested from *in vitro* cultured *F. tularensis*. It is hoped that the elucidation of genes that are expressed intracellularly will provide detailed information regarding virulence determinants of *F. tularensis* to aid future vaccine research.

1600 Cross-talk between two type-III secretion systems in *Escherichia coli* O157 and regulatory links to other potential virulence factors

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Genomics sequence analysis reveals that there are two gene clusters encoding components of type III secretion systems (TTSS) in enterohemorrhagic *Escherichia coli* O157:H7: the well-characterised LEE region and a gene cluster encoding a second type-III secretion system, ETT2. Within the ETT2 region, there are at least three putative transcriptional regulator genes, ecs3709 (or YgeH), ecs3720 (or EtrA) and ecs3734 (or EivF). Using molecular techniques, we have knock these genes and characterised the gene-deletion mutants in comparison with the parent strain in terms of protein secretion and transcriptional profile. Several prominent bands on a 1-D SDS-PAGE gel showed alterations in secretion in two of the mutants and five of these proteins were identified using Mass Spectroscopy as substrates of the LEE-encoded system. This effect on LEE substrate secretion was also confirmed by Western blotting using specific antibodies. Microarray studies have shown that two of the ETT2-encoded regulators influence transcription of the LEE, with Ler also showing a less pronounced effect on transcription of some ETT2 genes. These findings indicate that there is cross-talk between these two TTSSs. In addition, our microarray studies show transcriptional effects across many other genes, not just those confined to the TTSS clusters.

POSTERS

MI 01 Systemic *Candida parapsilosis* infection and antibody response in an experimental model

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Candida parapsilosis, a fungal pathogen which can cause systemic candidiasis infection, has reportedly been isolated from significant numbers of susceptible individuals. In this study systemic *Candida parapsilosis* infection and antibody response during infection was evaluated in a rat model of infection. Sprague-Dawley rats were infected by intravenous injection with either 1×10^6 or 1×10^8 *C. parapsilosis* blastoconidia. The severity of infection was evaluated through organ culture, carried out weekly. The animals' IgM response was determined by immunoblotting sera against *C. parapsilosis* cytoplasmic protein antigens. No deaths occurred in the animals given either infective dose. The liver was the main target organ for this infection. Recovery of *C. parapsilosis* from liver, kidney, spleen and heart decreased over time with a more rapid decrease seen in the animals given the lower infective dose. Low levels of IgM were produced against *C. parapsilosis* cytoplasmic antigen, with a slight increase in IgM production noted around 9 to 14 days post-inoculation. A 55 kiloDalton antigenic component appeared to be immunogenic in the infected animals. Our results indicate that *C. parapsilosis* has low virulence and its cytoplasmic proteins do not elicit a major antibody response in infected animals.

MI 02 Optimised Random Amplification of Polymorphic DNA (RAPD) for the specific detection of *Peptostreptococcus magnus* in polymicrobial infections

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Peptostreptococcus magnus (*P. magnus*) is a slow growing, gram-positive anaerobic cocci (GPAC) and is an opportunist pathogen. It is usually found in infections of the skin, urinary and urethral systems. Increasingly, many cases of infection with *P. magnus* have been isolated in conditions such as endocarditis and prosthetic joint infections, which are usually polymicrobial in nature. So far, no rapid method of identification exists. In this study, we are trying to develop a specific method of detection of *P. magnus* using random amplification of polymorphic DNA (RAPD) and the identification of a specific RAPD marker.

Primarily, this molecular tool will help in the rapid diagnosis of infection with *P. magnus* but will also be used to the typing of the different strains that have been identified from prosthetic hip infections and pus samples. Dendrogram analysis will reveal the distinct clusters of *P. magnus* and their relatedness. The typing of the different strains will help increase our understanding of how the infection with *P. magnus* takes place and the correlation of the different genotypes and the type of infection.

MI 03 Persistence of *Mycobacterium tuberculosis* (MTB) DNA at extra pulmonary sites of people who were latently infected in their lung

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Objectives: The specific sites of persistence of MTB during latent tuberculosis infection (LTBI) in terms of both organs and cell types remain elusive. We have identified the cadavers of Mexicans who died of causes other than tuberculosis, but who nevertheless had MTB DNA in their lung. This work attempts to study the metastatic location of LTBI and if such sites were found, to pinpoint the cellular location of MTB DNA in different anatomical structures.

Methods: Multiple anatomical sites of six cadavers with lungs positive and three with lungs negative for MTB DNA were examined using conventional and *in situ* PCR.

Results: The most common extra pulmonary sites positive for MTB DNA were liver, spleen and kidney, however brain was also found to harbour MTB DNA. Except for the spleen from one case, all other anatomical sites were negative in those who were negative for MTB DNA in their lung. *In situ* PCR revealed positive signals in the cytoplasm of macrophages, epithelial cells, endothelial cells, fibroblasts, and interestingly in neurons in white matter. Occasionally positive signals were found inside the nucleus.

Conclusion: Latency is maintained not only in the lungs but also at extra-pulmonary sites in the macrophages as well as in the non-professional phagocytic cells. For the first time, MTB DNA has been located tentatively inside the nucleus.

MI 04 Are Type IV pili involved in the adherence of *Burkholderia pseudomallei*?

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Burkholderia pseudomallei is the causative agent of melioidosis, a disease of humans and animals that is endemic in the tropical areas of Southeast Asia and the northern regions of Australia. Melioidosis is a severe and fatal infectious disease for which there is no licensed vaccine. An essential step in most infectious disease processes is the adherence of the pathogen to host cells. Bacterial adhesion requires the specific interaction of surface proteins (adhesins) with host cell receptors. Type IV pili are adhesins and are widespread amongst Gram-negative bacteria. The chromosome of *B. pseudomallei* has been found to contain genes that are putatively involved in type IV pili biogenesis. Type IV pili are predominantly made up of a major pilin subunit. The putative pilin subunit (PilA) of *B. pseudomallei* is encoded by *pilA*. In order to ascertain the role of this type IV pili subunit in *B. pseudomallei*, an unmarked nonpolar *pilA* deletion mutant was generated. Using an *in vitro* adherence assay the mutant was found to have reduced adherence to a variety of cell lines when compared to wild-type. This suggests a possible role for type IV pili, specifically PilA, in the adherence of *B. pseudomallei*, raising the possibility of an anti-adhesive vaccine.

MI 05 Antimicrobial activity of amino terminal domain of SGP (*Streptococcus mutans* GTP-binding protein)

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GTP-binding proteins have now been identified in several bacteria. They appear to be essential for cell growth since attempts to mutate this gene were unsuccessful. In the oral bacterium SGP (*Streptococcus mutans* G-protein) has been identified in *Streptococcus mutans* and reported that this protein complements an era mutation in *E. coli*. Evidence has been also presented that SGP plays a role in the stress response of *S. mutans*. Although GTPases are known to be involved in a number of bacterial cell functions including cell cycle regulation, the precise role of these proteins has yet to be determined. A decapeptide P1 corresponding to a homologous region of the SGP and the Era protein of *Escherichia coli* was synthesized and examined for growth inhibitory effects. When P1 (10 mg/L) was added to *E. coli* DH5a the viability of the cells was reduced 13%. However, the addition of lauric acid enhanced the bactericidal effects of P1 (68% killing in the presence of P1 plus lauric acid). Similar enhancing effects were also observed for MLG (mono

lauroyl-*rac*-glycerol) and SDS (Sodium dodecylsulfate). Furthermore, the addition of P1 together with the detergent MLG resulted in a number of elongated cells compared to cultures treated with detergent or peptide alone. As with *E. coli*, the bactericidal effects of P1 on *S. mutans* were also significantly enhanced in the presence of the detergent lauric acid. The combination of the two effecters produced more than 90% killing of *S. mutans*. Likewise, the combined action of P1 plus lauric acid reduced the viability of *Listeria monocytogenes*. However, P1 did not appear to be toxic to human gingival epithelial cells when added at concentrations as high as 1,000 mg/L. Therefore, P1 has properties which could be utilized as a novel antibacterial agent.

MI 06 The identification of iron-regulated proteins in *Francisella novicida*

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It is widely established that iron plays a central role to bacterial metabolism, acting as a cofactor to numerous cellular enzymes. Bacteria can obtain iron from the extracellular environment via several mechanisms, usually by expressing iron-chelating proteins that bind iron with a high affinity, enabling cell uptake. Extensive research has shown that such iron acquisition systems undergo strict regulation, to ensure cells satisfy their nutritional requirement whilst protecting against the build-up of toxic iron levels. During infection, bacteria often increase expression of systems that obtain iron due to the limited availability of free iron within the host. Therefore, such mechanisms can be regarded as important virulence factors that promote an established bacterial presence. The induction of iron-regulated proteins has offered an improved protection against certain pathogens, such as the fish pathogen *Aeromonas salmonicida*, allowing the production of vaccines against a number of diseases. Therefore, the aim of this research is to characterise the iron-regulated proteins of *F. novicida* and to investigate whether such proteins can serve as sub-unit vaccines against the facultative intracellular bacterium *Francisella tularensis*, the causative agent of tularemia. Our initial approach has been to analyse the protein profiles of *F. tularensis* subspecies *novicida*, grown under different iron conditions. We report here the differences in protein expression of *F. novicida* observed by 2D PAGE analysis, following growth in media of differing iron concentrations.

MI 07 The construction of a defined purine auxotroph in *Francisella tularensis* species

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Francisella tularensis is a Gram negative, intracellular pathogen, which causes the debilitating disease tularemia in humans and a wide range of animals. The attenuated *F. tularensis* live vaccine strain (LVS) can induce protective immunity against tularemia in humans, but the basis of attenuation is not understood at the molecular level, thus preventing its licensing as a live vaccine. Therefore we aim to construct a rationally defined mutant that would be suitable for licensing as a vaccine. The *purA* gene, which is part of the purine biosynthetic pathway, has been identified as a potential target for mutagenesis. A defined deletion was introduced into the central region of the *purA* gene and a chloramphenicol resistance gene inserted at the deletion site. The inactivated gene was cloned into the pUC18 plasmid. The *sacB* gene was also cloned into the polylinker region of the construct to provide an additional means of selection. The construct was introduced into *F. novicida* U112 by cryotransformation. Transformants were recovered and were identified as either integrants or mutants. A *F. novicida purA* mutant was characterised and found to be unable to grow in mouse macrophages. It was also highly attenuated in the Balb/c mouse model of infection. The *purA* mutant did not induce a protective immune response against challenge with *F. novicida* U112, possibly due to over attenuation. This was confirmed by rapid clearance during *in vivo* studies.

MI 08 Characterization of the O-antigen gene cluster of *Francisella tularensis* subspecies *tularensis*

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A gene cluster encoding enzymes involved in LPS O-antigen biosynthesis was identified from the *F. tularensis* Schu S4 partial genome sequence. All of the genes within the cluster were assigned putative functions based on sequence homology with genes from O-antigen biosynthetic clusters from other bacteria. Ten pairs of overlapping primers were designed to PCR-amplify the O-antigen biosynthetic cluster from 17 strains of *F. tularensis*. Although the gene cluster was present in all strains, there was a difference in size of some of the PCR products between subspecies *tularensis*, *holarctica* and *mediaasiatica* strains. The GenBank [/EMBL/DDBJ] accession number for sequences reported in this poster is AY217763.

MI 09 Antibiotic sensitivity of respiratory tract pathogens in the presence of lactoferrin and mucin

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Sputum contains lactoferrin, which limits iron availability to bacteria. Lactoferrin also has a direct effect by damaging the outer membrane of Gram-ve bacteria and thus increase their susceptibility to antibiotics. Presence of lactoferrin *in vivo* enhances the activity of antibiotics, an effect that would not be predicted by standard antibiotic testing methods. In this setting it may be useful to incorporate lactoferrin in antibiotic sensitivity testing methodology. Although such tests would be more expensive than the standard tests, but might have more beneficial results that lead to more effective treatment of the patient.

We determined the MIC and MBC of various antibiotics in presence and absence of lactoferrin and / or mucin for different strains of *H. influenzae* from chronic bronchitis patients and different strains of *S. maltophilia* plus the mucoid and non mucoid strains of *Ps. aeruginosa* isolated from CF patients. Lactoferrin used in two concentrations that found in chronic bronchitis patients (0.14 mg/ml) and CF patients (0.9 mg/ml).

Lactoferrin increased the susceptibility of the tested strains to rifampicin, Chloramphenicol and doxycycline, but not to erythromycin and fusidic acid. Mucin abolished the above mentioned effect on rifampicin and chloramphenicol.

This study didn't rule out the beneficial effects of lactoferrin, but pointed to do more investigations about the role of mucin before addition of an expensive lactoferrin to the susceptibility testing media can be justified.

MI 10 Rapid identification of *Arcobacter* species and *C. jejuni* and *C. coli* using melting temperatures of adjacent hybridisation probes

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The twenty species and eight subspecies of the genera *Arcobacter* and *Campylobacter* are Gram negative, microaerophile, curved, spiral or S-shaped cells, and members of the order *Campylobactreiales*, class *Epsilonproteobacteria*

phylum *Proteobacteria.* Though most members are pathogenic causing gastrointestinal illness, *C. jejuni, C. coli* and *A. butzleri* are the most frequently species. The current methods for their detection, identification and differentiation are cumbersome, time consuming and lack specificity.

A new, complex, single tube system, termed multi FITC adjacent hybridization Real Time PCR assay, was developed for the simultaneous detection and differentiation of *C. coli, C. jejuni, A. butzleri, A. skirrowii* and *A. nitrofigilis,* from other members of the family *Campylobacteriaceae.* The FRET method relied on the use of three specific adjacent downstream FITC-labelled probes and an adjacent universal CY5 labelled probe (Cy5-5'AGGTGITGCATGGITGTCGTTGTCG-P$O_4$3'), three different FITC labeled probe used termed as [probe *coli-jejuni*(5`-GTGCTAGCTTGCTAGAACTTAGAGA-FITC-3`)specific for *C. coli* and *C. jejuni*; probe butzleri [5`GTGTCTGCTTGCAGAAACTTGCATA-FITC-3] specific for *A. butzleri* and probe SkirCry specific for *A. skirrowii* and *A. cryaerophilus* [5`GTGTCTGCTTGCAGAAACTTATATA-FITC-3`]) were used). The binding of the FITC labelled probes in conjunction with the universal CY5 labelled probe to their specific target sites produced an increase in fluorescence and was measured in a LightCycler. The subsequent dissociation of the probes to produce specific melting temperatures (Tm) identified and differentiated the targeted species: Tm for *A. butzleri* was 67 ^{o}C, for *A. skirrowii* 63 ^{o}C, for *C. coli* and *C. jejuni,* 65 ^{o}C and for the other *Campylobacter species* 56 ^{o}C. 162 isolates out of a 193 isolates were differentiated as *C. coli* and *C. jejuni* group, *A. butzleri* (20 isolates) *A. skirrowii* (1 isolates) and other species (10 isolates).

MI 11 Rapid detection and identification *C. jejuni* from chicken sample by Real Time PCR

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The sixteen species and eight subspecies of the genera *Campylobacter* are Gram negative, microaerophile, curved, spiral or S-shaped cells, and members of the order *Campylobactreiales,* class *Epsilonproteobacteria* phylum *Proteobacteria.* Though most members are pathogenic causing gastrointestinal illness, *C. jejuni* is the most frequently specie. The current methods for their detection, identification and differentiation are cumbersome, time consuming and lack specificity.

A two-stepped Real Time assay, developed in a LightCycler™, detected, identified and differentiated the pathogens, *C. coli* and *C. jejuni*; from other members of the family *Campylobacteriaceae.* In the first step of the assay, the continuous monitoring of Fluorescence Resonance Energy Transfer (FRET) signal acquired from the hybridisation of two adjacent fluroprobes, [a universal probe Cy5 (5`-Cy5-AGGTGITGCATGGITGTCGTTGTCG-P$O_4$-3`) and a specific downstream FITC probe (5`-GTGCTAGCTTGCTAGAACTTAGAGA-FITC-3`)], to the 681 base pair amplicon target of the 16S rRNA gene (*E. coli* position numbers 1024 to 1075) produced form the amplification of primer pair, F2 (ATCTAATGGCTTAACCATTAAAC, *E. coli* position 783) and Cam-Rev (AATACTAAACTAGTTACCGTC, *E. coli* position 1464), enabled the identification of both *C. coli* and *C. jejuni.* As expected, a Tm of 65 ^{o}C was derived from the temperature dependent probe – DNA strand disassociation data. In the second step of the assay, an increase in fluorescence due to binding of the intercalating dye SYBR Green I to the DNA amplicons of the hippuricase gene (hipO) (produced by the primer pair Hip2214F and Hip2274R) was observed for *C. jejuni* but not for *C. coli* which lacks hipO genes well as the Ceu E gene for *C. coli.* Furthermore, a Tm of 85±0.5 ^{o}C and 56 ^{o}C derived from temperature dependent dye – DNA disassociation identified *C. jejuni* and the non-specific primer dimers and / or non-specific PCR products respectively, in line with our expectation. The two stepped assay was used successfully to identify and differentiate 20 *Campylobacteriaceae* isolates from fresh chicken pieces.

