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**Society for
General
Microbiology**



**UNIVERSITY
OF
LEEDS**

7–10 September 1999

ABSTRACTS

144th Meeting

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1015 Overview: transport of molecules across microbial membranes - a sticky business to get to grips

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Abstract not submitted

1100 Multidrug resistance efflux

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Multidrug resistance pumps (MDRs) are present in all organisms studied to date and belong to several unrelated families. The common feature of all these transporters is the ability to extrude chemically unrelated toxins from the cell. MDR substrates are amphipathic molecules, which allows them to discriminate between toxins and more polar cellular compounds. The simplest MDRs belong to the SMR family of drug/proton antiporters. MDRs of the MF family are 12-14 TMS transporters related to specific drug/proton antiporters. An ATP-dependent ABC MDR has been described in *L. lactis*. Gram negative bacteria have complex MDRs belonging to the MF and RND families that form multicomponent structures traversing the entire cell envelope. Transcription of the complex envelope MDR of *E. coli*, EmrAB/TolC is controlled by a multidrug sensor EmrR that binds ligands of the pump. A multidrug sensor BmrR controls the BMR MDR in *B. subtilis*, and a QacR sensor controls the QacA pump of *S. aureus*. Artificial substrates of most MDRs are hydrophobic cations. The only known natural group of these substances are berberine alkaloids that appear to be good substrates for MDRs. Plants that make these alkaloids additionally produce MDR inhibitors \bar{n} 5-methoxyhydrnocarpin and pheophorbide A. It is suggested that MDRs evolved to protect cells from natural hydrophobic cations such as berberine alkaloids.

1145 Regulation of solute accumulation in bacteria and its physiological significance

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Bacterial solute transport systems can be subdivided in three categories on the basis of their energetics: (i) *primary transport* systems use the free energy that is released upon the hydrolysis of ATP; (ii) *secondary transport systems* use the free energy that is stored in the electrochemical gradients of protons, sodium ions or other solutes across the membrane; (iii) *group translocation systems* chemically modify the substrate concomitant with translocation. The latter group constitutes the bacterial phosphoenolpyruvate sugar:phosphotransferase systems (PEP-PTS).

The primary ATP-dependent solute uptake systems in bacteria all belong to the class of ATP-binding cassette (ABC) family and use a binding protein to capture the substrate and to deliver it to the translocator complex. For long it was thought that the use of binding proteins was restricted to these systems, but recently it has been shown that some secondary transport systems also employ such accessory proteins. In general, the energetics and kinetics of the different types of transport mechanisms are known quite precisely from *in vitro* studies in membrane vesicles or proteoliposomes. Within the context of the cell there can be regulatory mechanisms superimposed on the systems, which then determine the solute accumulation levels as well as the actual rates of transport. This will lead to solute concentration gradients that are far from thermodynamic equilibrium. The focus of this paper is on these regulatory mechanisms and their specific physiological functions in bacteria.

1400 Arsenic transport systems from *Escherichia coli* to humans

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Resistance to arsenicals and antimonials evolved at least three times. 1) The *ars* operon of *Escherichia coli* plasmid R773 encodes both the ArsC arsenate reductase that reduces As(V) to As(III), and the ArsAB pump that extrudes arsenite and antimonite. The ArsA ATPase is the catalytic subunit of the pump. ArsA homologues are present in members of every kingdom, including prokaryotes, archaea and eukaryotes. ArsA has homologous N-terminal (A1) and C-terminal (A2) halves, indicating an evolutionary origin by gene duplication and fusion. ATP hydrolysis is allosterically activated by As(III) or Sb(III), ensuring that the pump does not hydrolyze ATP without coupled anion translocation. The crystal structures and mechanisms of ArsA and ArsC will be discussed. 2) In *Saccharomyces cerevisiae* the *ACR* gene cluster encodes an independently-evolved arsenic resistance. Acr2p is an arsenate reductase, and Acr3p is a secondary carrier for arsenite extrusion. 3) Finally, the *S. cerevisiae* Ycf1p, an ABC transport ATPase, pumps As(SG)₃ and other metal-thiol complexes into the vacuole, conferring resistance. *Supported by US Public Health Service Grants GM55425 and GM52216*

1445 Type II protein secretion: the main terminal branch of the general secretory pathway

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Bacteria have evolved several secretory pathways to release proteins into the extracellular medium. In gram-negative bacteria, the exoproteins cross a cell envelope composed of two successive hydrophobic barriers, the cytoplasmic and outer membranes. In some cases, the protein is translocated at once across the cell envelope, directly from the cytoplasm to the extracellular medium. In other cases, secretion occurs *via* a two-step process, and outer membrane translocation involves an extension of the signal peptide-dependent pathway for translocation across the cytoplasmic membrane. With respect to the so-called General Export Pathway (GEP), this route was designated as the General Secretory Pathway (GSP) and is widely conserved among gram-negative bacteria. The type II secretion mechanism or main terminal branch (MTB) of the GSP, involves 12-14 different Gsp proteins, which is the general term used to describe those proteins of the type II secretory apparatus. Surprisingly, the deduced amino acid sequences and initial characterisation of the proteins indicated that only two of the fourteen are effectively located in the outer membrane. All but one of the other proteins seem to be anchored in the cytoplasmic membrane, with the remaining protein peripherally associated with the cytoplasmic side of the inner membrane. This particular cell envelope distribution of the Gsp proteins renders difficult the investigation of their roles in the molecular mechanism of outer membrane translocation. Multiple aspects of the GSP mechanism, including machinery assembly, exoprotein recognition, energy requirement and pore formation for driving through the outer membrane, will be discussed.

1600 Secretion and injection of Yop proteins by the Type III machines of *Yersinia enterocolitica*

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Yersinia enterocolitica export toxic proteins during animal infections via a type III secretion mechanism. Some type III secretion substrates are injected into the cytoplasm of the eukaryotic host cells while others are secreted into the extra-cellular environment, and still others remain associated with the bacteria in a manner that presumably aids in the injection of the effector Yops. All three destinations remarkably require the same type III secretion machine. The signal for secretion cannot be distinguished on the basis of amino acid sequence similarity of the substrates. We have shown that type III machines in *Yersinia* can recognize at least 2 independent signals for secretion. One signal has been found in all Yops analyzed to date and is located in the first 15 codons. This signal tolerates drastic amino acid mutations and thus appears to be located in the mRNA sequence of the secretion substrate. The translation and targeting of the substrate to the secretion loci can be coupled by the mRNA signal. Some of the Yops harbor a second secretion signal that is located in their amino acid sequences and requires the binding of secretion chaperones. Those Yops that contain this signal are all injected into the eukaryotic cell, though, not all injected Yops have been found to bind a secretion chaperone. The recognition of secretion signals during infection may require an ordered program of gene expression. We have begun to reveal the importance of post-transcriptional regulation of Yop expression. LcrH and YopD bind type III substrate mRNA, likely causing the repression of translation. The absence of this translational regulation leads to the massive secretion of all Yops during infection rather than their specific targeting to extra-bacterial locations. Thus, there may be an ordered sequence of protein expression that is required to facilitate the accurate targeting of Yops.

1645 Assembly of bacterial adhesins across the outer membrane via the chaperone-usher pathway

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Abstract not submitted

0900 DNA uptake by transformable bacteria

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Discovery of natural systems for DNA uptake by cells began about fifty years ago. A brief survey of mechanisms for DNA uptake in bacterial cells during viral infection, conjugation, and transformation shows them to be quite distinct, although the latter two processes have similarities, such as the conversion of donor DNA to single strands on entry into a recipient cell. Detailed examination of transformation by free DNA in three different bacterial species shows that they all undergo a transient phase of competence for DNA uptake. The mechanisms for inducing competence, however, are distinctly different in the three species. Nevertheless, the sets of proteins that are ultimately induced share considerable homology, which indicates that the structures and mechanisms for uptake are similar. There appears to be an extrusion through the cell membrane and cell wall of a protein complex composed of type IV pilin-like structural proteins. Apparently bound to this complex are functional proteins for binding double-stranded DNA, nicking one strand, degrading the other, forming a pore in the membrane, and pulling one strand into the cell. All except the degradative nuclease are induced during competence. Although the essential proteins for DNA uptake have been identified, the precise mechanisms of uptake are still conjectural.

0945 *Escherichia coli* signal recognition particle - a historical perspective

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Abstract not submitted

1100 Protein translocation across the ER membrane in yeast

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Translocation of proteins across the endoplasmic reticulum (ER) membrane is a decisive step in the biosynthesis of many classes of proteins in all eukaryotes. The mechanism by which this occurs is becoming increasingly understood at the molecular level and can be divided into two distinct stages, namely targeting and translocation. Proteins destined for the secretory pathway are synthesised with an ER-specific addressing signal which determines their fate. Signal receptor molecules identify these "molecular postcodes" and ensure the delivery of the polypeptide to the ER membrane. The targeted polypeptide then engages with the Sec61 protein complex which creates an aqueous transmembrane channel through which the polypeptide can pass as it exits the cytoplasm and enters the ER lumen. In yeast, the targeting step can occur either co- or post-translationally. As might be expected these different targeting mechanisms have been found to require distinct cytosolic factors. For example, SRP appears to be exclusively required for co-translational targeting whereas Hsp70s are involved in the post-translational reaction. In either case the precursor is translocated via the Sec61 protein channel. However, current evidence suggests that fundamentally different mechanisms drive the two translocation reactions and that these require specific accessory factors. Recent insights into these mechanistic differences will be discussed.

1145 Peroxisome biogenesis

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Peroxisomes are almost ubiquitous eukaryotic organelles with a diverse biochemical repertoire ranging from photorespiration (in leaf-type peroxisomes) through beta oxidation of fatty acids (most peroxisomes) to glycolysis (in glycosomes of *Trypanosoma*). The importance of peroxisomes is emphasised by the finding that a number of devastating human genetic disorders are caused by defects in peroxisome biogenesis. Peroxisomal proteins are encoded in the nucleus and synthesised in the cytosol. Matrix and membrane proteins use distinct targeting signals and import machineries, although a detailed understanding of import mechanisms is lacking. Approximately 20 genes (PEX genes) have been identified which are required for peroxisome assembly in fungi, plants and mammals. Peroxisome biogenesis has a number of unusual features such as the ability of these organelles to import folded and oligomeric proteins. Although peroxisomes are capable of importing proteins post translationally, recent evidence suggests that some proteins may be targeted first to the endoplasmic reticulum then sorted to peroxisomes. This raises interesting questions about the evolutionary origin of peroxisomes.