MI 12 Killed whole cell induced protection against *B. pseudomallei*

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Burkholderia pseudomallei is the causative agent of melioidosis and emerging as a significant cause of disease in tropical and sub-tropical areas of the world. No vaccine currently exists for use against this bacterium and treatment often involves protracted antimicrobial therapy. The development of an effective vaccine for use against melioidosis would be of significant economic and humanitarian benefit. We have investigated the use of killed whole cells of *B. pseudomallei* as potential vaccine candidates and as tools in order to investigate those components of the *B. pseudomallei* cell that are important in inducing a protective response in mammalian models of disease.

MI 13 The role of the *Dam* gene in virulence of *Yersinia pestis* and *Yersinia pseudotuberculosis*

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We have identified a DNA adenine methylation (Dam) system in *Yersinia pestis,* the causative agent of plague. Dam is responsible for controlling the timing of DNA replication, the regulation of the expression of a number of virulence genes and also has a role in post-translational mis-match repair. Mutants of *Salmonella enterica* serovar Typhimurium that lack Dam are avirulent and are suitable as live vaccine vectors. A Dam methylation system has previously been identified in *Yersinia pseudotuberculosis.* Previous reports have shown that Dam was essential for viability, and over-production of Dam led to a >6000 fold attenuation in virulence. We constructed a plasmid that constitutively expressed the *Y. pestis dam* gene. This plasmid was introduced into a virulent strain of *Y. pestis,* strain GB, resulting in aberrant methylation. Although the plasmid was stable *in vivo,* constitutive expression of the *dam* gene failed to attenuate *Y. pestis,* in contrast to results previously reported for *Y. pseudotuberculosis.* We then created isogenic *dam* mutants of both *Y. pestis* and *Y. pseudotuberculosis.* These mutants were attenuated in Balb/c mouse models of disease showing a key role in the regulation of virulence.

MI 14 Invasion of primary cultures of human endothelial cells by *Enterococcus spp*

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Enterococcus spp are normal components of the intestinal flora, known by their inherent resistance to several antibiotics. Colonization of cardiovascular endothelium by enterococci can result in infective endocarditis highly resistant to antimicrobial chemotherapy. One possible mechanism used by this bacterium might be evasion of drug action and host immune response by invading eukaryotic cells. Using a standard antibiotic protection assay we tested clinical isolates' ability to infect primary cultures of Human Umbilical Vein Endothelial Cells (HUVEC). Invasion was determined by Gram stain, electron microscopy and C.F.U counts. Over a period of two hours *E. faecium, E. faecalis* and *E. gallinarum* strains attached and invaded HUVEC without causing citotoxicity. Electron microscopy revealed enterococci adhered to HUVEC surfaces and bacteria in membrane encased cytoplasmic vacuoles. Assays to determine invasion mechanisms are currently underway.

MI 15 The investigation of a novel intercellular communication system in bacteria

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The presence of intercellular signalling mechanisms between bacterial populations has been demonstrated for many bacterial species and a variety of signalling molecules have been identified, including the peptide pheromones utilised by Gram positive species and the *N*-acylhomoserine lactones produced by Gram negative bacteria. A novel system of intercellular communication was recently identified in laboratory strains of *Escherichia coli*. On solid agar, stationary phase cells (the helper (H) population) were found to confer ampicillin resistance to a physically separated population of cells (the stressed (S) population). This growth promoting effect was proposed to result from the airborne transmission of a signalling molecule produced by stationary phase bacteria. The aim of our study is to further characterise this novel communication system. Using laboratory strains of *E. coli*, initial experiments confirmed that H populations were able to confer ampicillin resistance to physically separated S populations. Subsequently, *E. coli*, *Yersinia pseudotuberculosis* and *Bacillus subtilis* were used as H and/or S populations in stress experiments involving ampicillin or kanamycin. The H population promoted growth of the S population on ampicillin only when *E. coli* strains were used as the S population, although all the tested strains could act as the H population. No growth promoting effects were observed when kanamycin was used.

MI 16 Investigation into differential gene expression between planktonic and biofilm cells of *Pseudomonas aeruginosa*

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Bacteria attach to available surfaces in aquatic environments and undergo physiological alterations as a result of differential gene expression. Many techniques have been developed in recent years to investigate differential gene expression with varying levels of success and subtractive hybridisation is regarded as one of the most sensitive, although it has not yet been adapted to study prokaryotic genomes. The aim of this investigation was to develop the PCR Select cDNA Subtraction kit (Clontech, UK), to make it suitable for use with prokaryotic cells, particularly *P. aeruginosa*. It was also necessary to develop a novel mRNA extraction method that could be applied to both biofilm and planktonic samples. Modifications have included design of appropriate controls to assess the completion of each stage. Data from initial runs indicate that all five 16S rRNA operons were expressed at a greater level in biofilm than planktonic cells, findings consistent with other research groups. Methods used to investigate differential gene expression tend to give diverse results and it is clear that further research is required in order to develop a reliable, repeatable and relatively inexpensive method.

MI 17 Characterisation of O-antigen biosynthesis genes in *Bordetella*

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Background and objective of investigation: *Bordetella bronchiseptica* and *Bordetella parapertussis* are pathogens of the mammalian respiratory tract. These bacteria express lipopolysaccharide (LPS) containing an O-antigen, necessary for wild-type (w.t.) virulence and encoded by the *wbm* locus. This project aims to characterise the roles of *wbm* genes in LPS O-antigen biosynthesis.

Methods: *Wbm* genes were disrupted by *in vitro* mini-Tn*5* transposon mutagenesis of a *wbm* gene-containing cosmid. Bacterial mutants were generated by allelic exchange, and LPS phenotype was assessed by SDS-PAGE.

Results: W.t. O-antigen is expressed with a narrow range of repeat units suggesting that chain length is tightly regulated. Mutation of several different *wbm* genes resulted in expression of O-antigens, identical to each other by SDS-PAGE analysis, which had a broader distribution of repeat units, suggesting deregulation of O-antigen chain length.

Conclusions: This data supports the role of *wbm* genes in O-antigen biosynthesis. It is possible that w.t. O-antigen structure is required for recognition by *Bordetella* O-antigen chain-length-determining machinery. Full understanding of *wbm* gene function will require structural analysis of the mutant O-antigens. The strains generated in this study will be useful for investigating the role of O-antigen in pathogenesis.

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MI 18 Comparative expression profiling in the three defined forms of ovine paratuberculosis

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Paratuberculosis (Johne's disease) is a chronic intestinal condition of ruminants caused by *Mycobacterium avium* subspecies *paratuberculosis (Map)*. *Map* gives rise to three different forms of intestinal pathology, in sheep: infected but asymptomatic, paucibacillary or tuberculoid form and multibacillary or lepromatous form. The response of the macrophage is thought to be pivotal to the outcome of the bacterial infection.

We have designed a Ruminant Immuno-inflammatory Gene Universal Array (RIGUA), which measures the expression of 512 different immuno-inflammatory genes in the three forms of the disease. In developing this microarray, we produced a first generation chip; the Ruminant Immuno-inflammatory Gene Reference Array (RIGRA).

This technology is being used to test two hypotheses: 1) There are intrinsic differences in the way macrophages respond to *Map* infection in the three forms of the disease (asymptomatic, paucibacillary and multibacillary); 2) Differences in the immuno-inflammatory gene expression in gut associated lesions are directly related to the different pathological forms of the disease.

Defining a *signature* of immuno-inflammatory gene expression in relevant tissues from the three forms, will also provide a foundation for the development of preventative measures against *Map* and possible targets for selective breeding.

MI 19 Cytotoxic activity in *Campylobacter jejuni* strains

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The most common cause of bacterial diarrhoea in many industrialized countries is *Campylobacter jejuni* infection, which represents a major public health and economic burden. Effective control of this organism in the food chain and the design of disease prevention strategies are hindered by a poor understanding of the physiology, virulence and genetics of this bacterium. Production of toxins by *Campylobacter* is an area that is poorly understood.

We have investigated cytotoxin production by different strains of *C. jejuni* by determination of the capacity of cell-free extracts of the bacteria to inhibit mitochondrial dehydrogenase activity in Vero or Caco-2 cells using a dye-reduction assay or by determination of the viability of the tissue culture cells by measurement of ATP (CellTiter-Glo™ Luminescent Cell Viability Assay). Little cytotoxin activity was detected in culture

supernates and, when cell-free extracts were investigated, most activity was membrane-associated. Differences in the extent of cytotoxin production was found between strains. The type strain 11168 and a *cdt*B mutant, lacking cytolethal distending toxin (CDT) activity, were used to assess to what extent CDT contributed to cytotoxicity. Extracts of the mutant strain were as cytotoxic as the wild type strain, indicating that toxin activity measured in the cytotoxicity assays was not due to CDT. The characteristic morphological changes of Vero cells after 72h incubation, due to CDT, were apparent only with the wild type strain, and not with the *cdt*B mutant. Work aimed at further characterization of the cytotoxin(s) will be presented.

MI 20 A *hexA* homologue from *Photorhabdus* regulates pathogenicity, symbiosis and phenotypic variation

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Photorhabdus is a genus of entomopathogenic Gram-negative bacteria that belong to the family *Enterobactericeae*. Remarkably, at the same time as being pathogenic to insect larvae, *Photorhabdus* also have a mutualistic relationship with entomophagous nematodes of the family *Heterorhabditiae*. *Photorhabdus* can be isolated in one of two phenotypically distinct forms, termed the primary and secondary variant. Both variants grow equally well and are equally virulent when injected into insect larvae. However, only the primary variant can support nematode growth and development. The primary variant produces several characteristics that are absent from the secondary variant, including bioluminescence and the production of extracellular enzymes, pigments and antibiotics and we hypothesised that these primary-specific phenotypes are required for symbiosis and the production of these factors is repressed in the secondary variant. To test this we subjected the secondary variant of *Photorhabdus temperata* strain K122 to transposon mutagenesis and we isolated two bright mutants. Both of these mutants were interrupted in the same gene that is predicted to encode a protein with homology to HexA from *Erwinia*. We show that the *hexA* mutants also produce the other primary-specific phenotypes and support nematode growth and development. Therefore, HexA represses symbiosis in the secondary variant of *Photorhabdus*. However, the *hexA* mutant was significantly attenuated in virulence to insect larvae. This suggests that, during a normal infection, pathogenicity and symbiosis must be temporally separated and HexA is involved in the regulation of this pathogen-symbiont transition.

MI 21 Identification and characterisation of immunogenic putative virulence factors of *Actinobacillus pleuropneumoniae*

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Actinobacillus pleuropneumoniae is the etiological agent of porcine pleuropneumonia: a highly contagious, severe and often fatal pulmonary disease. Current vaccines have been found to be ineffective, and few virulence factors are known. The aims of the project were to identify and characterize novel virulence factors and vaccine candidates of *A. pleuropneumoniae*. To do this a λ phage genomic expression library of *A. pleuropneumoniae* was constructed and screened with antiserum from convalescent pigs, and a rabbit polyclonal antiserum raised against preparations enriched in outer membrane and secreted proteins. Forty-one immunogenic clones were isolated: 36 detected using the rabbit sera; and three by their immunogenicity to pig sera. A single clone was detected by both. The Phagemids were excised, partially sequenced and mapped onto contigs of the unfinished serotype 1 strain 40325 genome sequence. One hundred and twenty open reading frames (ORF) were found close by or within the homologous regions. Each ORF was analysed *in silico*. A number of ORFs predicted to encode outer membrane or secreted proteins were selected. Several of these have now been cloned and are in the process of being characterised for further study.

MI 22 Structure and function studies of the type III secretion system proteins of *Yersinia pestis*

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Yersinia pestis, the causative agent of plague, affects both humans and animals. Successful treatment with antibiotics is dependent on a quick diagnosis. Current vaccines are either detrimental to humans or do not protect against the more severe forms of the disease, thus there is a clinical need for a more effective vaccine.

Yersinia is able to resist primary host cell immune responses by inhibiting phagocytosis by macrophages, using a type III secretion system, which translocates a number of proteins into the host cell. One of these proteins, LcrV, is directly linked to virulence and is therefore thought to be an ideal vaccine candidate. A titration model has been proposed which explores the interactions of LcrV and other *Yersinia* proteins (Yops) and places them on either the inner or outer membrane surface of the bacterial cell wall.

A number of vector constructs were made to enable the production of recombinant LcrV, LcrG and LcrQ with either an enhanced green (EGFP), yellow (EYFP) or cyan (ECFP) fluorescent protein, fused at either the N- or C-terminal. Secretion assays were performed under both secretive and repressive conditions to test the integrity of the constructs and confocal microscopy was performed to visualize the type III secretion system under real time.

MI 23 Identification and characterisation of two novel autotransporter proteins, designated *Campylobacter* autotransporter proteins A and B (CapA and CapB), in *C. jejuni*

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Two putative autotransporter proteins were identified *in silico* in *C. jejuni* strain NCTC 11168, and were designated *C*ampylobacter *a*utotransporter *p*rotein A (CapA; Cj0628/0629) and B (CapB; Cj1677/1678), respectively. Both *capA* and *capB* genes contain frame-shift mutations suggesting that expression may be phase-variable. The reconstructed *capA* and *capB* genes are predicted to encode proteins of *c.* 120 and *c.* 118 kDa, respectively, which are 82% identical. CapA has been cloned and expressed as a fusion with maltose-binding protein. Rabbit polyclonal serum was raised and electron microscopy with immunogold labelling demonstrated that CapA is accessible to antibodies on the surface of the organism. Cell fractionation studies showed that CapA is translocated to the outer membrane but is not secreted. Expression of CapA was demonstrated in Cj11168 and in 4/10 clinical isolates examined. Moreover, sera from patients convalescing from *Campylobacter* gastroenteritis contain CapA-specific antibodies, suggesting that CapA is immunogenic and expressed during infection. Mutation of the *capA* gene resulted in a reduced adherence and invasion phenotype *in vitro* and also caused a pronounced reduction in chicken colonisation. These findings suggest that CapA is a phase-variable, immunogenic, outer membrane protein, which plays a role in adhesion and invasion. Expression of CapB, on the other hand, could not be detected.

MI 24 Identification of new two-component systems involved in environmental sensing in *Bordetella bronchiseptica*

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The animal pathogen *Bordetella bronchiseptica* has the ability to adapt to growth in a variety of different niches that include low-nutrient conditions, the mammalian respiratory tract and an intracellular location within eukaryotic cells. Most virulence factors of *B. bronchiseptica* respond to environmental change via two-component regulatory systems (TCS) called BvgAS and RisAS. Comparative bioinformatics has been used to identify novel TCS homologues in *B. bronchiseptica* and two loci from different regions of the *Bordetella* chromosome were cloned and annotated *bagAS* and *bhoAS*. Homologous DNA exchange mutagenesis was used to create null mutants in these TCS in order to investigate a potential environmental-sensing role. To assess if these TCS(s) played a role in *Bordetella* virulence, mice were challenged intransally with wild-type and mutant strains. Both mutants showed a markedly increased rate of clearance from the murine lung, with the Bho null mutant being essentially avirulent and incapable of lung colonisation. Both mutants also displayed reduced intracellular survival in a murine macrophage-like cell line, compared to the wild type.

In order to investigate which gene products might be affected by the mutations, the proteome of the wild-type/mutant strains was analysed. Over 20 protein spots showed differences between the mutants and wild-type profiles. Using mass spectral and peptide analysis, the chaperone-like protein Cpn60, a GroEl homologue, was found to be up-regulated in the Bag mutant compared to the wild-type. Other differentially-regulated proteins have also been identified to help establish the role that these TCS(s) might play *in vivo*. Interestingly, both mutants showed, to different degrees, resistance to the penicillin derivatives piperacillin, ticarcillin, ceftazidim and cefepim. Exposure to the oxidant hydrogen peroxide indicated that the Bho mutant had a significantly increased sensitivity compared to the wild type. The work establishes two other TCS regulatory loci, besides *bvg* and *ris*, that play a role in the response of *B. bronchiseptica* to the external environment.