1445 Transport of proteins into and across the thylakoid membrane

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A surprising variety of pathways are used for the transport of proteins into and across the thylakoid membrane. Thylakoid lumen proteins, such as plastocyanin and the 33, 23 and 16 kDa photosystem II proteins, are synthesised with bipartite presequences in which an envelope transit sequence is followed by a cleavable thylakoid-targeting signal. The latter signals specify transport by two very different translocation systems in the thylakoid membrane. Plastocyanin and the 33 kDa protein are transported by an ATP-dependent Sec system that resembles bacterial Sec systems. The transport of other proteins, such as the 23 and 16 kDa proteins, requires neither stromal factors nor ATP, but depends on the thylakoidal delta pH. This system is highly unusual because the substrate proteins can be transported in a fully-folded conformation. Moreover, there is now clear evidence that a related system operates in a wide range of bacteria, primarily for the export of cofactor-containing proteins. Thylakoid membrane proteins are also targeted by distinct routes. Some require a form of stromal signal recognition particle (SRP) for insertion into the thylakoid membrane, together with GTP and protein transport machinery. Others appear to insert spontaneously by mechanism that has been demonstrated for only a few proteins in other membrane systems.

1530 Evolutionary origins of transmembrane transport systems

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Our lab has analyzed twenty completely sequenced genomes for known and putative transport systems, and we have used this information to classify transport proteins on the basis of (1) mode of transport and energy coupling mechanism, (2) phylogenetic family and subfamily, and (3) substrate specificity. Using rigorous phylogenetic criteria, over 200 families have been defined. The resultant information is currently available on our two web sites: (1) <http://www-biology.ucsd.edu/~msaier/transport/titlepage.html>, and (2) <http://www-biology.ucsd.edu/~ipaulsen/transport/titlepage.html>.

These studies have allowed us to establish evolutionary facts as follows: (1) Certain permease families have evolved independently, at different times, following different routes. (2) Several families arose by intragenic tandem duplication, triplication or quadruplication events, but evidence for such events during the evolution of other families is lacking. (3) In some cases, extragenic duplication events gave rise to increased permease complexity. (4) Some permease families have altered their numbers of transmembrane α -helical spanners (TMSs) during evolution. (5) A few families have drastically altered their transmembrane topologies during evolution. Thus, while homology strongly suggests common structure, it does not prove it. (6) While transport mode and energy coupling mechanism are highly conserved traits, a few families provide examples where these traits have not been strictly conserved. (7) While most families arose from a single ancestral system, some superfamilies have mosaic origins. These families are usually constructed on a modular design.

These and other observations will be discussed.

0900 *Mycoplasma pneumoniae* cytoadherence: some assembly required

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Mycoplasma pneumoniae causes primary atypical pneumonia and bronchitis in humans. This cell wall-less wonder has what is described as a minimal genome, yet possesses a remarkably complex terminal structure that mediates adherence to host respiratory epithelium. This polar, tapered attachment organelle is an extension of the mycoplasma cell and is defined ultrastructurally by an electron-dense core that enlarges to form a terminal button. Both the electron-dense core and terminal button are components of the mycoplasma cytoskeleton. Duplication of the attachment organelle is thought to precede cell division, with the tip structure believed to be important in chromosome segregation. This raises the possibility that mutations affecting cytoadherence might also confer a defect in cell development. Several protein constituents of the attachment organelle have been identified through the analysis of non-cytoadhering mutants. Genomic manipulation of mycoplasmas is possible by means of transposon delivery of recombinant genes, making it possible to elucidate the assembly and regulation of the terminal organelle and conduct structure-function analysis of its component proteins. Our studies have focused recently on proteins HMW1 and P30. HMW1 is a cytoskeletal protein localized primarily to the filamentous extensions of the mycoplasma cell. HMW1 is essential for proper development of the attachment organelle and localization of the cytoadhesin protein P1 to this structure. Furthermore, HMW1 may be subject to proteolytic regulation during cell development. Protein P30 is strictly associated with the attachment organelle and is thought to function in receptor binding. Loss of P30 results in striking morphological changes, including ovoid or multiple-lobed cells having a poorly defined tip structure. Nucleoid staining with DAPI suggests that these morphological abnormalities reflect a defect in cell division. Hence, P30 appears to have roles in both cytoadherence and cell development.

0945 Induction and evasion of host defences by type 1 pillared uropathogenic *Escherichia coli*

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Uropathogenic strains of *Escherichia coli* are the primary causative agents of cystitis and upper urinary tract infections. Filamentous surface adhesive organelles called type 1 pili are encoded by virtually all strains of uropathogenic *E. coli*. Using high-resolution electron microscopy we have shown that the adhesive tips of type 1 pili, which contain the adhesin molecule FimH, can interact directly with host receptors on the luminal surface of the bladder epithelium. Attached pili mediated intimate contact of the bacteria with the bladder surface, which is coated with hexagonal arrays of integral membrane proteins known as uroplakins. Bacterial attachment facilitated by FimH, either alone or in concert with other as of yet unidentified bacterial factor(s), triggered the exfoliation of host bladder epithelial cells as part of an innate host defense system. Exfoliation occurred via an apoptosis-like mechanism requiring caspase activation and involving host DNA fragmentation. Using various biochemical, genetic, and microscopic assays, we have found that the type 1 pilus adhesin can mediate the internalization of bacteria into bladder epithelial cells. Internalized uropathogenic strains of *E. coli* can replicate and microscopic studies of infected mouse bladders have indicated that the intracellular bacteria can eventually break out of infected host cells and colonize surrounding tissue. Fluxing in and out of bladder epithelial cells may provide a means for uropathogenic *E. coli* to resist clearance from the bladder by both innate and adaptive host defenses and may also facilitate the dissemination of uropathogens within the urinary tract.

1100 General properties of bacterial s-layers and cell walls: do they promote adhesion?

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The surfaces of bacteria are important because they are the first structure to interact with the external environment and they dictate whether or not the bacterium will adhere to an external surface, whether it be another cell or an inanimate material. New microscopical techniques are providing a better understanding of the molecular organisation of microbial surfaces so that their physical properties can be better understood. The surfaces of Gram positive cell walls (such as those of *Bacillus subtilis*) are profoundly affected by 'cell wall turnover', whereas those of Gram negative bacteria (such as those of *Pseudomonas aeruginosa*) by the O-sidechains of lipopolysaccharide. Generally, at pH~7.0 and temperatures between 18-37C, these bacterial surfaces are polar (i.e. hydrophilic) and the macromolecules and polymers, of which they are constructed, highly motional. The peptidoglycan network also has some motional aspects, is easily deformed and is perfectly elastic. S-layers are frequently encountered above typical Gram-positive and Gram-negative cell walls. As S-layer proteins (or glycoproteins) are secreted, they self-assemble into planar paracrystalline arrays (i.e. S-layers) having either oblique (p1,2), square (p4) or hexagonal (p3,6) lattices and, in so doing, usually make the cell surface more hydrophobic so that hydrophobic-hydrophobic interactions can predominate in adhesive processes. Yet, strategic polar groups can remain exposed on S-layer surfaces so that some adhesion must occur to select hydrophilic interfaces (e.g. *Synechococcus* GL can bind to calcite and gypsum minerals). All of these general aspects of bacterial surfaces can determine their capacity for adhesion and they shall be discussed as such.

1145 Adherence of *Helicobacter pylori* to the gastric mucosa

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Infection with *Helicobacter pylori* has been shown to be the cause of chronic active gastritis and duodenal ulcer disease. Exactly how *H. pylori* causes disease is not yet known but adherence to the gastric mucosa is thought to be an important virulence mechanism of the organism. *H. pylori* exhibits a very specific tropism for gastric mucous secreting cells in vivo and this makes it a particularly interesting organism to study in terms of its adherence properties. Surface molecules of *H. pylori* that are putative adhesin molecules include flagella, lipopolysaccharide and outer membrane proteins. Recently mutants have been generated defective in production of two outer membrane proteins that show decreased adherence to gastric tissue. The Lewis b blood group antigen has been proposed as a specific receptor for some strains of *H. pylori* and an adhesin BabA2 has

been identified. However it has been shown that there is no correlation between Lewis B expression and adherence of *H. pylori* to viable gastric cells. Other putative receptors for *H. pylori* include phosphatidylethanolamine, extracellular matrix proteins and MHC class II molecules. Specific adhesins and receptors for *H. pylori* have however yet to be identified. In addition, the nature of the attachment of the pathogen to the human gastric mucosa is controversial. Some workers have reported that adherence of *H. pylori* causes actin polymerisation and tyrosine phosphorylation of host proteins but others have been unable to confirm these findings. The molecular characterisation of putative adhesin molecules and colonisation studies with isogenic mutants in appropriate animal models are required in order to identify factors which mediate adherence and to clarify the mechanism of adherence of this organism to the gastric mucosa. Such studies could lead to the development of adhesin analogues for use in the inhibition of colonisation and improved therapy for ulcer disease.

1400 Proteinase:adhesin complexes of *Porphyromonas gingivalis*

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Porphyromonas gingivalis is an anaerobic Gram-negative rod which is frequently isolated from sites of destructive disease in the periodontal tissues. The organism produces several extracellular proteolytic enzymes which are thought to play an important role in the aetiopathogenesis of periodontal disease. The major activities produced *in vitro* have specificity for arginyl- and lysyl- peptide bonds which are derived from *rgpA*, *rgpB* (the arg-gingipains) and *kgp* (lys-gingipain). RgpA and Kgp are multi-domain translation products comprising a propeptide typical of other bacterial protease precursors, a catalytic domain and a long C-terminal extension with regional similarity to adhesins/haemagglutinins from other micro-organisms. Proteolytic processing of these precursors leads to the formation of non-covalently linked complexes of the catalytic domain of each enzyme in association with polypeptide(s) derived from the C-terminal extension. This association leads to a targeting of the proteolytic activity of these complexes to proteins of the extracellular matrix which are ligands for the adhesin polypeptide(s). For example the rate of degradation of fibronectin by the RgpA complex is almost two logs greater than the rate of degradation of this matrix protein by the catalytic domain of RgpA alone. Several other gene products of *P. gingivalis* contain domains which share sequence similarity to the C-terminal extensions of RgpA and Kgp although their precise roles have not been defined. However this family of proteinase/adhesin gene products appears to play a critical role in the biology of *Porphyromonas gingivalis*: passive immunisation with a monoclonal antibody to an epitope within the common adhesin domain is able to block recolonisation of periodontal patients by *P. gingivalis* for up to 9 months. The development of antimicrobial strategies which target the adhesin domain of RgpA and Kgp may provide a significant method of control of *P. gingivalis* in the treatment of periodontal disease.