MI 25 Characterisation of the *Burkholderia pseudomallei* proteome

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Burkholderia pseudomallei is capable of infecting a wide range of mammals, including humans, and is the causative agent of the disease melioidosis. Treatment of the disease is prolonged, the fatality rate can be as high as 40% and relapses are common. There is currently no licensed vaccine. The genome has recently been sequenced by the Sanger Institute and the availability of which has enabled the identification of proteins by MALDI-TOF mass spectrometry.

B. pseudomallei was cultured under a variety of conditions. Following cell lysis, the proteome was fractionated by serial extraction with reagents of increasing solubilising power. The first fraction was extracted in 40 mM Tris, while the second fraction was extracted with 8 M urea, 4% (w/v) CHAPS, 40 mM Tris and 0.2% (w/v) Bio-Lyte 3/10 ampholyte. The third fraction was extracted with 5 M urea, 2 M thio urea, 2% (w/v) CHAPS, 2% (w/v) SB-3-10, 40 mM Tris and 0.2% (w/v) BioLyte 3/10 ampholyte. The samples were then analysed by two dimensional SDS-PAGE. After staining, protein spots were excised using the GelPix robotic spot cutter (Genetix Ltd, UK). In gel trypsin digests were performed using a Multiprobe HT II liquid handling station (Packard Biosciences, UK) and Montage Zip plates (Millipore, UK). Protein identifications were assigned by MALDI-TOF peptide fingerprint analysis.

MI 26 Identification of the virulence factors of *Orientia tsutsugamushi* by 2D-PAGE

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Orientia tsutsugamushi is a member of Gram-negative, obligate intracellular bacterium. Infections by *Orientia tsutsugamushi* virulent strains are characterized by fever, rash, eschar, pnuemonia, myocarditis, and disseminated intravascular coagulation. *Tsutsugamushi* disease can only be transmitted to humans by trombiculid mite larva. *O. tsutsugamushi* invades host cells by induced phagocytosis and usually infects endothelial cells, macrophages, and polymorphonuclear leukocytes (PMN). However the virulence factors related to the phagosomal membrane lysis have not been identified.

In order to know effector molecules in this process, we compared proteom profiles of *O. tsutsugamushi*- infected host cells, *O. tsutsugamushi*, and host cells by 2D-PAGE. Several candidate proteins expressed from *O. tsutsugamushi* and involved in the infection were detected by this method.

Our results show that 2D-PAGE could be used for the identification of the virulence factors of the intracellular bacterium.

MI 27 Functional role of major surface proteins of group IV oral spirochetes in periodontitis

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Group IV oral spirochetes consist of *Treponema lecithinolyticum* and *Treponema maltophilum*. They belong to the most frequently found spirochetes in subgingival plaques of periodontitis patients. In order to know the pathogenetic role, the full-length genes encoding the major surface proteins (MspTL and MspA) were cloned and expressed by using the expression vector pQE30. Recombinant MspTL(rMspTL) and MspA(rMspA) were purified and their effects on host cells were examined.

Cultured periodontal ligament cells were stimulated with rMspTL or rMspA. The mRNA levels of proinflammatory cytokines and matrix metalloproteinases(MMPs) were measured by RT-PCR. Blocking experiments were performed by adding anti-MspTL and anti-MspA antibodies to cultures stimulated with rMspTL and rMspA, respectively.

Incubation of PDL cells with MspTL and MspA resulted in upregulation of IL-1ß, IL-6, membrane type 1(MT1)-MMP, and MMP-9 mRNA expression. The effects of the proteins on PDL cells were significantly inhibited by anti-MspTL and anti-MspA antibodies.

Our results suggest that major surface proteins of group IV oral spirochetes contribute to pathogenesis of periodontitis by induction of proinflammatory cytokines and MMPs involved in tissue destruction.

MI 28 Cloning, expression and characterisation of immunophilins from *Burkholderia pseudomallei*

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Burkholderia pseudomallei is a Gram-negative, intracellular, opportunistic pathogen that causes the disease melioidosis in man and many animals. Melioidosis is endemic in Southeast Asia and Northern Australia. Infections are particularly debilitating and can lead to abscesses in the lungs and other organs with accompanying septicaemia. *B. pseudomallei* is a recognised bio-terrorism agent and is particularly dangerous as there is no vaccine currently available and treatment with antibiotics is

prolonged and often ineffective, with mortality rates of up to 40%.

Immunophilins exhibit a peptidyl prolyl isomerase (PPIase), activity which catalyses the isomerisation of peptide proline residues between *cis* and *trans* conformations, an action that influences protein folding. Immunophilins have been shown to be conserved and essential for virulence in *Legionella pneumophilia* and three putative immunophilins have been identified in the *B. pseudomallei* genome. One of the imunophilins, MIP2, has been cloned, recombinant protein purified and has been shown to exhibit PPIase activity.

MI 29 Structure of lipid A in relation to sensitivity of *Burkholderia cepacia* complex organisms to antimicrobial peptides

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Burkholderia cepacia is an important respiratory pathogen in immunocompromised patients and those with cystic fibrosis. Treatment is complicated by its intrinsic resistance to a broad range of antibiotics and antimicrobial peptides (APs). Resistance of the organism is associated with the outer membrane structure, in particular the structure of the lipid A component of the lipopolysaccharide (LPS).

The aim of this study was to determine if *B. cepacia* genomovars differ in sensitivty to APs, and to examine the relationship between genomovar, lipid A structure and AP sensitivity. The sensitivity of 15 strains to 15 APs was examined in double layer agarose diffusion and broth microdilution assays. Six strains were resistant to all APs; SMAP29 inhibited the growth of 9 strains and 7 of these were also inhibited by D2A-22. No strain was inhibited by human APs. Lipid A, purified from representative strains, were subjected to electrospray mass spectrometry. Reproducible mass spectra were generated and strain-strain differences were discernible. Analysis of the data to identify moieties associated with AP resistance, including the effect of altered environmental conditions on the lipid A structure is being conducted. This will enable us to determine if a relationship between lipid A structure and AP sensitivity exists.

MI 30 Characterisation of *Burkholderia cepacia* mutants sensitive to polymyxin B

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A major problem in combating *Burkholderia cepacia* infection in cystic fibrosis patients is the organism's intrinsic resistance to a range of antibiotics, including antimicrobial peptides (APs). The aim of this investigation was to generate *B. cepacia* mutants sensitive to polymyxin B (PMB) in order to study AP resistance mechanisms. Strain LMG 16659 was selected for Tn*5* mutagenesis as it showed sensitivity to tetracycline allowing the selection of transconjugants. Approximately 3,000 colonies obtained following transconjugation with *Escherichia coli* harbouring pOT182 were screened 14 of which exhibited increased sensitivity to PMB. PCR analysis of 50 randomly selected mutants using Tn5 specific primers confirmed that >90% harboured the transposon, whilst the presence of single-copies of the transposon was confirmed by Southern Blotting and DNA hybridisation. Following self-cloning, the site of Tn*5* insertion within each mutant was determined by nucleotide sequencing. BLAST searches indicate that several of the sequences share homology with characterised outer membrane proteins. This may be significant, as outer membrane structure in Gram-negative bacteria has been linked to altered AP sensitivity. Further analysis is required to fully characterise the roles of the genes identified, and also to determine whether these mutants demonstrate a similar increase in sensitivity to other APs.

MI 31 Withdrawn

MI 32 Towards high throughput multi locus sequence typing of *Pseudomonas aeruginosa*

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Multi-locus sequence typing (MLST) is a sequenced based typing method, which uses data from seven housekeeping genes. It provides molecular typing data that is highly discriminatory and electronically portable between laboratories. MLST is therefore a suitable tool to investigate the relatedness of large populations of microorganisms and has been validated using important bacterial pathogens including *Neisseria meningitides* and *Campylobacter jejuni*. A method was designed for the analysis of large numbers of isolates using high throughput capillary sequencing. This protocol shows the discriminatory nature of MLST in comparison to serotyping methods employed by the Laboratory of Hospital Infection. As MLST allows the easy comparison and exchange of results obtained in different laboratories, a future application of this new molecular method could be as a useful tool for *Ps. aeruginosa* surveillance systems, by allowing the identification and distribution of analysis of *Ps. aeruginosa* clones in the environment. As MLST allows the easy comparison and exchange of results obtained in different laboratories, a future application of this new molecular method could be as a useful tool for *Ps. aeruginosa* surveillance systems. This technique could allow the identification and distribution of analysis of *Ps. aeruginosa* clones in the environment.

MI 33 Design, generation and validation of a multi-strain *Staphylococcus aureus* whole genome microarray

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Staphylococcus aureus is a leading cause of a range of diseases; from skin infection and food poisoning to life threatening infections, such as sepsis and infectious endocarditis. *S. aureus* is currently the most common cause of nosocomial infection in western hospitals, including 18,000 cases of bacteraemia in England per year, with a 20% mortality rate. Recently, strains have emerged that are increasingly resistant to antimicrobials, including the antibiotic of last resort, vancomycin. Seven strains of *S.aureus* have been sequenced, making this the most sequenced bacterium to date; these include E-MRSA-16 (strain 252), MRSA N315, VISA Mu50, MRSA MW2 MRSA COL, NCTC 8325 and MSSA 476. Examination of the sequences reveal a core of conserved genes that composes ~75% of each genome, yet alone the differences in the sequences are unable to explain the variation in pathogenicity and antibiotic resistance exhibited.

We have designed, constructed and validated a *S. aureus* microarray that represents all seven strains and also three plasmids. The E-MRSA-16 strain is a recent hospital acquired strain, involved in the substantial increase in MRSA infections in the UK over the last 5 years and as such was chosen as the reference strain. PCR products were designed to represent all E-MRSA-16 open reading frames (ORFs) and also the strain-specific ORFs for the remaining six strains. Numerous potential PCR products were designed for each gene and then one was selected to ensure inter-strain representation and minimise intra-strain cross hybridisation. PCR was completed using a MWG Biotech RoboAmp 4200 and amplicon sizes were verified using agarose gel electrophoresis. After purification, these products

were spotted on to poly-L-lysine coated slides using a BioRobotics Microgrid II. Initial investigations confirmed the effectiveness of the design process in generating a microarray that can be used to investigate multiple strains of *S. aureus* and further work has now begun to screen strains in order to identify genes associated with invasive disease.

MI 34 Linkage of erythromycin and tetracycline resistance genes in oral bacteria

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Oral bacteria have the opportunity for rapid spread between individuals and to interact with bacteria from other locations; they are therefore likely to be an important reservoir of antibiotic resistance genes. The aim of this study was to determine which erythromycin resistance genes were present in the oral bacteria of a healthy population and if these genes were linked to tetracycline resistance genes. Twenty saliva and plaque samples from healthy adults were screened for the presence of erythromycin-resistant bacteria and 122 were isolated. All the resistant isolates were screened for possession of the most common erythromycin-resistance genes by PCR. Forty-two isolates (mostly Gram-positive) contained resistance genes. The *mef* gene was the most commonly isolated followed by *erm* (B). Out of 12 isolates that were resistant to both tetracycline and erythromycin 11 carried the *erm* (B) and *tet*(M) genes and 5 transferred both genes in filter matings with *Enterococcus faecalis*. Linkage of multiple antibiotic resistance genes located on the same mobile element is of importance because use of any one of the antibiotics can select for resistance to different antibiotics.

MI 35 Absence of *Y. pestis*-specific DNA in human teeth from European excavations of putative plague victims

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Following recent detection of *Yersinia pestis* DNA in human teeth from medieval plague victims in France, a collaborative study based in two laboratories was undertaken to confirm and extend these observations over five different European burial sites believed to contain plague victims dating from the late 13th to 17th centuries. DNA was extracted from 121 teeth belonging to 66 individuals. No specific *Y.pestis* DNA was found in these DNA extracts with several different sets of primers, including those previously documented to yield positive results on DNA extracts obtained from 18th and 14th century teeth from plague victims. Non-specific PCR for 16SrDNA detected bacterial DNA in 23 out 27 teeth tested for which previously described tooth manipulation methods were used and 5 out of 44 tested for which a novel contamination-minimising embedding technique was used. Human mitochondrial DNA was found in 19/23 valid (i.e. not contaminated) PCRs on extracts obtained with old methods and 29/50 valid PCRs on extracts obtained with the new method. Thus with the novel method we describe, teeth may provide a relatively contamination-free nidus for DNA extraction, but we found high levels of contamination by environmental bacterial DNA with previously described methods of tooth manipulation. The absence of *Y.pestis*-specific DNA in an exhaustive search with specimens from multiple presumed European plague burial sites does not support the identification of *Y. pestis* as the aetiological agent of the Black Death and subsequent plagues. The utility of a published tooth-based ancient DNA technique to diagnose fatal bacteraemias in historical epidemics still awaits independent corroboration.

MI 36 Phenotypic differentiation of methicillin resistant *Staphylococcus aureus* isolates and their vancomycin intermediate and resistant progeny by intact cell mass spectrometry

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Methicillin resistant strains of *Staphylococcus aureus* (MRSA) are increasingly prevalent as nosocomial pathogens. The glycopeptide class of antibiotics are the mainstay of treatment for MRSA infections, but recent vancomycin treatment failures have been reported. Microscopic analyses of the vancomycin intermediate and resistant *S. aureus* (VISA/VRSA) strains causing such infections, suggests that their reduced susceptibility is due to phenotypic changes in the cell wall of the organisms.

Mass spectrometry (MS) is an established method for rapid phenotypic identification and typing of bacteria. Matrix-assisted laser desorption ionisation time-of-flight analysis (MALDI-ToF) is a particularly useful form of MS that produces a characterisitc mass/charge spectrum from intact bacterial cells (ICMS).

We have used ICMS to examine a collection VISA and VRSA isolates, derived from epidemic MRSA strains by serial passage in vancomycin. The isolates have MIC values in the range of 4 to 32 mg/ml vancomycin. By using ICMS, derivative organisms were clearly differentiated from their parents. Examination of the spectra produced by ICMS revealed a number of peaks specific to VISA/VRSA strains. This would indicate the upregulation of a cell surface molecule, the identity of which may be obtained following future proteomic analyses.

MI 37 Mosaic structure of VPI-2 encoding the *nan-nag* region among *Vibrio cholerae, V. mimicus and V. vulnificus*

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Vibrio spp. are natural inhabitant of estuarine systems, both as a free-living bacteria and as the natural bacterial flora of zooplankton. However, several species are pathogenic to humans. *V. vulnificus* infects open wounds, causing in some cases fatal septicemia . In immunocompromisedindividuals ingestion of raw shellfish infected with *V. vulnificus* can cause death. *V. cholerae* is the etiological agent of the profuse diarrhoeal disease cholera. The consumption of *V. mimicus*-contaminated shellfish has been linked to the development of gastroenteritis and cholera-like symptoms. The genes involved in N-acetylglucosamine utilization (*nan-nag*) and neuraminidase (*nanH*) are encoded on a novel 57.3 kb pathogenicity island, VPI-2, that is mainly associated with toxigenic isolates in *V. cholerae*. To study the evolutionary history of VPI-2, we examined its distribution in a variety of clinical and environmental *Vibrio* spp. isolates. Our analysis revealed the sporadic distribution of VPI-2 among *Vibrio* spp. *V. mimicus* isolates contain an 11.8 kb region encoding the *nan-nag* and *nanH* genes, whereas *V. vulnificus* isolates contain an 8.9 kb region encoding *nan-nag*. Comparative nucleotide sequence analysis of the *nanH* gene from *V. cholerae* and *V. mimicus* isolates revealed that the average nucleotide divergencebetween these species was 3.1%. In contrast, comparative nucleotide sequence analysis of a housekeeping gene, *mdh*, revealed an average nucleotide divergence between the two species of 10%. This suggests that the region was recently horizontally transferred between these *Vibrio* species.

MI 38 Single-strand conformational polymorphism analysis of clinical and environmental *Vibrio cholerae* isolates

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Cholera caused by toxigenic *Vibrio cholerae* is a severe and sometimes lethal human diarrheal disease that can occur as spreading epidemics. *V. cholerae* belonging to the O1/O139 serogroups are generally considered to be the only causative agent of epidemic cholera. *V. cholerae* non-O1 and non-O139 serogroups are associated mainly with sporadic cases of diarrhoea and extra intestinal infections. PCR restriction fragment length polymorphism analysis (RFLP) and PCR-Single-strand conformational polymorphism analysis (SSCP) were carried out on the highly conserved groEL gene found on both chromosomes of toxigenic and environmental *V. cholerae* isolates. Forty-eight strains encompassing two species and twelve serogroups were analysed in this work. Although different RFLP profiles were produced for both species, PCR-RFLP failed to discriminate between strains. PCR-SSCP analysis identified several primary clusters, and differentiated environmental non-O1/nonO139 from O1/O139 clinical isolates. Furthermore, PCR-SSCP could differentiate between strains from within a serogroup. This technique provides a rapid reproducible method for detection of mutations within gene sequences and may complement classical typing methods.