1445 Regulation of *Escherichia coli* capsule expression

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The production of an extra cellular polysaccharide capsule is a common feature of many bacteria. The capsule, which often constitutes the outermost layer of the cell, mediates the interaction between the bacterium and its immediate environment and plays a crucial role in the survival of bacteria in hostile environments. One such environment is the human host where interactions between the capsule and the host's immune system may be vital in deciding the outcome of an infection. Polysaccharide capsules may also promote the formation of biofilms and the colonisation of a variety of ecological niches, including indwelling catheters, prostheses and the formation of alginate-rich biofilms in the lungs of cystic fibrosis patients. In such instances the polysaccharide may present a permeability barrier to antibiotics and hinder the effective eradication of the bacteria.

Escherichia coli produces more than 80 chemically and serologically distinct capsules, called K antigens. These capsules have been separated into four groups (1-4) on the basis of chemical composition, molecular weight, intergenic relationships and regulation of expression. The majority of extra-intestinal isolates of *E. coli* associated with invasive disease express group 2 capsules, with particular capsules being associated with certain diseases. Typical of many virulence factors, expression of group II capsules in *E. coli* is regulated by temperature with group II capsules only being expressed at temperatures above 18°C. The temperature regulation is mediated by a complex regulatory hierarchy involving Hns, TypA, RfaH and mRNA processing. The regulatory circuit will be described in detail and its relevance to the expression of capsules *in vivo* discussed.

1600 Adhesive fibrils of oral *Streptococcus gordonii*

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Oral streptococci frequently express large surface structures (fibrils or fimbriae) that have been implicated in adhesion of bacteria to host surfaces and to other oral micro-organisms. For most streptococcal species, however, it has proved difficult to define precisely both the biochemical composition of these surface structures, and their role in adhesion. We have previously characterised in some molecular detail the involvement of CshA, a large (259 kDa) cell-wall-anchored protein of *Streptococcus gordonii*, in the adhesion of streptococcal cells to fibronectin and to *Actinomyces* spp. It is now clear that the expression of CshA by *S. gordonii* is associated with the production of sparse peritrichous fibrils of length 60.7 nm +/- 14.5 nm that are absent from *cshA* mutant strains. To determine if CshA comprised adhesive surface fibrils, we constructed an *Enterococcus faecalis* JH2-2 strain that expressed full length CshA on the cell surface. Electron micrographs of negatively-stained recombinant *E. faecalis* showed peritrichous fibrils (70.3 +/- 9.1 nm) that were absent from the control *E. faecalis* cells. The fibrils bound CshA-specific antibodies as demonstrated by immunoelectron microscopy. *E. faecalis* cells expressing CshA also showed increased cell surface hydrophobicity and increased adhesion to fibronectin compared with the control strain. In contrast, recombinant *E. faecalis* cells did not adhere to *Actinomyces naeslundii*. The results demonstrate that CshA polypeptide is the structural and functional component of *S. gordonii* adhesive fibrils.

1620 Interactions of *Staphylococcus aureus* with human endothelial cells *in vitro*

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Staphylococcus aureus is a major cause of community-acquired and nosocomial bacteraemia, with clinically apparent bacterial metastasis to tissues such as bones, joints and solid organs occurring in up to 53% of affected individuals. Bacterial seeding must involve interactions between circulating bacteria and vascular endothelial cells, to which *S. aureus* adheres *in vitro*. Using isogenic mutants, plasmid complementation, mono- and polyclonal antibodies and recombinant soluble bacterial fibronectin-binding protein, we have shown that the interaction between the *S. aureus* fibronectin-binding proteins and endothelial cell-associated fibronectin represents the dominant pathway for the adherence of *S. aureus* to resting, live, human endothelial cells *in vitro*. In the presence of the plasma protein fibrinogen, agglutinates of bacteria formed that nevertheless required functional fibronectin-binding protein to adhere to endothelial cells. Once adhesion has occurred, *S. aureus* cells undergo a process akin to phagocytosis. Our data indicate that *S. aureus* fibronectin-binding proteins are required for internalisation. Expression of fibronectin-binding proteins has been shown to be highly conserved among a large panel of recent clinical isolates of *S. aureus*, suggesting an important biological role for this property.

1640 Regulation of the fim switch that controls type 1 fimbriation in *Escherichia coli*

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Phase variation of type 1 fimbriation in *Escherichia coli* is controlled by site-specific recombination driven by two recombinases, FimB and FimE. Phase switching is regulated by amino acids alanine and leucine, and occurs at much higher frequency from the fimbriate-to-afimbriate phase (up to 0.7 per cell per generation) than in the opposite direction. Furthermore, the recombination substrate acts in *cis* to control *fimE* expression, apparently to impose temporal control on phase variation from the fimbriate-to-afimbriate phase. The molecular mechanisms responsible for the controls observed, and the possible physiological significance of the regulation, will be discussed.

POSTERS:

C1 Characterisation and adherence mechanisms of *Escherichia coli* strains causing infections in patients with a reconstructed bladder

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Withdrawn

C2 Multiplex cloning of binding domains using phage display and DNA isolated from cells in the human oral cavity

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The intimate association of microorganisms with epithelial cell surfaces presents an interesting paradox - why doesn't the commensal flora elicit an immune response from the host? The commensal bacterial almost certainly engage in a complex two-way communication with the immune surveillance cells of the host and many surface and secreted proteins from the commensal bacteria may be involved. We are investigating this bacterial-host interaction in a number of ways one of which is the isolation of binding-proteins from the commensal bacteria. We have used phage display to isolate phage carrying binding-domains of proteins from oral bacteria. Direct plating for the isolation of bacteria from an environmental sample is known to access only a few percent of the organisms in such a sample. To circumvent this problem we have used direct isolation of total DNA from mouth washes and used this DNA to form libraries in the phage display vector pG8H6. This multiplex cloning approach allows access to all the genome equivalents in a sample including organisms which are hard or impossible to isolate in pure culture.

The phage library was panned against immobilised human IgA and phage which bound were eluted and the cloned DNA was sequenced. The protein sequence of some of these binding domains have no counterparts in the current databases. The phage library was also panned on a human epithelial cell line after first removing phage which bound to collagen, then fibronectin and fibrinogen, in that order. The phage which bound to the epithelial cells after this treatment contained protein domains with some similarity to calyculin-associated proteins.

C3 Features of the *caf1m* periplasmic chaperone essential to assembly of *Yersinia pestis* polypeptide capsule

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In contrast to other extracellular export/ assembly systems, assembly of the *Yersinia pestis* capsule would appear to be remarkably simple. Only two specific *caf* gene products - periplasmic chaperone (Caf1M) and outer membrane secretin (Caf1A) - are required for export and assembly of the single gene product (Caf1). Caf1M belongs to the FGL subfamily of periplasmic chaperones involved in surface adhesin biogenesis. The following features identify this subfamily: (i) possession of a disulphide bond adjacent to the subunit binding site (ii) an additional variable sequence (FGL) between the assigned F1 and G1 β -strands (iii) an additional sequence at the extreme N-terminus of the mature polypeptide and (iv) in general, assembly of a simple structure composed of a single subunit. The significance of these unique features to subunit binding and export is being investigated in an *E coli* model system. Formation of the disulphide bond was shown to be essential to in vivo folding of Caf1M, but neither for maintenance of the finally folded structure nor for chaperone function. Analyses of deletion derivatives demonstrated that the FGL sequence is required for stable chaperone:subunit interaction but does not contribute significantly to the stability of the final conformation of Caf1M. Mutagenesis of selected residues of the FGL sequence identified specific residues essential for subunit binding. These results provide evidence for the first time that the variable sequence preceding the conserved subunit binding G1 β -strand plays a major role in subunit binding. Because of the variability in sequence and length of the FGL region in different chaperones, this region may contribute to the specificity and affinity of subunit binding.

C4 Flagella phase variation in *Salmonella typhimurium*

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Salmonella is the only species of Enterobacteria that has been found to express two flagellar antigens alternatively and to possess two flagellin genes *fliC* and *fliB* regulated by a complex gene switching mechanism. *Salmonella typhimurium* expresses flagella in either phase 1 (i antigen) or phase 2 (1,2 antigen). One cell can only express flagella carrying either i or 1,2 antigens but not both. In a given population of bacteria the proportion of cells expressing each phase can vary from 0 to 100 % and depends on the frequency of gene switching between phases. Whilst the genetic mechanism controlling the expression is very well understood, the environmental factors making the cell to switch phases, if any, are not well described.

We produced a range of monoclonal antibodies specific to antigens of phase 1 and phase 2 of *S. typhimurium* flagella which were used to develop an indirect ELISA to quantitatively measure the expression of flagellar phases and to study the expression of phases under different growth conditions.

To validate the quantitative ELISA, populations of single phased *S.typhimurium* were mixed in different proportions and theoretical calculation of the percentages of expression were compared with the results obtained by ELISA.

In a survey of 40 strains of *S. typhimurium* isolated from clinical specimens most strains expressed both phases in similar proportions, whilst some strains preferentially expressed one phase only. A selection of strains was subjected to varying growth conditions in order to study the effect of growth factors on phase expression. The conditions studied were media composition, solid versus broth media, growth in aerobic and anaerobic environments, pH and temperature. None of the growth conditions tested appeared to alter the predominant flagella phase of the strains. The experiments showed that some strains existed predominantly in one phase and continued to express this phase over many generations despite environmental stress such as low pH. The strains which expressed equal amounts of both phases were more likely to change their expression in favour of phase 1. The effect of long term storage was also studied. Nearly 70 % of strains stored on Dorset eggs slopes for 5 years increased the expression of phase 1 over phase 2.