MI 39 The use of intact cell mass spectrometry for rapid species level identification of clinically significant coagulase negative staphylococci

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Coagulase negative staphylococci (CNS) are emerging nosocomial pathogens that are predominantly associated with infections of indwelling medical devices. The majority of CNS causing infection are strains of *Staphylococcus epidermidis*, though a number of other species have been identified as human pathogens.

Clearly, rapid typing and identification of CNS is required for efficient infection control practice, though this is often hampered by the contamination of clinical samples with commensal organisms.

Intact cell mass spectrometry (ICMS) is becoming recognised as a rapid and reliable method for the phenotypic identification of human pathogens, producing a fingerprint of characteristic peaks representing molecular fragments from the bacterial cell surface. We have used ICMS to analyse a collection of CNS recovered from cases of prosthetic joint infection. Isolates were typed by internal transcribed spacer polymerase chain reaction (ITS-PCR) profiling, a validated method for species level identification of CNS and representatives were identified using ID32Staph™ kits. Examination of the mass:charge spectra allowed identification of species specific biomarker peaks. On the basis of the congruence between the data sets, it is suggested that ICMS has the potential to be a rapid, accurate and cost effective means of species level identification of CNS from cases of human infection.

MI 40 Molecular characterization of *Salmonella paratyphi* A causing bacteremia in Pakistani children

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Background: *Salmonella paratyphi A* (SPA) are now responsible for causing 20-30% of all cases of enteric fever in children in Pakistan. An increase in the number of cases has been observed in Karachi since 1999. Outbreaks associated with this organism have also been reported in other regional countries. Molecular typing techniques such as pulsed field gel electrophoresis (PFGE) which analyze bacterial chromosomal DNA have been shown to be very useful in epidemiological investigations of bacterial relatedness among endemic and epidemic strains.
Objective: The objective of our study was to use PFGE to epidemiologically characterize blood isolates of SPA from children suffering from enteric fever in Pakistan over a 5 year period (1998-2002).
Methods: SPA blood isolates from all children (age 0-18 years) suffering from enteric fever who had blood cultures submitted to the Aga Khan University laboratory or collection points throughout the country from April to December 2002 were analyzed. Randomly selected isolates of SPA from 1998 – 2001 were also analyzed. Chromosomal DNA from 107 SPA isolates (obtained from Karachi. Lahore, Islamabad, Rawalpindi, Quetta, Peshawar and Hyderabad) was subjected to enzymatic digestion with *xba* I (5´-TCTAGA-3´), a rare-cutting enzyme which yields large fragments of chromosomal DNA of varying molecular weights. These fragments were then analyzed by PFGE.
Results: PFGE analysis of the 107 SPA isolates revealed all strains to be clonal (identical) or closely-related (one to three band differences only). Strains obtained from different years as well as from different cities exhibited PFGE patterns that were almost identical to each other. PFGE could not discriminate between antibiotic sensitive and antibiotic-resistant strains.
Conclusion: This study demonstrates significant genetic homogeneity among circulating strains of SPA in Pakistan, indicating a point-source origin and epidemic spread. These findings are comparable to the clonal origin of multiple-drug-resistant strains of typhoid in Karachi demonstrated in earlier studies.

MI 41 Comparison of gene expression profiles of intracellular *Salmonella enterica* isolated from bone marrow derived macrophages and from a macrophage-like cell line

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The intracellular pathogen *Salmonella enterica* must adapt to the antimicrobial intracellular environment in the phagocytic cell. Whole-genome *Salmonella* microarrays have been used to determine the expression profile of *Salmonella enterica* serovar Typhimurium (*S. typhimurium*) during infection. This revealed a global modulation of bacterial gene expression in the vacuolar environment of the murine J774-A.1 macrophage cell line. We have been studying the more relevant murine bone marrow derived macrophage model (BMDM), and have compared *S. typhimurium* gene expression within the BMDM and J774-A.1 cells. We observed many similarities between the expression profiles, suggesting that the essential adaptation of *S. typhimurium* occurs in both an immortalised cell line and in bone marrow derived macrophages. A number of changes in *Salmonella* gene expression were observed and will be discussed.

MI 42 FRIVET: *fim* recombinase *in vivo* expression technology

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This study is focused on the construction and application of a novel form of *in vivo* expression technology (IVET) using the *fim* switch in *Escherichia coli* O157:H7. The system is therefore named FRIVET (*fim* recombinase *in vivo* expression technology).

Classic IVET and RIVET (recombinase *in vivo* expression technology) select for genes that are induced *in vivo* using random promoter trap methods. FRIVET will differ in that the promoters to be studied have already been defined in *E. coli* O157:H7. The FRIVET system can be used to analyse these promoters' activities and temporal expression patterns *in vivo*. By using a selectable reporter system based on antibiotic resistance bacterial promoter activity can be analysed at specific time points after inoculation. The use of the *fim* switch and its recombinase FimB allows for detailed fine-tuning of the FRIVET system to accurately measure promoter activation within set parameters. FRIVET will initially be tested *in vitro* on cell lines, before being used *in vivo* in an ovine model.

MI 43 Co-ordinate variable expression of espa filaments, translocated intimin receptor (Tir) and intimin in *Escherichia coli* 0157:H7

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Aim: This research aimed to examine the expression of EspA filaments and the subsequent production of Translocated Intimin Receptor (Tir) and Intimin.

Background: *E.coli* 0157:H7 expresses a type three secretion system (TTSS), enabling the translocation of effector proteins into host cells. The EspA protein, encoded for on the LEE4 *EspABD* transcript, forms filamentous organelles that are produced on the surface of the bacteria allowing translocation of effector proteins into the host. LEE5 encodes the translocon, Tir, and the bacterial adhesin, Intimin. Tir is translocated through the EspA filament into the host cell where it inserts into the plasma membrane.Tir can then bind Intimin present on the surface of the bacteria leading to intimate adhesion and the formation of the attaching and effacing lesions.

Methods: Indirect immunofluorescent microscopy was used to observe the EspA filaments on the surface of the bacteria and the production of Intimin in the extracellular membrane. Translational reporter fusions were constructed for the visualisation of the LEE operons 1-5, using green fluorescent protein (GFP).

Results and discussion: Production of EspA filaments was observed on a variety of strains and under a variety of growth conditions. It was found different strains produced variable levels of expression, which could be influenced by different growth media. Minimal media (M9) suppressed LEE expression enabling a known 'off' state to be achieved and switching assays were carried out. The inducing conditions showed proportional expression of EspA filaments through exponential phase, until stationary phase where expression was turned off.
The variable expression of EspA filaments correlated with Intimin production in the same proportion of bacteria, synchronising the two protein expressions.

Analysis of promoter activity from LEE operons 1-4 showed no transcriptional variation, however LEE5 did. This led to co-expression studies being performed. It was found that the variable expression of EspA and Intimin production also correlated with LEE5 expression.

This research demonstrates that while specific environmental conditions are required to induce LEE1-4 expression, a further check point exists before EspA filaments are produced. This post-transcriptional trigger is co-ordinately regulated with the transcriptional control of Tir and Intimin.

MI 44 Characterization of novel putative surface structures encoded by chaperone-usher systems in *Yersinia*

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Adhesins that mediate bacterial binding to host cells are frequently essential during early stages of infection. For the highly pathogenic bacterium *Yersinia pestis* few adhesins have been identified so far and none of these adhesins have been shown to be absolutely required for virulence. Interestingly, the genome sequencing of *Y. pestis* revealed eight operons belonging to the chaperone-usher family of adhesins. Two of the operons contained deletions or insertions which rendered these operons non-functional. Therefore, six of these operons potentailly encoded novel putative surface structures that could be involved in the pathogenesis of *Yersinia pestis* infections. All these six operons were also conserved in the closely related pathogen *Y. pseudotuberculosis.*

The aim of this work was to characterise these putative adhesins in *Y. pseudotuberculosis* and to evaluate their role in pathogenesis. Using suicide-plasmids, polar insertion mutants were generated in all six operons. In addtion, *luxAB*-fusions were also generated in all operons to allow monitoring of transcription of all the individual operons. All insertion mutants and the luxAB-fusion strains showed similar growth phenotype as the corresponding wild-type strain in vitro. Several of the chaperone/usher systems were found to be transcribed in a nutrient poor medium at 26°C, while transcription in vitro was significantly lower at 37°C. The type III secertion system that secretes and delivers Yop effectors into host cells is a key virulence determinant of pathogenic *Yersinia* species. None of the insertion mutants of these chaperone-usher systems were affected in Yop expression or secretion. However, some of the mutants were still attenauted in oral mouse infection model for *Y. pseudotuberculosis.* This indicates that these chaperone-usher systems encode novel adhesins, which are required for full virulence of *Yersinia.*

MI 45 A bioinformatic approach to identify novel virulence associated genes in bacterial pathogens

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Antibacterial therapy is becoming less effective due to the spread of multiple antibiotic resistant bacterial strains. This has lead to a concerted effort to develop novel antimicrobial agents based on the information in the data bases generated by genome projects. About 80 bacterial genomes including several important pathogens have been sequenced, revealing around 25% of the ORFs to be conserved hypothetical genes without known function. Presumably among this group of ORFs, protein classes could be identified that constitute suitable targets for novel therapeutics. Homologous gene classes from different bacteria that encode products of unknown or hypothetical function have also been identified. Some of these genes have been identified to be essential for in vitro viability and constitute potential targets for the development of novel antibacterial agents.

We used a bioinformatic approach to identify genes of unknown function common to several human pathogens that affect growth in vivo but not outside the animal host. Results: The hypothetical genes of six human pathogens including *Yersinia pestis* were compared. This comparison identified 17 different genes with homologues in the six pathogens as well as in many other pathogens including the potential BW agent *Francisella tularencis.* The virulence phenotype of each gene knock out was examined in a murine model of infection using the enteropathogen *Yersinia pseudotuberculosis* serotype I as a model for a systemic infection. Of the 17 genes/operons , 9 were found to be associated with virulence.

Using our strategy we could identify novel virulence associated genes. Analysis of the widespread involvement of these novel factors in virulence of other pathogens and whether these proteins can be targeted by different antibacterial drugs will be the focus of future investigations.

Wednesday 10 September 2003

DNA structure

0900 Structure and dynamics of branched DNA, and its recognition by proteins

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Branched DNA structures are important intermediates in the processes of DNA repair and recombination. The four-way (Holliday) junction folds by pairwise coaxial stacking of helical arms. Folding processes are dominated by electrostatic interactions, which must be lowered (eg by ion binding) to allow formation of the relatively compact stacked X-structure. This can occur in one of two conformers, depending on the choice of stacking partners. Use of single-molecule spectroscopy shows that junctions undergo a dynamic exchange between stacking conformers in solution. A natural junction formed from homologous sequences can also undergo step-wise exchange of basepairing (branch migration), and a unified energy landscape can be proposed for these processes.

Four-way junctions are specifically recognised by a series of structure-selective proteins. These may also be enzymes, such as the junction-resolving nucleases. In general these proteins distort the structure of their substrate. Recognition, distortion and catalysis are intimately linked, and thus the structure of the DNA is a key player in these important biological processes.

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0945 Non-B DNA and its physiological consequences

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Short segments of microsatellite DNA sequences have the capacity to exist as an orthodox right-handed B structure as well as several unusual conformations including triplex DNA, nodule DNA (bi-triplex), sticky DNA, tetraplexes, cruciforms, slipped structures, bent (curved) DNA, left-handed Z-DNA, anisomorphic DNA, flexible and writhed DNA, and underwound conformations. Certain types of sequence motifs enable the formation of these conformations at specific loci in genomes. A substantial and growing literature reveals a role for these conformations in DNA replication, recombination/repair, chromatin organization, and gene expression related to a variety of hereditary diseases. Also, the participation of flexible and writhed triplet repeat sequences and their slipped structures in the etiology of certain hereditary neurological diseases (myotonic dystrophy, fragile X syndrome, and Friedreich's ataxia) will be described. Moreover, our recent results on the involvement of cruciforms, triplexes, tetraplexes, slipped structures, and left-handed Z-DNA in the formation of long deletions in *E. coli* will be presented as well as the analyses of a number of human hereditary diseases caused by large deletions whose endpoints are dictated by these DNA conformational features.

Recombination

1130 Links between recombination, replication and chromosome segregation

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DNA replication and recombination are interlinked processes in all organisms. Furthermore, segregation of replicated chromosomes can be dependent on recombination processes. The functional reassembly of broken or stalled replication forks is universally dependent on homologous recombination proteins, although these may function in the absence of a complete recombination reaction. In a parallel fashion, homologous recombination is dependent on DNA replication. Indeed the initial functions of all recombination systems were probably to facilitate the replication of DNA and its transmission to daughter cells. In bacteria with circular chromosomes, homologous recombination can threaten chromosome segregation at cell division, because crossing over generates circular dimeric chromosomes. A conserved site-specific recombination system, mediated by the recombinase XerCD, acting at the recombination site *dif*, functions in chromosome dimer resolution. Dimer resolution uses the 1329 aa protein FtsK as an essential cofactor. FtsK action at the division septum may coordinate segregation with cell division. FtsK may also minimise sister chromosome entanglement and act in decatenation of newly replicated chromosomes.

1215 Holliday junction resolution – from bacteria to man

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The mechanism of homologous recombination and double-strand break repair (DSBR) involves invasion of the ends of a broken DNA molecule into homologous duplex DNA to form an intermediate structure in which duplexes are linked by a double crossover. The nature of the crossover, or Holliday junction, is now well characterized at both the biological and structural level. One remarkable feature of the Holliday junction is its ability to move along DNA and so generate increasing lengths of heteroduplex, a step that is critical for recombination.

In prokaryotic organisms, the enzymes that promote movement of the junction have been isolated and studied in detail. Although the 'branch migration' process is intrinsically isoenergetic, since DNA strands are exchanged by the breakage and reformation of hydrogen bonds in DNA duplexes, the *E. coli* RuvAB proteins promote branch migration in an ATP-dependent reaction that exhibits processivity and directionality. The reaction is driven by two molecular pumps (hexameric rings of RuvB) which lie oppositely oriented across the RuvA-bound Holliday junction. In addition to their role in the catalysis of branch migration, the RuvAB proteins cooperate with RuvC protein, a junction-specific endonuclease, to promote resolution of the Holliday junction. These reactions occur by a coupled mechanism leading to the separation of recombinant products.

The RuvABC-Holliday junction complex, or resolvosome, provides the paradigm for studies of similar activities in higher organisms. Human cell-free extracts have been fractionated to reveal analogous branch migration and resolution activities. After many years of effort, several key components of the resolvosome have now been identified. Cell lines disrupted for these Holliday junction processing activities exhibit a severe chromosomal instability phenotype, evidenced by defects in DNA break repair, replication fork instability and telomere dysfunction.

1400 Transposition

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Genomes are populated by discrete DNA segments whose capacity to translocate within and between replicons provides a powerful motor for generating large scale genetic diversity. These genetic objects are found in most eukaryote and prokaryote genomes and are themselves quite diverse. Some, such as plasmids and phage and other more exotic elements such as conjugative transposons, are capable of undergoing transfer between individuals and sometimes between species. Others rely on these transmissible elements for their dispersion.

This talk will focus on one large class of mobile genetic element, the insertion sequences (ISs). These are small (0.7-2.5kb) segments of DNA which include a single or sometimes two open reading frames (*orf*) which constitute nearly the entire length of the element and encode an enzyme, the transposase (Tpase), which catalyses the steps necessary for mobility.

More than 1000 different ISs have been identified to date and this does not include many which have been revealed in the increasing number of sequenced bacterial genomes. They have been loosely divided into about 20 families. Their distribution, both in terms of number and type is highly variable from species to species. Some bacterial genomes may carry more than one hundred IS copies made up of members of different families and different members of the same family.

The vast majority of ISs, like the retroviruses and many transposons, encode a Tpase which carries a characteristic constellation of amino acids called the DDE motif from three highly conserved residues which are involved in catalysis. The chemistry of the transposition reaction of these diverse elements must therefore be similar. However, the types of transposition product may vary from element to element. Some elements remain attached to donor DNA during transposition and their translocation results in a fusion between target and donor replicons. Other elements are separated from their flanking donor DNA prior to their insertion into a target site. This requires processing both strands at both ends of the element. While cleavage of the first strand may appear similar, recent results have shown that different strategies have been adopted for cleavage of the second, complementary, strand to liberate the transposon. An outline of these strategies will be presented together with an overview of the transposition mechanism adopted by one of the major IS families.

1445 Chromosome segregation and cell division in *Bacillus subtilis*

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Most cells segregate their chromosomes with extremely high fidelity when they divide. Bacteria do not have an overt machinery equivalent to the mitotic spindle of eukaryotes. However, they do have active mechanisms of segregation because GFP fusions to proteins that associate with specific sites in the chromosome have revealed that newly replicated sister sites separate from each other rapidly after they are formed. Sporulating cells of *Bacillus subtilis* provide a powerful tool for studying segregation in bacteria because they undergo asymmetric cell division; one chromosome therefore needs to move an extreme distance to reach the small polar (prespore) compartment. Five proteins are now known to be involved in prespore chromosome segregation. DivIVA is a multifunctional protein, which acts as an anchor at the cell pole, to which the *oriC* region of the chromosome becomes bound. Soj, Spo0J and RacA are DNA-binding proteins that interact with the *oriC*-region of the chromosome and organise the region, deliver it, and bind it to DivIVA at the cell pole. SpoIIIE is a DNA transporter that is targeted to the leading edge of the division septum and moves a large portion of the chromosome through the septum to complete the prespore chromosome segregation process. All of the proteins, except RacA, are also involved in vegetative cell cycle functions. Chromosome segregation also controls the positioning of the cell division septum and a new protein that is responsible for the well known "nucleoid occlusion" effect has now been found.

1600 Relating structure to mechanism in helicases

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In order to try to understand more about the mechanism of helicases, we have determined crystal structures of two different helicases complexed with DNA substrates. The mechanism that we proposed for the Superfamily 1 (SF1) helicase, PcrA, involved ATP-dependent translocation along single-stranded DNA. This suggestion was demonstrated directly by two different biochemical methods in addition to the crystallographic evidence. However, we wished to test the general applicability of this mechanism to helicases from other superfamilies.

To this end, we have now determined the structure of the Superfamily 2 (SF2) helicase, RecG, in a complex with its DNA substrate, a stalled replication fork. The structure reveals how RecG recognises this class of DNA junctions. Furthermore, the protein has been trapped in the initial stages of unwinding DNA allowing us to propose a mechanism for this process. This mechanism suggests that RecG, and potentially other SF2 helicases, are capable of ATP-dependent translocation along *double-stranded* DNA.

1645 DNA as an almost equal partner in regulation: DNA looping

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The discovery that a repressor could block access of RNA polymerase to a promoter–the principle of impenetrability--should have jolted us to the realization that DNA in cells does more than serve as a computer tape containing lots of stored information. DNA looping could then have been predicted, but wasn't. Accidental discovery, honest reporting of paradoxical data, and much hard work are all behind the discovery of DNA looping. DNA looping is generated by binding a protein or protein complex to two different and well separated sites on DNA. Multiple benefits accrue by tethering a multivalent, and it must be multivalent, protein in the vicinity of its functional target as occurs in DNA looping. Most of these can be traced to the fact that looping allows effective regulation by very low protein levels.

In the arabinose operon of *Escherichia coli*, regulation of the DNA looping state of the regulatory protein, AraC, versus the protein's nonlooping state utilizes rigidity of both AraC and of the DNA itself. Cells capitalize on DNA's rigidity in other systems as well. We see this in the multitude of DNA transactions in which a DNA bending protein is required and whose level is often regulated. When Ara C binds to p_{BAD} in a nonlooping state, it stimulates both the binding of RNA polymerase and the transition of polymerase from a closed complex to an open complex. Current work on AraC and regulation of the arabinose operon is exploring the interactions between the two domains of the proteins and the role of these interactions on DNA binding by the protein.

POSTERS

PBMG 01 Silver sulphadiazine, TSST-1 production & SaPI-1 excision in *Staphylococcus aureus*

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The Toxic Shock Toxin-1 (*tst*-1) gene of *Staphylococcus aureus* is carried on an integrated pathogenicity island (SaPI). These

elements are capable of excision & circularisation. The antimicrobial agent silver sulphadiazine (AgSD) has been shown to enhance TSST-1 production in some strains of *S. aureus* (responder strains). We postulated that because AgSD interacts with DNA it might induce SaPI excision and explain our previous observation that AgSD induces a shift in the position of *tst*-1. A SaPI-1 strain, RN4282, was cultured in the presence of AgSD (0, 25, 50 μg ml^{-1}) & free SaPI-1 detected by circularised SaPI-1-specific PCR. Recovery of circularised SaPI-1 was enhanced following growth with AgSD, which supports our hypothesis. A variant SaPI1-like amplicon was isolated from some strains, including the menstrual toxin shock syndrome strain T1. It has limited sequence homology with the RN4282 amplicon, but some homology with bacteriophage sequences, highlighting the common origin of these elements. The *tst*-1 regions of 20 clinical isolates were sequenced. Twelve strains had a serine for leucine substitution at position –30 of the signal peptide region. This difference does not correlate with AgSD-enhanced toxin production, but may be related to the origin of the *tst*-1 elements rather than their function.

PBMG 02 Recombinant production and purification of a pneumococcal IgA1 protease

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Streptococcus pneumoniae produces a protease which specifically cleaves human immunoglobulin A1 in the hinge region. This IgA1 protease is a metallo proteinase of approximately 200 kDa. The protease is an extremely robust enzyme. After denaturation in 1% SDS at 100 degrees it easily regains activity when SDS is replaced by Triton X100. In order to study this intriguing enzyme in detail I have cloned the *iga* gene encoding the IgA1 protease into an expression plasmid that adds six histidines to the protein. After transformation of the plasmid into *E. coli* the protease was produced in large amounts within the cells from where it was purified in its active form using affinity chromatography. However, the N-terminal part of the protease which contains repeat structures was subject to autoproteolysis and, therefore, a truncated version of the protein devoid of the repeats was expressed and found to be active and stable at room temperature. This should enable us to study this putative virulence factor in further detail.

PBMG 03 Regulation of phosphonate degradation genes in *Sinorhizobium meliloti* 1021

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In *Sinorhizobium meliloti* 1021 some phosphonates (e.g. methylphosphonate: MeP) are degraded by C-P lyase pathway and C-P lyase is induced in the presence of those phosphonates. Structural genes (*phnGHIJK*) for the enzyme C-P lyase have been sequenced and characterized. A gene similar to *phnF* of *E. coli*, a member of the GntR family of regulatory proteins, but divergently transcribed from *phnG* lies upstream of *phnG*. We investigated the role of the *phnF*-like gene in *S. meliloti* by measuring transcriptional activity in strains containing *phnG*::*lacZ* fusions. PhnF appears to repress transcription from the *phnG* promoter but when MeP was added to cultures the repression was lifted. A Δ*phnF* mutant of *S. meliloti* was constitutive for *phnG* expression but surprisingly did not grow well in minimal medium containing MeP or low (10 μM) inorganic phosphate as sole P source. Therefore, *phnF* may be required for the metabolism of phosphonates and also other P sources when present at low concentrations. The PhnF protein was overexpressed in *E. coli* as a thioredoxin fusion protein. Electrophoretic mobility shift essays showed that PhnF binds immediately upstream of *phnG* to a 13bp pseudopalindromic sequence TATAAGATCTATA.

PBMG 04 Restriction alleviation by plasmids and phages revisited: artful dodgers of bacterial defense systems

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This year marks the 50th anniversary of the DNA helix, but also the first paper on restriction alleviation[1]. This early observation provided the first evidence for inhibition of the *E. coli* K-12 nuclease, EcoK, after UV-induced DNA damage. Elucidation of the mechanism behind this process, intricate post-translational control involving ClpXP-mediated proteolysis, had to wait till the turn of the century (see for review[2,3])

Time brought unexpected results with respect to the manifold ways, in which bacteriophages and conjugative plasmids, the foreign invaders of bacteria, avoid destruction of their DNA by host restriction enzymes. Sofar no evidence has emerged for a similar induction of Clp-mediated proteolysis by either phages or plasmids, as mentioned above. Instead unusual modifications (glycosylation, methylation or acetamidation) of DNA bases are employed in some cases, yielding protection against many different restriction systems. The lambda Ral protein is unusual, as it appears to be a 'modification enhancer' rather than 'restriction alleviator'[4,2], while acidic proteins like phage T3/T7 Ocr[5,6] (overcoming classical restriction) and Ard (alleviation of restriction of DNA)[6, 7] may combat many members of the EcoK enzyme family via physical interference with the DNA binding site. Surprisingly, Ocr protein resembles size and shape of a bent piece of DNA, thus mimicking the normal DNA substrate for EcoK[8]. Conserved sequence motifs in Ocr, Ard and several others leads to the speculative conclusion of a common interference mechanism via highly charged surface residues, thus allowing nucleotide sequence-independent binding of the DNA pocket.

This presentation summarises the known mechanisms of these artful dodgers of host restriction, including the ways, in which their gene(s) manage to express protective protein products, before the sequence that encodes it, is attacked by the restriction enzyme.

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PBMG 05 Antibiotic production by *Photorhabdus temperata* subsp. *temperata* isolate K122

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Photorhabdus temperata is a motile gram-negative bacterium that forms a mutualistic association with entomopathogenic nematodes of the family *Heterorhabditidae*. The bacterium lives in the gut of infective juvenile nematodes that seek out and penetrate the open blood system of insect larvae. The bacteria are regurgitated and multiply rapidly causing the host to die of bacterial septicemia within 24 – 48 hours. During exponential growth, and in stationary phase, *Photorhabdus* produces a variety of different antibiotics believed to prevent other bacteria from

occupying their ecological niche. *Photorhabdus* also produces a number of pigments causing the insect cadavar to become deep red following death. Stilbene derivatives and anthraquinone derivatives are the major metabolites thought to be produced by *Photorhabdus*. Both appear to be pigmented, both are thought to be antimicrobial and both occur simultaneously during *in vitro* and *in vivo* growth. Our work has focused on the biosynthesis and functions of these two metabolites by identifying and characterising *Photorhabdus* transposon mutants that either lack pigment, antimicrobial activity or both. Work has also been undertaken to assess the environmental conditions that induce pigment and antibiotic production in wild type *Photorhabdus*.

PBMG 06 Acyl carrier proteins in the biosynthetic cluster of the antibiotic mupirocin

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Mupirocin or Pseudomonic acid A, a polyketide antibiotic synthesized by *Pseudomonas fluorescens* NCIMB10586, is an important agent for controlling MRSA. Its core structure is synthesized by a series of modular multifunctional megaproteins while finishing is achieved by individual tailoring enzymes. The presence of 16 acyl carrier proteins (mACPs) makes mupirocin biosynthesis unique among the known systems. This study investigated whether all the ACPs encoded in the tailoring region (*macps12-16)* are essential, and why there are tandem copies (*macp3/4* & *macp5/6/7*) in the modular steps and at what stage the essential ACPs act. For the tandem clusters while deletion of the whole cluster abolished mupirocin production, deletions of any of macp3/4 and *macp5/6/7* or pairs of *macp5/6* or *macp6/7* but still carrying one *macp* did not. However HPLC analysis of mutant strains showed reductions in mupirocin production compared to wild type. Deletion of discrete *macps* (12-15) in the tailoring region gave complete loss of antibacterial activity but the *Dmacp16* retained some activity. HPLC analysis of *Dmacp16* revealed an intermediate, which had a mass of 516.2 Da corresponding to pseudomonic acid B. Thus there may be a single ACP for each step of the biosynthetic pathway and use of appropriate ACPs will be necessary when modifying the pathway. Tandemly repeated *macp* genes may be an efficient strategy to achieve maximum throughput during the manipulation of novel pathways.

PBMG 07 Regulation of formation of the intracellular beta-galactosidase activity in *Aspergillus nidulans*

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Intracellular ß-Galactosidase of the filamentous fungus *Aspergillus nidulans* was not formed during growth on glucose or glycerol, but was rapidly induced during growth on lactose or D-galactose. L-arabinose, and D-xylose also induced ß-galactosidase activity. Addition of glucose to cultures growing on lactose led to a rapid decrease in the ß-galactosidase activity. In contrast, in cultures growing on D-galactose, addition of glucose decreased the activity of ß-galactosidase only slightly. Glucose inhibited the uptake of lactose, but not of D-galactose, and required the carbon catabolite repressor CreA for this. In addition, CreA also repressed the formation of basal levels of ß-galactosidase and partially interfered with the induction of ß-galactosidase by D-galactose, L-arabinose and D-xylose. Phosphorylation of D-galactose was not necessary for induction. Interestingly, a mutant in galactose-1-phosphate uridylyl transferase produced ß-galactosidase activity at a low, constitutive level even on glucose and glycerol, and was no longer inducible by D-galactose, whereas it was still inducible by L-arabinose. We conclude that the biosynthesis of the intracellular ß-galactosidase of *A. nidulans* is regulated by CreA, partially repressed by galactose-1-phosphate uridylyl transferase, and induced by D-galactose and L-arabinose in independent ways.

PBMG 08 A reductive pathway of galactose catabolism in *aspergillus nidulans*

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Galactose catabolism in yeast proceeds via the Leloir-pathway, that involves phosphorylation by galactokinase (encoded by *galE*). While yeast *galE* mutants are unable to use D-galactose as a carbon source, *galE* mutants of the filamentous fungus *Aspergillus nidulans* could grow on D-galactose in the presence of ammonium – but not nitrate ions – as nitrogen source. Mycelia of the wild-type *A. nidulans* accumulated intracellular galactitol (50 mM), whereas the *galE* mutant accumulated a 10-fold higher concentration. Unlike an *A. nidulans* mutant in L-arabitol-dehydrogenase, the accumulated galactitol was catabolized lateron in both the wild-type and *galE* strains. Further, an *A. nidulans* mutant in hexokinase (*frA2*) was unable to grow on galactitol, and a *galE / frA2* double mutant was unable to grow on either galactose or galactitol. Mycelia of *A. nidulans frA2* accumulated intracellular L-sorbose on galactitol, indicating it as an end-product of galactitol oxidation. Both the *frA2* and the *galE / frA2* mutants were unable to grow on L-sorbose, indicating that its catabolism involves phosphorylation by the hexokinase. The results therefore provide evidence for a second pathway of D-galactose catabolism in fungi, which involves reduction of the galactose into galactitol, NAD^+-dependent oxidation by an arabitol dehydrogenase to L-sorbose and phosphorylation by hexokinase.

PBMG 09 A novel transcriptional repressor required for sporulation in *Streptomyces coelicolor* A3(2)

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Streptomycetes are filamentous soil bacteria, which exhibit a complex life cycle. A striking feature of this life cycle is the erection of differentiated hyphae, which grow into the air away from the surface of the colony. These aerial hyphae eventually septate to form uninucleoid, grey pigmented spores. Developmental mutants in streptomycetes fall in to two main classes; those lacking aerial hyphae, the so called bald (*bld*) mutants, and those which form aerial hyphae that are unable to differentiate in to spores, these are characterised by their white appearance (*whi* mutants). Many of the known developmental loci characterised to date encode regulatory proteins, and little is know of the accessory proteins controlled by them, indicating the need for further screens to aid our understanding of the developmental process. In the present study we have utilised *in vivo* transposition to identify a novel transcriptional repressor of the GntR family involved in development of *Streptomyces coelicolor* A3(2). It is located in a cluster of genes, which when disrupted individually, each null mutant exhibits a developmental phenotype, demonstrating this cluster plays an important role in the differentiation of *S. coelicolor.*

PBMG 10 Evidence for phase variation in the *phnE* gene for phosphonate transport in *Escherichia coli K-12*

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Phosphonates (Pns) are compounds which contain a direct C-P bond. Both natural and synthetic examples are important in agriculture, medicine and manufacturing industries. They are metabolised by relatively few organisms. Strains of *E. coli* break down a restricted range of Pns via the CP-lyase pathway encoded by the *phn* gene cluster. However, *E. coli* K-12 is cryptic for the utilisation of methylphosphonate (MePn) as a P source because of an 8 bp insertion in the *phnE* gene potentially encoding the membrane component of an ABC-transporter. The octameric sequence is repeated directly 3 times in the *phnE* gene suggesting that slip strand replication may have caused the mutation. We show that MePn-utilising variants of *E. coli* K-12 occur at high frequency in the population ($\sim 10^{-1}$) through loss of the 8bp insertion in *phnE*. Populations no longer required to use MePn as sole P source, switch back to the crypticity in MePn utilisation at an equally high frequency. The system behaves as a high frequency ON⇔OFF phase switch. We have explored the mechanism of the switch activity and its presence and potential significance in other *E. coli* strains.