C5 The role of fimbriae and flagella in the persistence of *E. coli* O78:K80 in the chick

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Escherichia coli is the aetiological agent of avian colibacillosis with infection occurring via the respiratory tract usually following a primary bacterial or viral infection or as a result of poor husbandry practices. The most severe and economically important manifestation of avian colibacillosis is colisepticaemia which is characterised by airsacculitis, pericarditis, perihepatitis and salpingitis.

Adhesion is a necessary prerequisite for bacterial colonisation and pathogenesis and is often mediated by proteinaceous surface appendages known as fimbriae. Type 1 and curli fimbriae are expressed by most *E. coli* strains of importance in colibacillosis, but their role in avian disease remains equivocal. Avian *E. coli* also commonly elaborate flagella which confer motility and are considered to play a role in facilitating adhesion.

Persistence is defined as a state where micro-organisms can be found continuously in the host and it is considered that bacterial adhesion to host tissue is an aid to overcoming host defences such as urination, desquamation and peristaltic propulsion. It is widely accepted that persistence is essential for the success of bacterial pathogens and it is often accompanied by the shedding of the organism into the environment through the faeces and urine.

Mutants defective for the elaboration of type 1, curli and flagella were constructed by insertional inactivation of fimbrial and flagellar genes in a well characterised pathogenic *E. coli* O78:K80 isolate. Persistence characteristics of the knockout mutants were compared to the wild-type progenitor strain in *in vivo* persistence and persistence competition experiments. These studies showed that flagella followed by curli > type 1 were important in the persistence of *E. coli* O78:K80 in the chick.

C6 Adhesion of Verocytotoxic *Escherichia coli* O157:H7 to tissue culture cells and gut explants

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Verocytotoxic *Escherichia coli* (VTEC) O157:H7 have become the most common cause of juvenile renal failure since their recognition as a foodborne pathogen in 1982. Although, most cases of foodborne illness are associated with the consumption of contaminated undercooked ground beef, illness from consumption of unpasteurised milk, cheese, apple cider and salad vegetables has also been reported. One of the main virulence factors of O157:H7 strains is their ability to form attaching and effacing (A/E) lesions on the host cell surface. An outer membrane protein intimin (*eaeA*) allows the intimate attachment of the bacterium to the host cell surface, however, the mechanism of initial adhesion to the host cell surface, prior to microvillus effacement, is not well established in VTEC strains. Mutants defective for the expression of intimin did not form A/E lesions, but using a quantitative assay and scanning electron microscopy, were still adherent in a Hep-2 tissue culture assay. In addition, intimin mutants were still adherent in calf and chick explant studies. Adherence of isogenic fimbrial mutants to type 1 and curli fimbriae was also indistinguishable to those of wild type cells. These data indicate that intimin and fimbriae are not involved in the adherence *per se*. We are currently engineering additional isogenic mutants in other surface antigens (flagella and *espA*) to determine whether these molecules are involved in the initial attachment of VTEC to the host cell surface.

C7 The role of SEF14 and SEF17 fimbriae in the adherence of *Salmonella enterica* serotype Enteritidis to inanimate surfaces

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To gain an understanding of the role of fimbriae and flagella in the adherence of *Salmonella enterica* serotype Enteritidis to inanimate surfaces, the extent of adherence of viable wild-type strains to a polystyrene microtitre plate was determined by a crystal violet staining assay. Additionally, elaboration of surface antigens by adherent bacteria was assayed by fimbriae and flagella specific ELISAs. Wild-type Enteritidis strains adhered well at 37°C and 25°C when grown in microtitre wells in colonisation factor antigen broth but not other media tested. At 37°C adherent bacteria elaborated copious quantities of the SEF14 fimbrial antigen whilst at 25°C adherent bacteria elaborated copious quantities of the SEF17 fimbrial antigen. Afimbriate and aflagellate knock-out mutants were assessed in the adherence assay also. Mutants unable to elaborate SEF14 and SEF17 adhered poorly at 37°C and 25°C, respectively, but adherence was not eliminated. Non-motile mutants showed reduced adherence whilst type 1, PEF and LPF fimbriae appeared not to contribute to adherence in this assay. These data indicate that SEF17 and SEF14 mediate bacterial cell aggregation upon inanimate surfaces under appropriate growth conditions.

0905 "There is no such thing as safe food"

JOHN HERITAGE

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Member of the UK Government's Advisory Committee on Animal Feedingstuffs

Over recent months, the UK Press has become pre-occupied with the perceived problems of 'genetically-modified' (GM) foods. The British public has lived with food scares for more than a decade. *Listeria hystera*, salmonella in eggs and, of course, BSE. The GM food debate is the latest, high profile scare. What, then, *are* the concerns? An alternative title could well have been "GM foods - a case for resistance". The role of antibiotic resistance markers in GM foods is one where scientific opinion is divided. Their use in GM foods is examined in the broader context of the safety evaluation of foods under European regulations. Particular attention will be paid to the role of the *bla* gene in GM foods; other significant resistance markers will also be discussed.

0945 Microbiology for non-microbiologists: the challenges of interest and assessment

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There are many levels involved in being a 'non-microbiologist'. These may range from students on a Biology degree who do not see microbiology as their chosen specialism, through part time students for whom microbiology is a necessary option, though not relevant to their job, to primary school science teachers and undergraduates on any course who have chosen a science elective. The knowledge base and intended outcomes may vary considerably, as should assessment criteria. However, for 'non-majors', emphasis on the relevance of the subject to everyday life, and assignments which enable the participants' own subject skills to be used appear to be more successful than the more traditional approaches. The presentation will describe and critically review experiences with a range of units/assignments applicable to 'non-majors'.

1025 The use of computers in teaching modular courses in microbiology

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Modular courses pose both challenges and opportunities for the deployment of Communications and Information Technology (C&IT). For example, students may be more diverse in terms of their microbiological background, their motivation towards the subject and their facility with C&IT. The solutions provided by C&IT are not necessarily novel but in an appropriate context they are easy to construct and deploy and have the significant advantage of being largely independent of time and space. The advent of the Web and commercial products has increased the range of options available to teachers.

In principle, Computer-Assisted Learning can be customised to the requirements of the individual student though this is rarely the case and might in any case be seen as making the learner less rather than more autonomous. Providing the student with variety and choice, however, is generally to be encouraged. A simpler strategy may be to use Computer-Assisted Assessment in a formative manner to help students identify possible areas of weakness and to indicate resources covering such topics. Freely available packages from sources such as the Teaching & Learning technology Programme can be used to provide alternatives or precursors to laboratory exercises. There is probably also some scope for the use of standard IT productivity applications, e.g. spreadsheets, in classes as well as the integration of exercises based on more specifically scientific software such as sequence analysis and systems modelling packages.

Finally, teachers should consider adopting a holistic C&IT model that embraces administrative as well as pedagogic needs. The communications element of C&IT may usefully underpin Web-based exercises for groups as well as providing interaction with and between individual students. Institution-wide support for provision of course information, communication and submission of work is likely to become prevalent over the next few years with the arrival of global standards. At the same time, greater knowledge as to how students use such systems will hopefully refine the next generation of learning technology.

1125 Teaching Diversity through small group activities in practicals: a case study from Zoology

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As a response to decreasing time and resources for studying diversity in invertebrate biology, practicals using a small group approach were devised in 1996 and have been running very successfully since. The problems leading to this development are not specific to zoology and the practical can be applied to any branch of biology. The presentation will review the problems leading to the redesign, the principles and practice of using this new approach and a critical review of the approach and its advantages and disadvantages.

1205 Sex, pies and videotapes: teaching microbiology to non-microbiologists

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This presentation will describe details of an 'elective' module 'Microbes & Man' delivered in the first semester to Level one students who have had little, or no previous exposure to microbiology. In recent years, the majority of students taking the module has been Biology students (although not exclusively so) and many have been studying for Microbiology awards. Experience has shown that students who have a genuine interest in a particular subject usually perform well academically in that subject. For this reason, the principal aim of 'Microbes & Man' is to stimulate amongst students an enthusiasm for microbiology and to place the subject in context. A variety of teaching approaches designed to achieve this has been employed and will be discussed.

1245 Teaching microbiology in a multi-disciplinary course - a German experience

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Fulda University of Applied Sciences („Fachhochschule“) offers a four years course (six taught semesters, 240 ECTS credits) in „Household Management, Nutrition and Food Quality“ („Oecotrophologie“). The Course qualifies for professional fields such as health protection and nutritionhousehold management and environmental issues quality management, product development and marketing in the food industry, catering units, hotels and restaurants. In each of these fields, it is essential that graduates are able to communicate efficiently with experts, consumers and business managers. For this, they require skills and knowledge in a wide array of subjects of natural, physical and social sciences as well as economics, but also key qualifications such as creativity, ability to work in teams and to manage projects. In this situation, a problem-orientated approach in teaching microbiology, and strong links to related subjects of the Course proved to be most appropriate, even in the first year. Examples of issues motivating the students to have a closer look at basic and applied microbiology include

- food safety
- genetic engineering and food processing
- hygiene related to waste treatment and recycling processes
- probiotic foods.

Problems related to these and similar issues are also dealt with in independent project work. This starts already in the first semester and comprises, in total, 30 credits (about 560 hours). Project work proved to be a very efficient way of providing key qualifications.