PBMG 11 Biochemical characterisation of a temperature-sensitive DNA ligase from *Escherichia coli*

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DNA ligases catalyse joining of DNA ends and are essential enzymes in all cells due to their requirement during DNA replication. As the first step in ligation, all DNA ligases form a covalent enzyme-adenylate intermediate, using either NAD+ or ATP as the donor of the adenylate group. The uniqueness of NAD+-dependent DNA ligases to eubacteria makes them a potential target for novel antibiotics.

Several temperature-sensitive mutant strains of *E. coli* have been described. Of particular value has been *E. coli* strain GR501, which has been used to confirm *in vivo* ligation activity of several over-expressed proteins. *E. coli* GR501 harbours a mutation in *ligA* (*ligA*$_{GR501}$) that renders the strain non-viable at temperatures of 42°C or above. Here, we show that the mutation is a cytosine to thymine transition at base 43, resulting in a substitution of leucine to phenylalanine at residue 15 (Leu15Phe). We observed that LigA$_{GR501}$ is synthesised at permissive and non-permissive temperatures, showing that the mutation does not affect expression of LigA$_{GR501}$. Purified LigA$_{GR501}$ has ligation activity *in vitro*, although the activity is reduced at temperatures > 42°C. We propose that the mutation in LigA$_{GR501}$ affects the structure of the protein at the non-permissive temperature, resulting in the observed loss of activity.

PBMG 12 A genomic analysis establishes the role of guanosine tetraphosphate (ppGpp) in the environmental regulation of *Salmonella* virulence gene expression

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Salmonella causes infection of mammalian hosts by co-ordinating the expression of a number of key virulence genes. In this work we used in-house constructed microarrays to define the ppGpp regulon in *Salmonella enterica* sv. Typhimurium using a strain deleted for the *relA* and *spoT* genes. We investigated the expression of several pathogenicity islands and virulence related genes because it had previously been shown that the mutant strain is severely attenuated in BALB/c mice, and that the strain exhibited reduced expression of both *invF* and *hilA*, the major transcriptional activators required for *Salmonella* Pathogenicity Island (SPI1) expression (K. Tedin, pers. comm.) The expression profile of *Salmonella* was defined in both the mutant and wild-type strains grown under conditions which are relevant to the gastrointestinal environment. We discovered that ppGpp plays a central and specific role in virulence gene expression and that the expression of all of the known regulators of SPI1, except *hilACD* and *invF* are either unchanged or elevated in the mutant strain relative to the wild-type strain. Based on these observations, we propose a model to explain how ppGpp regulates virulence gene expression in *Salmonella*

PBMG 13 Function and clinical significance of the staphylococcal antibiotic resistance determinant *msr*(A) and related genes

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The plasmid-borne gene *msr*(A) confers inducible resistance to 14-membered ring macrolides and type B streptogramins in staphylococci. The encoded amino acid sequence contains two nucleotide-binding domains typical of the ABC transporter superfamily, consistent with the apparent drug efflux mediated by Msr(A), but is not associated with any hydrophobic transmembrane domains. It is thus a member of the class 2 ABC transporters, a group that includes systems involved in antibiotic resistance and in cellular processes other than transport.

Preliminary evidence suggests that Msr(A) expression and/or function may be regulated as part of a stress response system. Msr(A) function is impaired under controlled conditions of physiological stress, including elevated growth temperature and salt concentrations.

Related antibiotic resistance determinants have been identified, including *vga*(A) and *vga*(B) in staphylococci, *msrC* in *Enterococcus faecium* and *lsa* in *E. faecalis*. We have investigated carriage of *msr*(A) and related genes in coagulase-negative staphylococci isolated from CAPD patients. *Msr*(A) has previously been detected in a relatively high proportion of clinical staphylococcal isolates, including a number which carry *erm* genes. This acquisition of more than one macrolide resistance gene may suggest an alternative role for *msr*(A) within the cell. We have additionally screened a collection of clinically significant enterococcal strains for the presence of such genes.

PBMG 14 Phenotypic variation in *Photorhabdus temperata*

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The enterobacterium, *Photorhabdus temperata*, has a symbiotic relationship with nematodes of the *Heterorhabditis* family. After prolonged *in vitro* growth, stable phenotypic variants that do not facilitate normal nematode development appear in the population. Such cells are termed secondary variants (distinct from primary variants - those that do support nematode development). Phenotypic variants can be distinguished by a variety of different phenotypes, for example, pigment production, dye adsorption, antibiotic production, protease production and bioluminescence. In general, primary variants are positive for these characteristics

whilst secondary variants show diminished or lack of production of these characters. To understand the correlation of phenotypic variation with symbiosis and to investigate the molecular mechanism controlling these factors in *P. temperata*, transposon mutagenesis was carried out on both primary and secondary variants. Secondary to primary variant mutagenesis yielded 2 mutants, both in *hexA*, a Lys-R type transcriptional regulator, confirming the importance of this regulator in controlling symbiosis. Primary to secondary variant mutagenesis yielded 9 mutants, which could be classified into 6 groups based on proposed function i.e. metabolism, respiration, environmental sensing, host cell recognition, and transcriptional regulation. Using this data we propose a model for the regulation of phenotypic switching, and therefore symbiosis, in *P. temperata*.

PBMG 15 Molecular characterisation of iron siderophore mutants in *Photorhabdus*

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Iron plays an essential role in many important bacterial processes. Therefore, bacteria employ specific mechanisms to obtain iron from the extracellular milieu, such as the utilisation low molecular weight molecules called siderophores. *Photorhabdus temperata* has a symbiotic relationship with its specific nematode partner *Heterorhabditis*, but also has a pathogenic interaction with insect larvae. To investigate the role of iron in the life style of *Photorhabdus* a mutant bank was screened for the production of siderophores on CAS agar. In this study we describe two mutants that were identified as hyper producers of siderophore. The first mutant was shown to be an interruption in the *exb*D gene, part of the ExbB-ExbD-TonB membrane energy translocation system, which is important in providing energy for active translocation of siderophores across the outer membrane into the periplasm. Pathogenicity assays revealed that the *exb*D mutant was significantly delayed in killing insect larvae and we show that this delay is related to an *in vivo* growth defect. The second mutant was shown to be an interruption of a gene encoding a siderophore permease but, remarkably, this mutant is not affected in pathogenicity. Therefore in *Photorhabdus* siderophore production and uptake *per se* does not affect pathogenicity but a functional *exb*D gene is essential for normal *in vivo* growth.

PBMG 16 Isolation and characterization of K4 type yeast killer protein

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Certain yeast strains termed killer yeasts produce and excrete into the medium proteins or glycoproteins which are lethal to sensitive yeasts. Killer toxins are classified into eleven types (K1 – K11) on the basis of their killing spectra and interaction with other killer yeasts. Toxin production confers considerable advantage to the yeast strains in competing with sensitive strains for nutrients available in their environment. Our previous studies revealed that some of these toxins were also inhibitory to gram (+) pathogenic bacteria. In this study we have aimed to purify and characterize the K4 type yeast killer toxin by using *Hansenula anomala* NCYC 432 as the source of this protein. Killer toxin production was tested at various pHs and temperatures by killer zone assay in a plate test and found optimum at pHs 4.0 – 4.5 and 22 – 24 ºC. The killer protein in the culture medium was concentrated by ultrafiltration and then applied to gel filtration chromatography (Bio CAD 700 E) The purified active fraction was then applied to discontinous gradient SDS PAGE and migrated as a doublet. The molecular weight was estimated to be in the range of 47,000– 49,000. The peptide map analysis of the two bands revealed that they represent isoforms of the same protein. Polyacrylamide gel electrofocusing of this protein showed two pH values for its isoelectric point (pHs 3.4 and 3.8) that arised from two isomeric forms. . The glyco-nature and amino acid sequence determinations are under investigation in our laboratory. As yeast killer proteins are promising antimicrobial agents these results will be of help in industrial scale purification of the K4 type yeast killer protein.

PBMG 17 Regulation of *fimB* in response to sialic acid

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The phase variation of type 1 fimbriation in *Escherichia coli* is controlled by the inversion of a 314 bp element of DNA, determined by FimB (switching in both directions) or FimE (switching from the ON-to-OFF orientation predominantly), and influenced by auxiliary factors IHF, Lrp and H-NS. The *fimB* gene is separated from the divergently transcribed *yjhATS* operon by a large (1.4 kbp) intergenic region of unknown function. We have shown that *fimB* expression is regulated by multiple *cis*-active sequences that lie far upstream (> 600 bp) of the transcription start sites for the recombinase gene. Two regions characterized further (regions 1 and 2) show limited identity, and each coincides with a methylation-protected Dam (5' GATC) site. Region 1 encompasses a 27 bp DNA sequence conserved upstream of genes known (*nanAT*) or suspected (*yjhBC*) to be involved in sialic acid metabolism, and we show that FimB expression and recombination are suppressed by N-acetylneuraminic acid. Methylation-protection at region 1, but not at region 2, requires the FadR-like regulator, NanR. We propose that *E.coli* recognizes the amino sugars as a harbinger of potential host defense activation, and suppresses the expression of type 1 fimbriae in response.

PBMG 18 ParF, a non-actin-type polymerizing protein that mediates procaryotic DNA segregation

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DNA segregation in procaryotes involves the action of evolutionarily unrelated ATPases belonging to either the Walker- or actin-type superfamilies. The ParF partition protein specified by the multidrug resistance plasmid TP228 is a Walker-type ATPase that assembles into extensive filaments, when incubated with ATP. The polymerization process was studied by light scattering experiments in parallel with bundling assays and the ultrastructure of the ParF polymers was investigated by negative-stain electron microscopy. The fibres observed by EM appear to be formed by multiple paired protofilaments and are reminiscent of actin rods. These bundles possess characteristic frayed ends. The formation of filaments occurs almost instantaneously upon incubation with ATP, but not ADP. Addition of the non-hydrolyzable analog ATPγS also triggers the assembly of ParF into fibres, indicating that polymerization does not require hydrolysis. The filaments are transient, dynamic structures undergoing depolymerization. The effect of mutations in two highly conserved residues of the ParF ATP-binding motif is under current investigation *in vivo* and *in vitro*.

PBMG 19 Structure-activity relations in the plasmid partition protein ParG

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The partition cassette of multidrug resistance plasmid TP228 is composed of two genes *parF* and *parG*, and an upstream region comprising the putative partition site (*parH)* and operator. ParG is a dimeric protein (8.6 KDa monomer) characterised by a highly structured C-terminal region and a non-structured N-terminal tail. NMR studies reveal the presence of a β-ribbon followed by 2 α-helices in the C-terminal region, which is characteristic of the Ribbon-Helix-Helix family of DNA-binding

proteins. Our aim is to establish the role of this N-terminal end during partition.

Derivatives of Par G that contain N-terminal insertions and deletions dimerise and are able to interact with native ParG and ParF *in vivo* and *in vitro*. Those N-terminal ParG mutants bind both *parH* and the operator site in a sequence-specific manner, similarly but not identically as native ParG. Particularly Δ19ParG displays a DNA-binding pattern markedly different from full-length ParG. Some aspects regarding the activity of the non-structured N-terminal tail of ParG during plasmid partition are yet to be ascertained. However, it seems evident that, as a significant part of the structure of the protein, it must hold a crucial role in its partition activity.

PBMG 20 Transcriptional networking between CreBC and CRP in *Escherichia coli* via a novel mechanism for regulating cAMP levels

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In *E. coli*, cAMP interacts with CRP and in affects the expression of a large regulon involved in carbohydrate metabolism. cAMP is made at a rate dependant upon the metabolic state of the cell, and can be degraded by phosphodiesterases, CpdA and CpdB. The CreBC two component system is another metabolic regulator. We have previously presented evidence that CreBC can regulate the expression of *cpdA*. CreBC over active (Cet) mutants were made from *E. coli* MG1655, and RT-PCR confirmed that *cpdA* expression is upregulated compared to the parent strain. cAMP assays confirmed that this had a dramatic effect on cytoplasmic cAMP levels with the Cet mutant having around 10% of that found in MG1655. This drop in [cAMP] is enough to significantly reduce CRP activity, as determined by LacZ assays in the presence of IPTG (which derepresses LacZ expression). To prove involvement of *cpdA*, the gene was disrupted by Km^R insertion, which resulted in cAMP levels and LacZ activity rising to levels found in an MG1655 *cpdA*::Km^R mutant, i.e. around double those in MG1655. These data confirm that as well as regulating the Cre regulon, CreBC are able to regulate the CRP regulon by transcriptional networking via altering cAMP levels.

PBMG 21 Identification of an amino acid residue that activates OxyR resulting in constitutive activation of target genes in *Xanthomonas campestris*

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The objective of this study is to characterize mutations in *oxyR5* which resulted in the transcriptional regulatory protein being locked in the oxidized form and constitutively activated genes in the OxyR regulon. OxyR5 from a *X. campestris* pv phaseoli H_2O_2 resistance mutant has two important mutations G197D and L301R. The protein exists in the oxidized-like form in uninduced cells as judged by the protein ability to activate the *ahpC* promoter. Analysis of footprint patterns from the DNaseI protection assay indicates that OxyR5 and OxyRG197D bind to the target site in the *ahpC* promoter as oxidized proteins under reducing conditions. Site-directed mutagenesis of the mutant gene shows that OxyR5 can exist in oxidized-like form, independent of the highly conserved C residues at positions 199 and 208 where in normal OxyR, a disulphide bond between these residues converts the protein from its reduced to the oxidized form. The D197 is absolutely required for the conversion of the protein into the oxidized-like form in uninduced cells. The mutation at D197A gives a protein with similar properties to wild type OxyR. In vivo, the OxyR5 probably lock in oxidized-like form resulting in continuous high levels activation of target genes in OxyR regulon.

PBMG 22 Biosynthesis of mupirocin by *Pseudomonas fluorescens*

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Mupirocin (pseudomonic acid A) is a polyketide antibiotic produced by *Pseudomonas fluorescens* NCIMB 10586. Mupirocin targets bacterial isoleucyl-tRNA synthase, as a competitive inhibitor of isoleucine. It is a potent inhibitor of methicillin resistant *Staphylococcus aureus*, and is used clinically as a topical treatment.

The 74kb mupirocin biosynthesis cluster is comprised of 6 multifunctional open reading frames (ORF), whose predicted amino acid sequences show homology to type I polyketide and fatty acid synthases, and 29 individual genes which have potential auxillary and tailoring functions specific to mupirocin biosynthesis.

It has been proposed that mupirocin biosynthesis involves assembly from C_{12}, C_5 (which together make monic acid) and C_9 (9-hydroxynonanoic acid) units. We have assigned sub-regions within the cluster each of which specifies the synthesis of one of the components of mupirocin and have started to carry out knockouts in the pathway to test our hypothesis. D inactivation of specific *mup* domains and ORFs results in either partial or complete loss of production of antibacterial activity. HPLC analysis has revealed products of these mutants that have altered retention times compared with pseudomonic acid A. The chemical nature of these putative intermediates in mupirocin biosynthesis will be determined by mass-spectroscopy and NMR to identify the role of specific proteins in the biosynthetic pathway.

PBMG 23 Blueprint for an enterobacterial plant pathogen: the complete genomic sequence and approach towards the functional genomics of *Erwinia carotovora* subsp. *atroseptica*

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The complete genome sequence of the enterobacterial plant pathogen, *Erwinia carotovora* subsp. *atroseptica* (*Eca*), is now available and is providing important new insights into how this close relative of the enteric human and animal pathogens causes disease in its natural host - potato. *Eca* has a genetic backbone essentially common to all enterobacteria and, in addition, carries a number of well characterise pathogenicity genes, such as the cell wall degrading enzymes. However, sequencing has also uncovered a number of genes novel to *Eca* but orthologous to pathogenicity genes found in various groups of phytopathogens. Such genes are currently being studied using various functional genomics tools including proteomics, microarrays, mutation grids and pathogenicity assays. Together with bioinformatic interrogation, these tools are allowing potential pathogenicity genes to be recognised, screened, and their disease roles determined. Comparison of the *Eca* genome sequence with that of the recently-sequenced *Erwinia chrysanthemi*, other enterobacteria and other plant pathogens, will contribute to unravelling the biology and pathogenicity of *Eca*, while serving as a blueprint for future molecular phytopathological research.