0910 Deep bacterial biosphere in marine sediments

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The presence of active microbial populations in subsurface environments and even into basalts and granite, has now been demonstrated. This includes subsurface, deep, marine sediments where substantial bacterial populations are consistently present at a range of sites in the Pacific and Atlantic Oceans and the Mediterranean Sea. The biomass in these sediments to 500 m (average global sediment depth) is equivalent to ca. 10% of surface biomass. The deepest samples analysed so far are 842 m and as populations don't decline faster in deeper layers bacteria should be present even deeper. DNA extracted from deep sediments is consistently high molecular weight and thus indicative of intact, viable bacteria. 16S rRNA gene sequence analysis demonstrates considerable biodiversity. In addition to sequences predominantly related to known anaerobic bacteria there are some unique sequences, such as a deep branching group of the d-proteobacteria. Consistent with this genetic biodiversity a range of different bacterial types have been enriched and the few pure culture isolates from these demonstrate that they are well adapted to their sub-surface environment (e.g. barophilic, enhanced fermentation and ability to use ferric iron). The rates and geochemical impact of deep bacterial activity in marine sediments demonstrates that these processes are substantial. As bacteria can grow at high pressures (>1000 Atm) and temperatures (113C), physical conditions should not become limiting in marine sediments for several kilometres. Thus the "classic" separation between biologically catalysed diagenesis (<60C) and thermal catagenesis may no longer be appropriate. As organic matter becomes increasingly recalcitrant during burial, bacterial activity, however, may still become limited by the lack of degradable organic compounds. Thus the presence of significant bacterial populations to even 842 m is surprising and even more so the increases in bacterial activities and populations which occur in the deeper layers at some sites. Laboratory heating experiments, to simulate the response of bacterial processes to sediment burial and heating, combined with analysis of microbial processes in gas hydrate sites have suggested new mechanisms to enable bacterial processes to continue at depth in marine sediments. In gas hydrate sediments on Blake Ridge bacterial activity is stimulated within the deeper layers of the gas hydrate zone (190 - 445 m) such that rates can exceed those close to the sediment surface. Below ca. 100 m, low molecular weight volatile fatty acid concentrations begin to increase and continue increasing with depth (>15mM at 691 m). This reflects increasing bioavailability of organic matter during burial and heating, and results in the large stimulation in bacterial activity. Although, gas hydrates may be an extreme example of this processes, as reflected in the high rates of methane formation, if it occurs even albeit much reduced, in other sediments it could explain the widespread presence of active bacterial populations in deep marine sediments and offers the prospect of an even deeper and hotter biosphere and one that may be intimately involved in deep diagenetic processes, including those that ultimately produce fossil fuels.

0950 Geochemical evidence for deep bacterial activity

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Microbes depend upon and modify the chemistry of their environment, and chemical measurements on subsurface samples can be used to deduce important aspects of the microbial ecosystem. These include the determination of the dominant microbial functional group, the limitations on other community members, and the presence of organisms that are non-culturable or may be assumed to be absent. Chemical measurements are useful in investigations of microbial ecosystems because of the chemical function of microbes; microbes facilitate chemical reactions to extract energy from organic carbon sources, and are therefore dependent on the concentrations of reaction components. Microbial activity alters the concentrations of these components, and an ecological succession of microorganisms may be revealed by the examination of spatial trends in reactant and product concentrations. The microbial succession is due to the isolation of the biogeochemical system from the surface, so that nutrients are not readily replenished. Microbial diversity along groundwater flowpaths often decreases because needed nutrients are consumed and not replaced. Broad spatial analysis of chemical variability may thus reveal large-scale biogeochemical processes operating along flowpaths. Finer scale chemical measurements across lithologic boundaries may also reveal whether distinct communities exist within separate strata and whether they are interdependent.

1100 Aquifer microbiology

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Microorganisms carrying all the major terminal electron-accepting processes have been recovered from both deep pristine aquifers and well as shallow aquifers contaminated with organic compounds. One of the most significant microbially catalyzed redox reactions in the subsurface is the reduction of Fe(III) to Fe(II) which greatly impacts on water quality and subsurface geochemistry. Although Fe(III) reduction had, until recently, been modeled primarily as an abiotic reaction, it is now generally recognized that most of the Fe(III) reduction in the subsurface is the result of microorganisms enzymatically transferring electrons to Fe(III). Evaluation of the microbial community with molecular techniques that avoid culture biases have demonstrated that *Geobacter* species are the predominant Fe(III)-reducing microorganisms in the Fe(III) reduction zone of many aquifers with temperatures of 10-20 °C. Hyperthermophilic Archaea that are capable of conserving energy to support growth from the oxidation of hydrogen or hydrocarbons coupled to the reduction of Fe(III) may be important catalysts of Fe(III) reduction in deeper, hotter subsurface environments. Phylogenetic and geological evidence suggests that Fe(III) reduction, possibly taking place in hot subsurface environments, was one of the first forms of microbial respiration.

1145 Microbial basalt weathering

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Abstract not submitted

1405 The role of SRB in forming major crustal geochemical anomalies: The Lower Carboniferous, Irish Zn+Pb Orefield

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The Irish Zn+Pb Orefield is the richest per square kilometre in the world. The Navan mine, located 30km north-west of Dublin, is Europe's biggest Zn producer, and a truly world-class base-metal deposit hosting reserves of 69.9Mt, grading 10.1 percent Zn and 2.7 percent Pb. The deposit occurs as a complex, sub-horizontal orebody hosted entirely by Lower Carboniferous (~350Ma) marine carbonates.

On the basis of over 250 sulphur isotope analyses, two sources of sulphur have been identified in the deposit – deep-seated/hydrothermal, and seawater sources. Baryte (BaSO₄) at Navan and elsewhere in the Orefield has been derived by direct precipitation of seawater sulphate with hydrothermally exhaled Ba: the S isotope signature ($\delta^{34}\text{S}$) of this baryte being identical to that estimated for Lower Carboniferous seawater sulphate ($\sim +20 \pm 2\text{‰}$). A proportion of sulphide in the deposit is derived from the bacteriogenic reduction of Lower Carboniferous seawater sulphate. This bacteriogenic sulphide has a distinctive low $\delta^{34}\text{S}$ ($\sim -15 \pm 10\text{‰}$) resulting from isotopic fractionation during the bacterial reduction process.

The deep-seated, or hydrothermal source, is leached from the underlying thick (>4km) rock package, and brought into the deposit along with the ore metals. The correlation of the relatively high $\delta^{34}\text{S}$ of hydrothermal sulphide ($\sim +10 \pm 5\text{‰}$) in the deposit with the sulphide $\delta^{34}\text{S}$ from drillcore in the underlying package elucidates the origin of this sulphur.

Using the isotopic signatures of individual minerals and bulk sulphide ore concentrates, we estimate that around 80% of the sulphide in the deposit is bacteriogenic. This equates to a deposit of 3.7 Mt of S at ore grade, and substantially more when non-ore grade sulphide is considered. A similar proportion of bacteriogenic sulphide is also seen at the Silvermines and other producing deposits in the Orefield, thus making Navan and the Orefield in general, a major crustal-scale geochemical anomaly attributable to the activity of sulphate reducing bacteria. Careful study of the ore-host rock relationships and sulphide textures at Navan indicates that the bulk of the ore was precipitated sub-seafloor, in cavities within the carbonate package. We estimate that fluids containing bacteriogenic sulphide may have been present to a depth of at least 150 m below the Lower Carboniferous seafloor during ore formation.

1450 A deep biosphere in oil reservoirs

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Abstract not submitted

1555 Molecular analysis of bacterial diversity in the deep subsurface biosphere

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This paper provides a brief overview of the application of molecular biological methods to analysis of microbial populations in the deep sub-surface biosphere, and focuses primarily on sub-seafloor sediments obtained from different geographical locations around the world. Polymerase chain reaction (PCR) cloning methods were used to amplify 16S rRNA genes directly from DNA extracted from Ocean Drilling Programme (ODP) sediment cores. Gene sequences were analysed to provide an estimate of microbial community diversity with depth profiles and to identify phylotypes that might be associated with processes shown to occur in the sediments. A variety of genera from the major phyla of Bacteria (*Proteobacteria*, *Cytophagales*, and Gram Positive genera) were identified as phylotypes in sediment 16S rRNA gene libraries. However, community diversity did not appear to change significantly with depth, from 0.5 to 234 m below the sea floor. PCR primers designed to amplify 16S rDNA from methanogens in sediment cores containing methane hydrates, resulted in the isolation of *Euryarchaeota* phylotypes clustering within the *Methanomicrobacteria* and *Methanobacteriales*. Molecular analysis was also used to monitor and direct enrichment cultures (from MPNs) for specific groups of prokaryotes associated with processes such as sulphate reduction, Mn- and Fe-reduction, methanogenesis, acetogenesis, and xenobiotic degradation. 16S rDNA phyloprobes have been constructed for the analysis prokaryotic communities in sediment samples, and for screening of enrichments through to the isolation pure cultures. The utility of these probes and this general approach for the purification of slow-growing organisms from deep sub-seafloor sediments is discussed.

1625 Isotopic evidence for deep bacterial activity

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Bacterial populations have recently been demonstrated in a range of deep sedimentary environments, however the geochemical impact of their presence has yet to be resolved. In this presentation this problem is addressed using stable sulfur and oxygen isotopic compositions of pore-water sulfate and rates of sulfate reduction (measured by $^{35}\text{SO}_4^{2-}$ turnover) in sediments from three Ocean Drilling Program sites from the Cascadia Margin accretionary wedge (Sites 888, 890/889 and 891). The uppermost 10m of all three sites exhibit decreasing sulfate concentrations with depth and corresponding increases in sulfate $\delta^{34}\text{S}$ and $\delta^{18}\text{O}$, as sulfate is removed by bacterial sulfate reduction. At site 891 low concentrations of sulfate persist in the sediment to 165m and there are low rates of sulfate reduction to this depth. At the other two sites sulfate concentration exhibits a minimum at ~10m depth and increases again below ~50m. The isotopic data show that this sulfate is formed by oxidation of isotopically light, early diagenetic pyrite and that the oxygen in the sulfate molecules is derived entirely from the pore-water. This is consistent with anaerobic oxidation of pyrite, with Fe^{3+} in the sediment the probable oxidizing agent. This contrasts with the assumption that pyrite, once anoxically buried, remains stable.

Increased sulfate concentrations stimulate bacterial sulfate reduction at depths of 70-250m and this locally modifies the sulfate and pyrite isotopic compositions. Changes in the isotopic composition of pyrite at depth are consistent with the oxidation of early-formed pyrite and the formation of new pyrite associated with deep stimulated sulfate reduction. These mineralogical changes are also reflected in the deep diagenesis of magnetic minerals. Elevated deep bacterial activity is of sufficient magnitude to have a major effect on bulk sulphur isotopic composition of the sediment. The reoxidation processes we describe are important in sustaining deep bacterial activity and they should continue with increasing depth, as there is a large solid phase ferric pool, even in ancient deposits. Bacterial processes may thus potentially persist to much greater depths than have previously been considered possible.

0905 Bacteria under pressure

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The study of high-pressure-adapted (piezophilic, previously termed barophilic) bacteria is providing insight into life in the largest portion of the known biosphere, the deep-sea. The deep-sea is characterized by hydrostatic pressures ranging from 10 MPa at a depth of 1,000 m to approximately 110 MPa in the Challenger Deep of the Mariana Trench at nearly 11,000 m. Several microbiologists have investigated the growth ability of deep-sea bacteria under varying pressure conditions. However, the question of what the maximum pressure at which bacterial growth is attainable remains unanswered.

Recently, we reported the isolation of two obligate piezophilic strains from the Challenger Deep. Optimal pressure conditions for the two strains were 70 – 80 MPa with pressure maximums near 150 MPa. Such pressure are the highest reported for bacterial growth however still higher-pressure environments exist in the deep-sea subsurface, the environment under the deep-sea bottom. Parkes and his co-workers reported the detection of many microbial activities in such environments by analysis of deep-sea subsurface core samples obtained through the Ocean Drilling Project. It is reasonable to suspect the bacteria living in such environments exhibit piezophilic growth perhaps well beyond 150 MPa. The study of deep-subsurface microbiology has profound implications with respect to microbial diversity, general physiological ecology, and biogeochemical cycling in such extreme environments. The continued study of deep-sea microbiology will contribute important information to deep-subsurface microbiology particularly with regard to growth under extreme elevated pressure conditions.

0930 Marine aquifer system

1010 Bacteria in post-glacial freshwater sediments

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The laminar sedimentary deposition of material in aquatic environments provides a chronological framework upon which to study the characteristics and ecology of bacteria persisting at depth. The sediments of Windermere permit the study of bacteria deposited in the post-glacial era. Prokaryote sediment communities were examined for culturability, viability, community structure and for the presence of mobile genetic elements. Direct counts of bacteria revealed that in excess of 10^9 cells (per g dry weight sediment)⁻¹ could be detected throughout the 6 m depth of core (representing 10000-12000 years of deposition). The potential for active geochemical cycles was inferred from the presence of specific groups of bacteria. Metabolically active bacteria were demonstrated fluorometrically after challenging sediment samples with the viability dyes CTC and CFDA. Bacteria were culturable over the full depth of core although numbers declined significantly with depth from 10^7 c.f.u (g dry wt sediment)⁻¹ at the surface to 10^1 - 10^2 c.f.u. (g dry wt sediment)⁻¹ below 3 m depth. Gram-positive and Gram-negative bacteria were isolated from all levels and spore forming heterotrophs dominated. Bacteria demonstrating denitrifying activity were detected at all depths, although sulphate-reducers were not detected below 20 cm. PCR was employed on total community DNA and amplified eubacterial and archaeal DNA and insertion sequence 1 (IS1; specific to Enterobacteriaceae) from samples encompassing the entire depth of the core, although *uidA* (specific to *E.coli/Shigella*) could not be detected below 2.5 m. Collectively, these observations show that bacteria and archaea are capable of long-term persistence and activity in deep, ancient freshwater sediments.

1105 Origins of halobacteria in ancient salt deposits

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Evaporation and subsequent burial of a vast hypersaline sea of the Permo-Triassic period (Zechstein Sea) has resulted in large salt deposits underlying northern Europe. Such ancient salt deposits provide 'island habitats' for extremely halophilic archaea (halobacteria), which cannot grow in media containing less than 10 % w/v NaCl. We have characterised several isolates from surface-sterilised rock salt and over 100 isolates from subsurface brine pools of different origins, which contain up to 2×10^6 viable cells per ml. Several lines of evidence support the notion that at least some of these halophiles are remnants of populations which inhabited the Zechstein Sea. For example, heterotrophic, aerobic eubacteria from salt deposits and from surface hypersaline environments differed, because the former needed at least 10 % w/v NaCl for growth - an indication of long-term adaptation to a permanently salt-saturated environment. Independently isolated halococci from three geographically different Zechstein salt deposits were shown to belong to the same species, which, to date, has not been found in surface environments. Further support for the long-term growth/survival of halobacteria in ancient salt deposits comes from investigating their ability to live inside fluid inclusions and from studying their nutritional requirements.

1145 Is the present the key to the past for microbially-produced minerals? Coupled interactions of microbial and inorganic systems

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Minerals formed in the geological past by microbial degradation of organic matter can be identified by their chemical and isotopic characteristics. Sediments in a well-vegetated saltmarsh in north Norfolk (England) are less than 60 years old and allow direct investigation of current microbial populations. The study involved porewater and mineral chemistry, environmental phospholipid and 16S rDNA analysis, isolation of *in situ* organisms with 16S rDNA analysis. The vegetation provides organic matter which is being buried, forming the primary electron donor for a complex set of redox reactions: sulphate and Fe(III) are the main electron acceptors. The most dramatic expressions of these processes are the nodular concretions up to 20 cm. across. They consist of fine-grained iron carbonate and iron sulphide with minor calcium carbonate. However, the nodules are not zoned. They do not seem to have any direct geological equivalent. In contrast, massive manganoan iron carbonate concretions of Carboniferous age can be inferred to record successive products of Mn(IV) and Fe(III) reduction with carbonate being provided by methanogenetic fermentation. It is unclear exactly what modern environment could produce similar minerals. Integrated microbial and mineral ecology is beginning to provide an understanding of complex environments, ancient and modern.

POSTERS:

EM1 *In situ* biodegradation in organically-contaminated aquifers: how important are protists?

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Subsurface protistan populations are mostly composed of heterotrophic nanoflagellates. In pristine, uncontaminated aquifers flagellate population densities are typically low. By contrast, in organically- contaminated aquifers they may reach about 10^5 cells per gram dry weight of aquifer material. Aquifer flagellates are mostly bacterivorous and frequently associate with sediment particles, known to harbour abundant bacterial populations which may be capable of biodegradation processes. Recently, bacterial grazing by subsurface protists has been shown to have a significant effect on the rate of *in situ* biodegradation of trichloroethylene and of monoaromatic hydrocarbons. This corroborates earlier reports that aquifer flagellates may be used as indicators of *in situ* biodegradation. We also hypothesize that flagellates are directly involved in biodegradation processes.

EM2 Diversity and biotransformation activities of nitrile-degrading rhodococci

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Nitriles are toxic organic cyano-group-containing compounds, widely distributed in the environment as a result of biosynthetic and industrial processes. Diverse microorganisms have been found able to catabolise these compounds by a number of enzymatic routes. The main focus of this work is to investigate the geographical diversity of nitrile transforming rhodococci that inhabit deep-sea sediments and to compare these to terrestrial counterparts. Nitrile-utilising bacteria have been isolated by batch and chemostat enrichments using acetonitrile, succinonitrile, benzonitrile or bromoxynil as a sole carbon source. Deep-sea isolates were recovered from sediment samples from various Pacific Ocean geographical locations at depths ranging from 1151 to 6475 meters. Terrestrial isolates were recovered from soil samples from 6 world-wide locations. Mycolic acid profiles suggests that most of these isolates belong to the genus *Rhodococcus*. Pyrolysis mass spectrometry (PyMS) analysis grouped some of these isolates with *Rhodococcus* type species. A total of 42 nitrile-metabolising rhodococci have been isolated and initially a sub-set of acetonitrile strains is being characterised. Kinetics of growth on 20mM acetonitrile suggests that most of these isolates use a nitrile hydratase/amidase hydrolysing pathway. Whole cells are being used to access enzyme activity towards acetonitrile. Use of specific enzyme inhibitors will help to understand the hydrolysing enzymes present.

EM3 Influence of aldehydes upon the activity of bacterial isolates recovered from drinking water sand filters

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Slow sand filtration is a common method used to produce microbiologically safe drinking water. The unique feature of the slow sand filter is the surface active layer or 'schmutzdecke' which acts as an interface for biofilm development and water purification. The role of the 'schmutzdecke' is to remove natural organic matter, transform synthetic organic compounds and retain pathogens. Water from upland sources can be discoloured during heavy rainfall due to the presence of natural organic matter (NOM). As NOM cannot be biodegraded, ozone can be used to remove this discolouration prior to filtration. Aldehydes are one class of compound that are by-products of ozonation. They act as substrates which feed directly into the TCA cycle. Currently experiments are being carried out to investigate the effect of aldehydes produced by ozonation upon the activity of bacterial isolates from the 'schmutzdecke'. Preliminary data indicates that these isolates effectively utilise the aldehydes produced by ozonation.

EM4 Sulphate reduction and its correlation with biodegradation of diesel and paraffin hydrocarbons in old drill cuttings piles in the North Sea

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In the deeper layers of the North Sea large piles have formed on the sea bed as a result of the traditional dumping of drill cuttings. In particular the older drilling fluids contained concentrations of components of diesel and paraffin, which are toxic to the sediment fauna and relatively recalcitrant to bacterial degradation. Previous chemical analyses of drill cuttings piles have shown a general decrease of the hydrocarbon load and the disappearance of single components over several years after discontinuation of drilling. Whether this decrease was due to bacterial degradation or abiotic weathering was unknown. It has been thought that biodegradation of hydrocarbons in marine sediments occurred solely under aerobic conditions. The oxic layer in a carbon-overloaded ecosystem such as this, however, is only a few millimetres thick and sulphate reduction becomes the major biological activity. Some recent reports have now linked anaerobic metabolism to the degradation of hydrocarbons. Here we present data correlating the occurrence of hydrocarbon-utilising sulphate reducing bacteria in drill cutting piles to chemical evidence suggesting *in situ* biodegradation of hydrocarbons.

EM5 Free-living amoebae in an organically-contaminated aquifer in Mexico

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Until relatively recently subsurface protozoa remained virtually unstudied. However, it is now known that protozoa, especially heterotrophic flagellates and free-living amoebae, are probably widespread in the deep subsurface. In pristine aquifers their numbers may be low or even zero whereas in organically-contaminated aquifers protozoan abundances are usually much higher. This is probably as a result of higher growth rates of bacteria which support larger populations of bacterivorous protozoa.