PBMG 24 Gene expression on broad host range IncP-1 plasmids

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A DNA array to monitor the expression of IncP-1 plasmid genes was designed and tested. Total RNA extracted from *E.coli* C600

cultures containing mutated and wild type IncP-1α plasmid, RK2, is being reverse transcribed into cDNA and labelled with CyDye. The labelled cDNA is then used to probe the microarray, and analysed using GeneSpring software. The effect of a variety of environmental conditions as well as mutations in key regulatory genes will be reported.

PBMG 25 Identification of α-mannosidase, β-N-acetylglucosaminidase and DNase activity in *Chlamydia trachomatis*

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Chlamydia trachomatis (CT) is an important sexually transmitted organism that causes pelvic inflammatory disease in females. Although the genome of CT has been published, the biochemistry of the organism is still not fully understood. Indeed, more than 20% of the genes in the CT genome have not yet been allocated function. Enzymatic studies have demonstrated the presence of α-mannosidase, β-N-acetylglucosaminidase and DNase, none of which have been previously described in this organism. A Bioinformatics approach has been used to identify the genes encoding these enzymes utilising BLAST. Signature sequences of these three enzymes were found using BLASTP and CLUSTALW and these were used to interrogate the CT genome. However, little homology was found raising the question as to the usefulness of a Bioinformatics approach in sequence identification.

PBMG 26 Comparative genomics of firmicutes and actinobacteria reveals a new family of proteins involved in stationary phase survival

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Growth and resuscitation of *Micrococcus luteus* from starvation-induced dormancy is controlled by an autocrine resuscitation-promoting factor (Rpf) secreted by actively growing cells. More than 40 *rpf*-like genes have been identified in the Actinobacteria. Many of their products contain LysM motifs and one contains a peptidase M37 domain, suggesting a possible role in peptidoglycan binding/metabolism. A strain lacking three of the five *Streptomyces coelicolor* genes grows normally. The genes have been assigned to several sub-families based on the domain structure of their products and their genomic context.

Members of the RpfB subfamily have an N-terminal region containing multiple copies of the DUF348 domain followed by the Rpf domain. The YabE protein of *Bacillus subtilis* has a similar structure with an unrelated domain at the C-terminus, which is found in more than 40 gene products from bacilli and clostridia. As with the Rpf family, the number of proteins per organism is variable and several sub-families are recognizable.

The four *B. subtilis* genes have been inactivated singly and in various combinations. The *yocH* mutant shows reduced post-exponential phase survival in nutrient-poor medium, a phenotype that is more pronounced in double, triple and quadruple mutants. This phenotype, and the presence of LysM domains in several family representatives suggest that the Firmicute proteins have a similar biological role to that of the actinobacterial Rpf-like proteins.

PBMG 27 Rpf-mediated resuscitation of stressed mycobacteria

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When stressed in several different ways (e.g. incubation in prolonged stationary phase / treatment with various antibiotics / persistence within murine macrophages) the great majority of cells within populations of *Mycobacterium smegmatis* and *Mycobacterium bovis* (BCG) lose the ability to form colonies on agar plates. The apparent viability of these cells is often somewhat higher when they are incubated in liquid medium for MPN determination. Resuscitation in liquid medium is stimulated by the provision of Rpf either exogenously or endogenously. Rpf is a protein, secreted by growing cells of *Micrococcus luteus*, which promotes the resuscitation of dormant, "non-culturable" cells and stimulates the growth of viable cells from small inocula under nutrient-poor conditions (Mukamolova *et al*, 1998, PNAS 95, 8916-8921). Rpf-like proteins are also found in both *M. smegmatis* and *M. bovis* (BCG).

We propose that adoption of a transiently "non-culturable" state, from which the bacteria can be resuscitated, represents a survival strategy adopted by bacteria that have experienced the gradual imposition of conditions inimical for growth. Rpf may assist recovery from injury following such stress, or it may be required for the resumption of active growth and cell division.

PBMG 28 Repression at a distance and cooperativity with other repressors by KorB of broad host range IncP-1 plasmid RK2

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The DNA-binding protein KorB has roles in both regulation of gene expression and plasmid partitioning at cell division. In regulatory mode, KorB acts as a global repressor on the plasmid genome via interactions with a range of protein partners. At some RK2 promoters KorB is known to exert its effect after binding an operator (O_B) that is distant from the promoter; at other promoters the KorB operator is located immediately upstream of the promoter. We have investigated the distance and orientation constraints on KorB acting both alone and cooperatively with repressors TrbA and KorA, by reporter gene assays on synthetic promoters where the O_B site has been moved away from its natural position. KorB was able to repress, and to interact with TrbA or KorA bound at the promoter, from a range of distances in both promoter-proximal and promoter-distal situations, and no spatial restraints were apparent. To further investigate this "action at a distance", DNA-binding protein "roadblocks" were placed between the *trbB* promoter and its distal O_B site. Introduction of operators for *lac* repressor or CRP between promoter and O_B site was found to reduce regulation by KorB at *trbBp*, and no helical periodicity in this effect was observed. These results are most consistent with polymerisation or scanning of KorB along the DNA to exert its effect on the promoter from a distant site.

PBMG 29 Getting to the root of the problem - expression and regulation of type III secretion genes in the plant growth-promoting rhizobacterium *Pseudomonas fluorescens* strain SBW25

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Plant growth promoting rhizobacterium *Pseudomonas fluorescens* SBW25 carries a type III protein secretion system (TTSS), although the biological and ecological significance of this system remains unknown. To gain an insight into the function of the *P. fluorescens* system we initiated a study of the mechanism of regulation. We have generated a range of strains carrying different combinations of transcriptional reporter (*uidA*) fusions to chromosomal TTSS genes, overexpressed TTSS regulators and TTSS regulatory mutants. Together our data show that transcription of the *P. fluorescens* TTSS is not elevated in response to environmental signals that activate the *P. syringae* TTSS and yet the enhancer binding protein gene *rspR* activates

the TTSS alternate sigma factor gene *rspL*, which activates the TTSS structural genes as is found in *P. syringae*. A low level of TTSS expression *in vitro* led us to search for negative regulators by screening for mutants that overexpress TTSS structural genes. From a screen of 80,000 mutants, we identified 12 that showed elevated expression of *rscU*. Genes implicated in negative regulation include genes involved in iron uptake and genes responsive to changes in turgor pressure. Together these suggest that in the rhizosphere, high osmolarity and iron limitation activate *P. fluorescens* TTSS gene expression.

PBMG 30 The structure and function of partition protein, ParB of *Pseudomonas putida*

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Many bacterial plasmids and chromosomes encode members of the *parA* and *parB* families that are needed to prevent plasmid or chromosome loss at cell division. ParB is normally a DNA binding protein specific for a *cis*-acting, centromere-like sequence (*parS*) needed for better than random segregation of the genetic element encoding it. The genome sequence in *P. putida* revealed that there are at least ten sequences similar to the ParB binding site predicted on the basis of the ParB binding site from *Bacillus subtilis*. These binding sites are distributed across more than half the genome. We are testing the function of these ten putative sites *in vivo* and *in vitro*. Two of the sites located near the replication origin are within the operon encoding *dnaN*, *dnaA*, *recF* and *gyrB*. DNA fragment gel retardation as well as crosslinking, immunoprecipitation and PCR have been applied to show that ParB binds specifically to these two sites. Binding at the other sites was not detected. Thus one function for ParB may be to modulate expression of *gyrB* and this may provide a connection between *par* gene function and control of supercoiling of the chromosome. ParB has been purified and is currently in crystallisation trials with the long-term aim of determining its structure by X-ray crystallography.

PBMG 31 The interaction of the ammonium transporter AmtB with the signal transduction protein GlnK

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The high-affinity ammonium transporters (Amt proteins) constitute a conserved protein family found in all domains of life from bacteria to man. In both eubacteria and archaea the Amt structural gene, *amtB*, is invariably co-transcribed with a second gene, *glnK*. GlnK is a member of the P_{II} signal transduction protein family; proteins which regulate many aspects of microbial nitrogen metabolism. Typically these proteins are covalently modified by uridylylation in nitrogen limiting conditions and de-uridylylated in nitrogen sufficiency e.g. after ammonium shock.

We have previously shown that *E.coli* GlnK interacts with the inner membrane in an AmtB-dependent manner suggesting that GlnK may control AmtB activity. We now report that AmtB and GlnK interact in a rapid and reversible manner in response to µM concentrations of extracellular ammonium. This "ammonia shock " leads to de-uridylyation of GlnK as a consequence of an increase in the intracellular glutamine level. This in turn results in sequestration of GlnK by AmtB and concomitant inactivation of the transporter. The conserved linkage of *amtB* and *glnK* suggests an ancient evolutionary origin. We therefore propose that the regulation of AmtB is the original and primary physiological role of GlnK and that other functions of this P_{II} protein have evolved subsequently.

PBMG 32 Structure-function studies on the *E. coli* ammonium transporter AmtB

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The Amt proteins constitute a family of high-affinity ammonium transporters that are ubiquitous, being found in eubacteria, archaea, fungi, plants and nematode worms. In higher animals, including man, the Amt family is represented by the erythroid Rhesus proteins. These polytopic membrane proteins show significant conservation of primary amino acid sequence and a combination of bioinformatic and empirical data indicates that they have 11 or 12 transmembrane helices with a cytoplasmic C-terminal tail. This tail can vary in length between 30 and 140 residues in different organisms with a conserved core sequence of around 30 amino acids.

In archaea and eubacteria the Amt structural gene is invariably co-transcribed with *glnK* which encodes a signal transduction protein responsible for sensing intracellular nitrogen. The primary role of GlnK appears to be the regulation of Amt activity. We have used site-directed mutagenesis to examine the role of the C-terminal region of AmtB, which in *E. coli* comprises just the core sequence. Our studies indicate that the region is required for full activity of the protein and for interaction with the signal transduction protein GlnK. We propose that the C-terminal region constitutes a discrete domain and plays a significant role in regulating Amt activity.

PBMG 33 The Canadian ophiostoma genome project

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Previous genetic studies of Ophiostomatoid fungi have focused on individual genes. With the development of high throughput automated DNA sequencing, bioinformatics, and microarrays, it is now feasible to identify and study large populations of genes controlling various aspects of cell metabolism. We are using the pathogen Ophiostoma novo-ulmi and the phylogenetically related saprobe O. piceae as model species for genomic studies on the Ophiostomatoid fungi with economic importance in forestry. We are conducting a large-scale investigation of genes involved in mycelial and yeastlike growth, protein glycosylation, pathogenicity, pigmentation and fruiting. These fungal genes are important for substrate colonization, host-pathogen interactions and propagation. Some of them may provide targets for control strategies. To date, 2000 EST library clones have been sequenced producing 600 hits by BLASTX analysis. *This 3-year project involves four different laboratories and is financed by the Natural Sciences and Engineering Research Council of Canada.*

Tuesday 9 September 2003

This competition is sponsored by the Society to encourage excellence in scientific communication by young microbiologists. Group Committees have now judged recent oral or poster presentations by members who are postgraduate students or postdocs who have gained their PhD in the past two years. The finalists from each Group go forward to compete for prizes at a special session of short oral presentations on their research. The best three entries win cash prizes and all entrants receive a free year's membership of the SGM.

1405 The structure and function of meningococcal T-cell stimulating protein A (TspA)

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Introduction: TspA is a high molecular weight, surface exposed T-cell and B-cell stimulating protein of *Neisseria meningitidis*, which has a highly positively charged N-terminus, a hydrophobic putative trans-membrane region and a highly negatively charged C-terminus. In this study the molecular features and function of TspA were investigated.
Results and Discussion: TspA was cloned, expressed, purified and used to raise rabbit polyclonal antiserum. An isogenic deletion mutant and truncated tspA mutants, lacking part of the N- or C-terminus were produced. Homology to fimV, the *Pseudomonas* gene involved in twitching motility, suggested a link between TspA and the type IV pilus. Since pili are important in the association of meningococci to human cells, the adherence of TspA mutant bacteria and their wild type counterparts were compared. The mutants were reduced in their ability to adhere to cell culture monolayers. There was also a reduction in transformation competence of the Null mutant.
Conclusion: These findings imply that TspA interacts with the meningococcal pilus. Further work on twitching motility and electron microscopy is underway to establish this link.

1420 The hepatitis C virus ion channel protein, p7: characterisation of a novel anti-viral drug target

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Hepatitis C virus (HCV) is a major cause of Hepatocellular Carcinoma and is now the leading cause for liver transplantation in the developed world. Treatment of the virus is currently limited to the use of type 1 interferon either alone, or in combination with the guanosine analogue ribavirin and this therapeutic regime is expensive, poorly tolerated, and effective in only 40 % of cases world-wide. Furthermore, resistance to this treatment is common in the viral genotypes found in the west. Recently, inclusion of Amantadine has been shown to improve the effectiveness of current drug regimes in clinical trials, though its mode of action is unclear. We have shown that the p7 protein of HCV oligomerises both in cells and *in vitro* to form an ion channel structure as determined by biochemical and biophysical methods. Ion channel activity was demonstrable both in artificial lipid bilayers and in cell-based functional assays. Amantadine was shown to specifically inhibit the ion channel activity of p7 at concentrations in the micromolar range, which also inhibits the M2 ion channel of Influenza A virus. Furthermore, mutation of a conserved region of charged amino acids abrogated ion channel activity without affecting intracellular localisation and this protein displayed a dominant negative phenotype. Given the absolute requirement for a functional p7 protein in the related Pestivirus, Bovine Viral Diarrhoea virus for the production of infectious progeny, HCV p7 presents a novel candidate drug target for anti-viral therapy.

1435 Identification of the homologues rhtX and fptX, novel genes that function in the utilisation of the siderophores rhizobactin 1021 and pyochelin, respectively

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S. meliloti 1021 is a gram negative bacterium found free living in the soil or in a symbiotic nitrogen fixing relationship with *Medicago sativa*. There is a high iron demand during symbiosis and consequently the iron acquisition mechanisms of rhizobia have been the subject of particular interest. *S. meliloti* 1021 produces one known siderophore termed rhizobactin 1021. Rhizobactin 1021 is a citrate hydroxymate siderophore, which is structurally similar to aerobactin that is produced by various pathogenic strains of *E. coli*. A regulon comprising the outer membrane receptor for rhizobactin 1021, *rhtA*, a biosynthesis operon, *rhbABCDEF* and an AraC type regulator, *rhrA* has previously been described. Analysis of the region directly upstream of the *rhbABCDEF* operon enabled the identification of a gene termed *rhtX*, the protein product of which in addition to RhtA is also required for transport of the siderophore. The expression of *rhtA* and *rhtX* in an *S. meliloti* strain that does not produce or utilise rhizobactin 1021 conferred upon this strain the ability to utilise the siderophore. Analysis in *E. coli* indicated that rhizobactin 1021 was transported via the aerobactin outer membrane receptor, IutA and the FhuBCD inner membrane transport system. Expression of *rhtX* in an *E. coli fhuB* mutant conferred upon the strain the ability to utilise rhizobactin 1021 but not aerobactin or ferrichrome indicating that RhtX can act as alternative transporter of rhizobactin 1021 in *E. coli*.

htX shows low level sequence homology to AmpG, a protein known to function as a permease in peptidoglycan recycling. RhtX also shows homology to a number of uncharacterised proteins in several bacterial genomes, all of which are located proximal to genes that are known to be, or are predicted to be involved in iron acquisition. A homologue of RhtX was identified in the opportunistic human pathogen *P. aeruginosa.* The gene, termed *fptX* was located in a region downstream of the outer membrane receptor for pyochelin, a siderophore produced by the bacterium. The *fptX* gene was mutated by allelic replacement and the mutant was found to be defective in pyochelin utilisation. It is proposed that RhtX and FptX are members of a novel family of permeases that function in siderophore utilisation.