Wastewaters are an important source of groundwater pollution, sometimes as a result of their use for irrigation. The world's oldest and largest scheme for agricultural irrigation using urban wastewater is in the Mezquital Valley, 50 km north of Mexico City, which has resulted in extensive pollution of the underlying aquifer. The aim of this study was to characterize the populations of subsurface FLA in the Mezquital Valley.

Freshly-drilled subsurface sediment samples and pumped groundwater samples from various depths between 11 – 35 m were screened for the presence of free-living amoebae. All samples contained amoebae with at least eight genera represented. The most commonly encountered genera were *Acanthamoeba* and *Hartmannella*, both of which contain species that are potentially pathogenic to man. It is not known whether these are indigenous forms or have been introduced with the wastewater. Current and future work will investigate the biodiversity and role of amoebae in the deep subsurface.

EM6 Differential screening of PAH-degrading bacteria for groundwater bioremediation

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Polycyclic aromatic hydrocarbons (PAHs) are well known pollutants of both soil and groundwater. In recent years, increasing attention has been paid to biotreatment of contaminated sites rather than the more traditional methods of excavation/removal to landfill and containment on site. One focus of research has been the role of micro-organisms in the degradation of pollutants. Microbes capable of mineralising PAHs in the environment therefore have potential application in the remediation of polluted soil and groundwater.

Bacteria capable of mineralising the PAH phenanthrene were isolated from a site heavily contaminated with fuel oils. Initial studies of their degradative abilities in a mineral salts medium indicated that the bacterium *Achromobacter cholinophagum* was able to degrade phenanthrene at a concentration of 1.3 ppm within 20 days.

It is the purpose of this study to more closely examine the degradative potential of these bacteria in a contaminated aquifer: by comparing degradation rates in mineral salts medium, artificial groundwater and natural groundwater. The degradative ability of bacteria in contact with rock in a range of particle sizes is also being examined as part of this study. Preliminary results of this experiment will also be discussed.

EM7 The geochemistry and geomicrobiology of relict hydrothermal sulphide deposits

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The diagenetic re-mineralisation of seafloor-sulphide deposits and the role of microbes in the metal-exchange processes were investigated in metalliferous sediments from the *Alvin* relict hydrothermal zone in the TAG area at 26°08'N (Mid-Atlantic Ridge). The solid-phase and concomitant pore water concentrations of Fe, S, Si, Cu, Zn, Mn, U and Au were measured in a 230 cm long gravity core from the southern periphery of the relict vent field. These measurements were complemented by detailed analysis of bacterial abundance and specific activity. The altered sulphidic sediments are capped with a ~30cm thick layer of carbonate-rich (~80% CaCO₃), Fe stained sediments. Two distinct sulphide layers interbedded with Fe-oxysilicates and overlain by a thin layer of Fe/Mn oxyhydroxides were found in this core. The dominant mineral-phase in both sulphide layers, which originate from mass-wasting of mound sediments, is pyrite with some goethite. The presence of secondary atacamite in the upper sulphide layer attests to the reaction of the exposed metal-sulphides with seawater, whereas in the lower sulphide layer Cu occurs primarily as chalcopyrite and chalcocite. Solid phase data and scanning electron microscope (SEM) analysis show dissolution of primary sphalerite in the upper sulphide layer and re-precipitation as secondary sphalerite directly above and below. In the lower sulphide layer, in contrast, pore-water profiles and SEM images indicate re-precipitation of sphalerite as coating on primary sulphide textures. Both U and Au display distinct diagenetic peaks near the top of the upper sulphide layer, whilst no such peaks were observed below. A sharp diagenetic peak of solid phase Mn was observed within the carbonate cap. This is matched by increased levels of dissolved Mn and the presence of Mn-reducing bacteria below this peak, indicating release of Mn at depth and re-precipitation at the redox-boundary. Microbial reduction and oxidation of S has been observed throughout the core, indicating that microorganism are particularly active in terms of S-cycling. High sulphate reduction rates (SRR) directly above the upper sulphide layer are a potential driving force for mobilisation of metals in this layer. At depth, SRR are negligible, thus explaining the contrasting geochemistry of the two sulphide layers. Alternatively, the penetration of seawater into the upper sulphide layer would cause oxidation of pyrites, thus producing acidity with subsequent dissolution of sulphide phases.

0905 Lysis in bacteria

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Spontaneous bacteriolysis normally does not occur. This is due to the presence, in the bacterial cell wall, of the tightly cross-linked polymer peptidoglycan (murein), which forms a covalently closed, bagshaped structure, called sacculus. However, autolysis may result from either a flaw in the proper synthesis of the murein sacculus or by the action of murein hydrolases ubiquitously present in bacteria. Murein synthesis inhibitors such as penicillins are among the most efficient and therapeutically widely used antibiotics. By way of contrast, although being extremely powerful antibacterial agents, the endogenous murein hydrolases are not fully exploited for inducing bacteriolysis. Yet, bacteria are filled with a wealth of different murein hydrolases of different specificities. As a matter of fact, in the case of *Escherichia coli* one or even more specific enzymes have been found including endopeptidases, carboxypeptidases, endo- and exo- glucosaminidases and muramidases for each type of covalent bond in the murein sacculus. The cellular regulatory systems that obviously very efficiently control these potentially suicidal enzymes (autolysins) are still far from being understood. However, the more we learn about the control mechanisms, the more experimental handles we can obtain that allow us to induce autolysis of growing cultures by triggering the endogenous murein hydrolases.

0950 Yeast autolysis: mechanism and interest - focusing on the lactic yeast *Kluyveromyces marxianus* var. *marxianus*

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For many years, a lot of data dealing with *Saccharomyces cerevisiae* autolysis have been reported : when this process was induced by energy source deficiency and thermal shock it seemed rather slow, but its rate could be increased by means of chemical inductors. It has been proven that the first step of *S. cerevisiae* autolysis was a disturbance of supramolecular intracellular structures (mainly cytoplasmic membrane), but cell-wall continuity was preserved.

Among the few lactose-metabolizing yeasts, *Kluyveromyces marxianus* var. *marxianus* seemed especially interesting since its autolysis has proven to be faster than *S. cerevisiae* one, the autolyzate was an attractive substitute for standard yeast extract for starter cultures, and it contained high valuable intracellular molecules like enzymes and B group vitamins. It has been demonstrated that the critical step of the process, consisting in the biosynthesis of lytic enzymes, is under tight control of the conditions of the prior culture.

Compared to that of *S. cerevisiae*, the bibliography about *K. marxianus* autolysis appeared less abundant. However, the enzymatic activities and the release kinetics of low molecular products have been modelled. It has also been demonstrated that the mechanism proposed for *S. cerevisiae* autolysis appeared also likely for *K. marxianus*.

In conclusion, among the number of physical, chemical and enzymatic methods for disrupting or weakening yeast cell walls, autolysis is the only method that appears practical on an industrial scale.

1100 Assessment of bacterial viability and permeability

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Fluorescent staining methods developed in our laboratories have led to a functional classification of the physiological state of individual bacterial cells based on reproductive activity, metabolic activity and membrane integrity. The multi-colour staining approach has improved our understanding of various dye properties (Nebe-von Caron *et al*, 1995) and has also highlighted the interference of dye efflux systems, a major cause of the erroneous interpretation of the mode of action of many fluorescent stains. Efflux systems relying on non-specific proton antiport pumps (Midgley 1986) can be harnessed and a deliberate measurement and can be used to discriminate for cells generating a proton gradient. Used in combination with membrane potential and membrane integrity stains this allows the simultaneous differentiation of four functional sub-populations in bacterial populations. It is therefore possible to resolve on individual cells physiological state beyond culturability, based on the functionality of these metabolic pumps and the presence or absence of an intact polarised cytoplasmic membrane, enabling assessment of population heterogeneity. Importantly, the reproductive growth capacity of intact cells can be demonstrated by cell sorting (Nebe-von Caron *et al*, 1998).

Such multi-staining flow cytometric techniques have been used for the 'at-line' study of bacterial fermentations (Hewitt *et al* 1998). It has been shown during the latter stages of small scale (5L), well mixed fed-batch fermentations that there is a considerable drop in cell viability, about 20%, as characterised by cytoplasmic membrane depolarisation and permeability (Hewitt *et al* 1999). These phenomena are thought to be due to the severe and steadily increasing stress associated with glucose limitation during the fed-batch process. Such severe effects are not found in either batch or continuous culture fermentations. The possibility of using these findings for improved process control using 'on-line' flow cytometry will be discussed.

Hewitt C. J., Nebe-von Caron G., Nienow A. W. and McFarlane C. M. (1999). The use of multi-staining flow cytometry to characterise the physiological state of *Escherichia coli* W3110 in high cell density fed-batch cultures. *Biotechnology and Bioengineering*, (In Press).

Hewitt C. J., Boon L. A., McFarlane C. M and Nienow A. W. (1998) The use of flow cytometry to study the impact of fluid mechanical stress on *E. coli* during continuous cultivation in an agitated bioreactor. *Biotechnology and Bioengineering*, Volume 59, No. 5, pp 612-620.

Midgley, M. (1986) The phosphonium ion efflux system of *Escherichia coli*: relationship to the ethidium efflux system and energetic studies. *J. Gen. Microbiol.* 132 (Pt 11), 3187-3193.

Nebe-von Caron, G. and Badley, R.A. (1995) Viability assessment of bacteria in mixed populations using flow-cytometry. *Journal of Microscopy-Oxford* 179, 55-66.

Nebe-von Caron, G., Stephens, P. and Badley, R.A. (1998) Assessment Of Bacterial Viability Status By Flow Cytometry And Single Cell Sorting. *J. Appl. Microbiol.* 84, 988-998.