1450 The cyanide insensitive oxidase protects the opportunistic pathogen *Pseudomonas aeruginosa* against cyanide

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Pseudomonas aeruginosa synthesises the potent respiratory inhibitor hydrogen cyanide at concentrations of up to 300µM. In addition it also possesses a branched electron transport chain, terminated by up to five terminal oxidases, one of which is the cyanide insensitive terminal oxidase (CIO). This oxidase is proposed to have a role in allowing aerobic respiration during

cyanogenic growth conditions. Furthermore, it has been proposed that the electrons derived from oxidative HCN synthesis are accepted by oxygen via the CIO terminated pathway. Therefore, we have investigated the relationship of the CIO to cyanide synthesis and cyanide sensitivity in *P. aeruginosa*. We demonstrate that the presence or absence of a CIO has no impact on cyanogenesis in liquid cultures, however minimum inhibitory concentrations for cyanide suggest the CIO has a protective role in the growth of *P. aeruginosa* under cyanogenic conditions. A number of further growth studies confirmed the importance of the protective role of the CIO to the growth of *P. aeruginosa* in the presence of physiologically relevant concentrations of cyanide. These data suggest that a functional CIO is required for active growth under cyanogenic conditions but that its presence is not required for cyanide synthesis *per se*.

1505 Identification of novel genes involved in *Salmonella* biofilm formation using DNA microarrays

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Opportunistic pathogens, such as *Salmonella* spp., are of particular importance for the medical and food industries as they cause diseases ranging from gastroenteritis to typhoid fever. The success of *Salmonella enterica* serovar Typhimurium to survive and overcome environmental stress is apparent from the frequent isolation of these bacteria from a large number of sources, ranging from soils to kitchen surfaces. It has been shown that survival and persistence is enhanced when *Salmonella* is attached to a surface as a biofilm, allowing bacteria to overcome limited nutrient availability, low pH and the low temperatures used in food storage. The formation of highly structured biofilms on surfaces during food processing allows *S*. Typhimurium to cause contamination while resisting antimicrobial treatments.

Currently, it is not clear which genes are required for biofilm formation and persistence, and whether they are differentially expressed in comparison to free living (planktonic) cells. To identify genes that are involved in *Salmonella* biofilm formation we have determined whole genome expression profiles using DNA microarrays. This approach has allowed us to quantify the difference between planktonic and biofilm-regulated genes of *S*. Typhimurium for the first time. Analysis of the microarray data revealed that more than 11% of genes were biofilm-regulated and that many genes involved in chemotaxis, motility and amino acid metabolism were affected. Our data was validatedby the identification of genes that encode proteins known to be involved in biofilm formation, such as fimbriae. Many genes located in *Salmonella* pathogenicity islands were found to be down-regulated. Interestingly, the expression of many unknown and uncharacterised genes also showed significant variation. Deletion mutants were constructed for selected genes identified by the microarray analysis to investigate their role in biofilm formation. These experiments have identified novel genes that play a role in biofilm formation in both static and flowing systems. The biofilm-regulated genes identified from the microarray data are being further investigated at the protein level.

1550 Detection of the protozoan parasite *Cryptosporidium parvum* by Nucleic Acid Sequence Based Amplification (NASBA) of the DNA Replication gene *Cp-RPA1*

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Background: The protozoan parasite *Cryptosporidium parvum* is the causative agent of cryptosporidiosis in several mammals, including humans, causing severe gastrointestinal distress. While usually self-limiting, the disease can become chronic in the immunocompromised, contributing to increased morbidity and mortality in these populations. The emergence of *C. parvum* as a globally significant human pathogen has led to a recognition of the need for sensitive and reliable methods of detection for the transmissable oocysts of this pathogen.
Methods: Nucleic acid sequence based amplification (NASBA) is an isothermal, RNA-based rapid amplification method, which utilises electrochemiluminescent probe techniques to detect the amplified target sequence. The gene *Cp-RPA1* encodes the large sub-unit of the *C. parvum* replication protein A (RPA1), a single-stranded-DNA binding protein essential for DNA replication.
Results: An optimal set of NASBA primers for a sequence within the *Cp-RPA1* gene was identified, with a detection limit of 50 oocysts. The ruthenium-tagged NASBA amplicon was subsequently directly sequenced to confirm the target.
Conclusion: This report presents the first application of NASBA to a DNA replication gene in any organism. NASBA of the *Cp-RPA1* gene is proposed as a sensitive molecular detection system for the presence of *Cryptosporidium* oocysts, with potential as a measure of viability.

1605 Mineral formation from toxic metals by sulphate-reducing bacterial

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Abstract not received

1635 Medics require speedy answers – rapid results from blood culture

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Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the most common causes of nosocomial infections and bacteraemia. Standard bacterial identification and sensitivity testing can take as long as 72 h to report results. Reduction in this delay would significantly improve the quality of patient care. This study aimed to increase the speed of diagnosis using PCR, which would improve therapy and infection control. A duplex PCR was developed to detect the *mecA* and *coag* genes, and detection of these respectively ascertained resistance to methicillin and differentiated between *S.aureus* and coagulase-negative staphylococci. The method was validated against clinical isolates, including methicillin-sensitive *S.aureus*, MRSA, methicillin-resistant coagulase-negative staphylococci and methicillin-sensitive coagulase-negative stphylococci and showed complete agreement with standard laboratory methods. Studies in clinical usage indicate that a species and sensitivity diagnosis is achieved one day earlier than conventional methods. The impact on patient care of the development is reviewed.

1650 A randomized, controlled trial comparing ganciclovir or ganciclovir plus foscarnet (each at half dose) for pre-emptive therapy of cytomegalovirus infection in transplant recipients

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[1]Dept of Virology and [2]Bone Marrow Transplant, [3]Liver Transplant[3] and [4]Renal Transplant Units, Royal Free and University College Medical School & Royal Free Hampstead NHS Trust, London

Whole blood from bone marrow, liver and renal transplant patients was tested twice weekly by polymerase chain reaction (PCR) for cytomegalovirus (CMV) DNA. Patients with two consecutive PCR positive samples were randomized to receive either full dose ganciclovir (5 mg/kg iv twice daily) or half dose GCV (5 mg/kg iv once daily) plus half dose foscarnet (FOS)

(90mg/kg iv once daily) for fourteen days. Separate randomisation codes were used for each patient group and the primary end point was the proportion of patients who became PCR negative within fourteen days (primary endpoint). Forty-eight patients were randomised. Their base line demographic characteristics were well matched as was the base line CMV viral load (4.28 $\log_{10}$ in the ganciclovir (GCV) group, compared to 4.01 $\log_{10}$ in the GCV plus foscarnet arm). In the GCV group, 71% (17/24) of patients became CMV PCR negative within 14 days compared to 52% (12/23) in the GCV plus FOS arm ($p = 0.23$). Eight patients required the study drug to become stopped or reduced; seven out of the eight patients were in the GCV plus FOS arm ($p<0.05$). Baseline viral load was messured in 42 patients, and the rate constant κ for viral growth or decay computed. Median baseline viral load was 0.8 $\log_{10}$ ($p = 0.02$) higher in patients who failed the primary endpoint. In addtion, replication rate was faster ($t_{1/2} = 1.5$ versus 2.7 days; $p < 0.001$) and viral decay slower ($t_{1/2} = 2.9$ versus 1.1 days; $p < 0.001$) in patients who failed in primarty endpoint.

This study does not support for ganciclovir plus foscarnet synergism in vivo and nearly all adverse side effects were seen in patients receiving this combination. Ganciclovir plus foscarnet treatment regimen may be of use in patients with impaired bone marrow function, where administration of full dose ganciclovir is precluded

Measuring baseline viral load and computation of viral replication identifies patients at risk of treatment failure who require a prolonged course of antiviral treatment.

Main Symposium

Monday 8 September 2003

1600 Global views of host/pathogen interactions; transcriptional changes in virally infected cells

PAUL KELLAM

Virus Genomics and Bioinformatics Group, Dept Virology and Dept Immunology & Molecular Pathology, University College London

Manifestations of viral disease are often the sum of the damage done by a harmful agent and the bodies' response. This means that to understand the totality of a pathogenic process a detailed understanding of diverse aspects of normal and diseased cellular physiology is required. To perform this on a genome wide scale requires many of the new advances in high throughput functional genomics. DNA microarrays have begun to provide a detailed global view of the transcriptional programs of cells as they respond to different viruses. Many of these small studies have re-identified common cellular antiviral mechanisms. However, with the use of larger microarray experiments and advanced computational analysis novel insights into both viral and host gene expression programs have been obtained. This is leading to a change in use of microarrays from exploratory to hypothesis driven research tools.

Some of the most extensively studied viruses using microarrays are the herpesviruses. In this talk the use of host and pathogen microarrays will be illustrated using the herpesvirus, in particular Kaposi's Sarcoma Associated Herpesvirus (KSHV) as an example. Herpesviruses are large, enveloped, double stranded DNA genome viruses that infect a wide range of animal species. Of the eight human herpesviruses, whole genome DNA arrays have documented the co-ordinated gene expression for representatives of each herpesvirus subfamily. However, the herpesvirus life cycle is intimately linked to the biological processes of the host cell and the viral necessity for combating the host antiviral response. When analysed in the context of host gene expression, focused microarray studies are able to identify novel latent cellular reservoirs and new therapeutic targets for the treatment of KSHV induced tumours.

Environmental Microbiology – Posters

EM 23 Isolation of integrons from Antarctic soils

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Integrons provide a mechanism for horizontal gene transfer, serving as genetic elements that permit acquisition and expression of genes contained within mobile cassette structures. The integron unit is a major contributor to the dissemination of antibiotic resistance among diverse Gram-negative bacteria. Using a PCR-based strategy, integrons have been isolated from environmental DNA extracted from pristine Antarctic mineral soils. The environments sampled have minimal anthropogenic disturbance with no history of exposure to synthetic chemicals. Sequence analysis of cloned integrons has revealed the presence of the *aadA* gene cassette in Antarctic environments, an adenyltransferase enzyme conferring resistance to the antibiotics streptomycin and spectinomycin.

EM 24 Proteomic investigations into copper resistance in the extremely acididophilic Archaeon *Ferroplasma acidarmanus*

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Ferroplasma acidarmanus is an extremely acidophilic archeaon isolated from the Iron Mountain superfund site in Northern California, USA; an environment characterized by the lowest pH and highest concentration of heavy metals reported to date. The sequenced isolate *Ferroplasma acidarmanus* 'Fer1' is believed to play an important role in the oxidization of sulfide minerals associated with the production of acid mine drainage, and may contribute significantly to localized geochemical cycling and the partitioning, mobility and toxicity of metals in this and other highly acidic environments. Fer1 grows optimally at 42° chemomixotrophically, utilizing ferrous iron and yeast extract or sugars, and is capable of growing at pH 0, representing one of the most extreme examples of acidophily ever reported. Fer1 exhibits remarkably high tolerance to copper ions (15g/l) when adapted by multiple-step culturing in the presence of 1g/l (.0157mol/l) copper. Exposure to sub-toxic concentrations of copper results in the detectable differential expression of cellular proteins as detected by 2D-PAGE. These proteins are currently being identified by mass spectrometry and genes coding for proteins of interest will be cloned for expression within a host bacterial system. These protein expression studies should provide fascinating insights into the genetic and biochemical mechanisms of metal homeostasis in this archaeal extremophile.

Food & Beverages Group – Poster

FdBev 17 Comparative study to extract and detect Cry9C in corn products

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Genetically modified crops and foods derived from them continue to ignite controversy and spur the interest of public advocacy groups, regulators and consumers. Recently in the USA one genetically modified corn product intended for animal use only, has made its way into the human food chain. This genetically modified corn contained the Cry9C gene derived from *Bacillus thuringiensis*. The Cry9C gene codes for the production of the Cry9C protein that acts as an insecticidal toxin protein. It is heat stable and resistant to digestion. The corn was not approved for human consumption because it might trigger an allergic reaction. Since the discovery that Cry9C was introduced into the human food chain through corn-derived products, several illnesses attributed to these products were investigated by the FDA and the CDC. In our study we compared two DNA protocols of extraction, the Promega Wizard®, a DNA purification Kit, and the cetyltrimetyl ammonium bromide (CTAB) extraction protocol, and two PCR based detection methods, a conventional method and a commercial one by DuPont Qualicon "BAX system PCR Kit for Cry9C"in detecting the genetically modified corn in 23 regulated food products. Using the Promega kit™ DNA was extracted from 20 corn products while DNA was extracted from 19 products using the CTAB protocol. In measuring the quality of DNA extracted using a spectrophotometer at A260/280 absorbance ratio both Promega wizard® and CTAB protocols provided good amount of DNA. Two products were found positive for Cry9C by the BAX PCR system and one was detected positive by conventional PCR. The use of the BAX PCR is simple, rapid and reduces pipeting and handling errors.

Microbial Infection Group – Posters

MI 46 Differential response of bovine macrophages to infection with members of the *Mycobacterium tuberculosis* complex

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Tuberculosis, a chronic disease of the lower respiratory tract, is caused by members of the *Mycobacterium tuberculosis* complex. All bacteria within this complex are closely related at the genetic level, however their ability to cause disease varies considerably from host to host. Key to the success of these pathogens is their ability to reside within macrophages. This study aimed to assess the differential response of bovine macrophages to infection with *M. bovis* (virulent), *M. bovis*-BCG (attenuated vaccine strain) and *M. tuberculosis* (avirulent in cattle). In particular the production of nitric oxide (NO) and TNFα was assessed. Macrophages infected with *M. bovis*-BCG produced little NO or TNFα, even when IFNγ was added as a co-stimulant. Infection with *M. bovis* similarly saw no TNFα secretion, however NO was detectable in culture supernatants and the amount of NO was enhanced by co-stimulation with IFNγ. Macrophages infected with *M. tuberculosis* produced the most NO, particularly in the presence of IFNγ, and also produced considerable amounts of TNFα. It is hoped that these studies in conjunction with the analysis of bacterial intracellular survival (using microarray technology) will aid the understanding of differences in the pathogenicity of these three species with regards to bovine infection.

MI 47 A serotype-specific role of PnpR/S in pneumococcal virulence and gene regulation

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The ability of bacteria to sense and adapt to environmental stimuli is often mediated by two component signal transduction systems (TCSs). Global analysis of the genome of *Streptococcus pneumoniae* revealed 13 TCSs. In this study we examined pneumococcal TCS04 which consists of a response regulator and a histidine kinase encoded by *pnpR* and *pnpS*, respectively. This TCS shares significant homology with the PhoP/Q systems of other bacterial pathogen. PhoP/Q systems have been shown to be important for the virulence of many pathogens and are known to respond to levels of divalent cations in the environment. This investigation examined the virulence and gene regulation patterns of *pnpR* mutants of three different pneumococcal serotypes. We identified a serotype-specific attenuation of virulence. Microarray technology was used to determine the transcriptional changes, which may be responsible for this attenuation. A significant down-regulation of a known pneumococcal virulence factor, pneumococcal surface protein (psa) was observed in the attenuated *pnpR* mutant. Further confirmation of *psa* downregulation in the *pnpR* mutant was made by semi-quantative RT-PCR and by analysis of other *psa*-associated phenotypes.

Young Microbiologist of the Year

Tuesday 9 September 2003

1605 Linked redox precipitation and sequestration of sulphur and selenium under anaerobic conditions by sulphate-reducing bacterial biofilms

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Biological sulphur cycling comprises both assimilatory incorporation into biomass as sulphide and dissimilatory oxidation-reduction transformations, carried out by bacteria for energy gain. Specific transformations between redox states are attributed to different microbial groups, ecologically defined by their roles in the carbon and sulphur cycles. Sulphate-reducing bacteria (SRB) are characterised by their capacity for dissimilatory reduction of sulphate (SO_4^{2-}) to sulphide (HS^{2-}). Reoxidation of sulphide to elemental sulphur is accomplished by anaerobic phototrophs, e.g. green and purple sulphur bacteria, or by aerobic chemotrophs. Thus biological production of elemental sulphur from sulphide generally requires either light, or oxygen.

The biological cycling of selenium is receiving attention, due not only to its importance as a trace element, but also to the recognition of selenium as a serious pollutant. SRB can enzymatically reduce small amounts of selenium oxyanions to selenide, or elemental selenium. Whilst the potential for indirect, SRB-mediated chemical reduction of selenium oxyanions has been identified, little attention has been paid to the biogeochemical, or biotechnological significance of such a reaction; indeed, the ability for SRB to participate in selenium cycling under low-redox conditions has been questioned. However, SRB are increasingly recognised as active into the oxic zone, whilst the reaction described can take place some distance from the site of SRB activity, where selenium oxyanions are more frequently encountered.

Here we report that SRB can mediate formation of elemental sulphur in the presence of selenite. The indirect, enzymatically-mediated coprecipitation of sulphur and selenium is a generalised ability among SRB, arising from sulphide biogenesis, and can take place under a range of redox conditions and in the dark. When SRB grow as attached biofilm under laboratory conditions (also their natural growth mode in most environments), the resulting sulphur-selenium mineral forms nanometre-scale aggregates that are effectively sequestered within the biofilm structure.

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