1145 Detection of autolysis in *Penicillium Chrysogenum*

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An industrial strain of *P. chrysogenum* was cultivated in batch, fed-batch and continuous submerged fermentation processes. Autolysis (enzymatic self degradation) was studied in the processes either as a naturally occurring phenomenon, towards the end of batch and fed batch time courses, or as a result of a variety of imposed nutrient starvation regimes in all modes of cultivation. Autolysis was monitored by following previously described indicators for the process - typically, biomass decline and ammonia release (the quantifiable consequences of enzymic degradation of cellular materials). In addition, profiles for the activity of key enzyme classes (proteases and glucanases) were obtained and new image analysis methods were developed for quantification of autolytic degradation in hyphal compartments. The key findings from the study were:

1. The extent of autolysis appears to be dependent on the nature of the nutrient limitation.
2. The processes of cell death and autolysis are distinct.
3. Autolysis in *P. chrysogenum* involves a succession of protease activities.
4. The process of autolysis may be followed using image analysis software.

By using a variety of methods, a greater understanding of the physiology of autolysis has been gained. Knowledge of the triggers and steps involved in fungal autolysis may serve as a starting point from which to develop control strategies to minimise the occurrence of autolysis in large scale fermentation processes.

1400 Autolysins of lactic acid bacteria and cheese manufacture

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Abstract not submitted

1445 Bioprocess monitoring at the speed of light

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Near infra-red spectroscopy (NIRS) is a rapid, non-destructive, technique requiring no sample preparation, which can monitor concentrations of several chemical species simultaneously. Monitoring typical bioprocesses by means of NIRS is more challenging than for simpler chemical reaction processes due to the complexity of the chemical matrix and to the pronounced changes in concentrations of reactants, products and by products with time. The application of NIRS for monitoring the cultivations of *Pichia pastoris* and *Streptomyces fradiae* was investigated. At-line models have been built for carbon sources, biomass and other crucial analytes. Various chemometric and validation approaches have been used to optimise these models and assess their performance in practical situations. Correlation coefficients of 0.95 and above have been

achieved for validation of models both internally and externally. Monitoring of biological parameters in bioprocesses by on-line / *in-situ* means is highly desirable since it can lead to significant improvements in process control. The development of these models is an important milestone towards this application. The ultimate aim would be to incorporate on-line NIRS allowing real time monitoring of biological parameters within a computer based expert system.

1545 Micromanipulation measurements of cell mechanical properties

COLIN THOMAS

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Micromanipulation is a relatively novel technique for characterising the mechanical properties of microscopic particles, including cells. As such it has the potential to provide data on the weakening of cells caused by nutrient exhaustion and the onset of autolysis.

General aspects of the method will be described, and some data will be presented on the effects of the strength of hyphae of *Saccharopolyspora erythraea* following nitrogen and carbon depletion.

1605 Use of lysis for cell disruption in bioprocessing

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Abstract not submitted

POSTERS:

FB1 Oxidative stress response in submerged cultures of a recombinant *Aspergillus niger*

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Oxygen plays a central role in submerged liquid fungal bioprocesses, however, the nature of oxygen is dual, it represents both an essential requirement as well as a potential threat. A recombinant strain of *Aspergillus niger* (B1-D), engineered to produce the marker protein hen egg white lysozyme, was chosen as a model strain to investigate fungal susceptibility to oxidative stress in submerged culture in bioreactor systems. The culture response to oxidative stress, produced either by addition of exogenous hydrogen peroxide or by high dissolved oxygen tensions has been examined in terms of the activities of two key defensive enzymes: catalase (CAT) and superoxide dismutase (SOD). Batch cultures in the bioreactor were generally found to have maximum specific intracellular activities of CAT and SOD in the stationary/early decline phase. Continuous addition of H₂O₂, starting in the early exponential phase, induced CAT but did not increase SOD significantly. Gassing an early exponential phase culture with O₂ enriched (25 vol%) air resulted in increased activities of both SOD and CAT relative to control processes gassed continuously with air. The profile of the specific activity of SOD appeared to correlate with dissolved oxygen levels in processes where no hydrogen peroxide addition occurred.

FB2 Fungal autolysis and programmed cell death in *Penicillium chrysogenum*

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The process of fungal autolysis in freely dispersed hyphal elements was examined using image analysis in addition to the agarose gel DNA fragmentation assay commonly used to distinguish between apoptotic and necrotic cell death. Autolysis in batch bioreactor cultures were also assessed in terms of biomass decline, changes in nutrient concentration and off gas analysis. Two batch processes, with high and low concentrations of the toxic penicillin G precursor phenylacetic acid (PAA) were compared in terms of the percentage of autolysed regions in freely dispersed hyphae, and the profiles of intracellular DNA fragmentation. The proportion of autolysed regions, as measured by the image analysis method, increased with time in both processes, although autolysis was more widespread in the process with high PAA levels. DNA fragmentation in this process occurred in an ordered, apoptotic manner, i.e. discrete DNA bands were observed in the gel, whilst the process fed with low concentrations of PAA showed smeared DNA on gels indicating non-specific exonuclease activity, characteristic of necrotic cell death. This study further associates micro-morphology of hyphal autolysis in *P.chrysogenum* with intracellular changes occurring during the ageing process, and perhaps in response to stress, via high levels of PAA addition.

FB3 Effect of oxygen starvation on autolysis in batch and chemostat cultures of an industrial strain of *Penicillium chrysogenum*

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An industrial strain of *P.chrysogenum* was subjected to short periods of oxygen starvation from between 1 and 5 h in both batch bioreactor cultures, and, glucose and nitrogen-limited chemostat cultures. The effects on fungal autolysis were assessed using previously described indicators of autolysis (McNeil *et al.*, 1998), with micro-morphological changes quantified using an image analysis. Despite previous reports that O₂ starvation was a likely contributory factor bringing about the onset of autolysis, levels of the indicators of autolysis were modest in this study both during and following periods of anoxia. Indeed, cultures remained active at a reduced rate following anoxia in batch cultures, and recovery from anoxia was found to be dependent on the nature of the nutrient limitation previously imposed in chemostat systems. The possible recovery of cultures from periods of anoxia was examined by inoculating aliquots of the bioreactor culture into shake flasks with complete medium. Cultures recovered readily after 1 h O₂ starvation in batch culture, while no recovery, or autolysis, was observed after > 3 h of O₂ starvation in any nutrient limited chemostat system. This study suggests that the processes of cell death (cessation of metabolic activity) and cellular autolysis are distinct phenomena.

FB4 Side chain cleavage of progesterone to produce androstenes by *Bacillus sphaericus*

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In recent years the demand for steroid drugs has increased. The increase is likely to continue because of the expanding demands of steroids as contraceptives, corticosteroids, anticancer and geriatric drugs.

Society for General Microbiology – 144th Meeting - Leeds – 7-10 September 1999

The present project involves the microbial production of steroids which is designed to investigate the side chain cleavage of the sterols to produce androstenedione and androstadienedione which can be used as precursors to a variety of pharmacologically important steroids. This is being achieved by the use of different microbial strains isolated from vegetable oil industry waste and progesterone as the raw material.

The aim of the project is to screen the microbial strains isolated from the vegetable oil industry waste on the basis of their capability of transformation of sterols and their subsequent conversion to C-19 steroids, purification of side chain cleavage enzyme and cloning the gene responsible for biotransformation. Based on the results of the preliminary investigations strain 2 was chosen for the further study because of its capability of *in vitro* and *in vivo* side-chain cleavage of sterols/ steroids.

FB5 Diversion of carbon flux through PEP carboxylase increases flux to glutamate production

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Metabolic modeling of carbon flux through the enzymes of the central metabolic pathways employed by *Corynebacterium glutamicum* for the production of glutamate during growth on glucose revealed that PEP carboxylase enjoys a relatively high 'flux control coefficient' and as such may be considered as 'rate controlling'. Further analysis revealed that increased gene dosage (overexpression) of PEP carboxylase was accompanied by increases in the intracellular level of α -ketoglutarate, the immediate precursor for glutamate biosynthesis, and as such is a legitimate target for metabolic intervention in order to maximise flux to glutamate production in industrial fermentation.

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Joint with THE PATHOLOGICAL SOCIETY
FOOD-BORNE INFECTIONS AND INTOXICATIONS
Wednesday 8, Thursday 9 & Friday 10 September 1999

0905 Overview of food-borne infections in the UK

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In 1998 over 90,000 cases of food poisoning were recorded in England and Wales. The incidence of food poisoning has risen steeply in the last 10 years and the possible reasons for this will be presented.

Laboratory diagnosed gastro-intestinal infections caused by food-borne organisms are indicative of the major trends. The figures for 1998 indicate that the number of *Campylobacter* infections have risen to almost 60,000 whereas those caused by *Salmonella* (23,400) and *E.coli* O157 (887) are lower than in previous year. Epidemiological typing of these three Genera has revealed changes in the distribution of the major "types" that caused infections during 1998. The possible implications of these changes will be discussed.

The incidence of outbreaks and cases associated with other food-borne pathogens, such as *Staphylococcus aureus*, *Clostridium perfringens*, *Bacillus spp* and *Listeria monocytogenes* will be reviewed. Data from food-borne outbreaks also assists in risk assessments by identifying potential vehicles of infection, risks associated with the setting/location, attack rates and the severity of illness.

Infection with viruses and enteric parasites may also be food-borne. The emergence and significance of these less traditional food-borne pathogens will be highlighted. The PHLS Food Hygiene Laboratory (FHL) also investigates and confirms food-borne outbreaks of *Scombrototoxin* poisoning, *Haemagglutinin* poisoning (red kidney beans) and shellfish poisoning. Data from the PHLS FHL will be reviewed.

Throughout this lecture examples of incidents and outbreaks will be presented to illustrate specific issues and emerging problems.

0950 Understanding pathogenic mechanisms of foodborne pathogens

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Basic understanding of the pathogenic mechanisms of foodborne pathogens is essential to the enhancement of public health. Information on pathogenic mechanisms is important to the development of: (1) intervention strategies at production and for humans, (2) treatments for infection/intoxication, (3) improved methods for detection and isolation, and (4) risk assessment and analysis for regulatory rulemaking. Using as an example enterohemorrhagic *Escherichia coli* (EHEC), attempts are being made to: (1) develop interventions through vaccines that incorporate identified virulence factors of EHEC such as intimin to reduce carriage of *E. coli* O157:H7 by cattle and protect humans from infection; (2) provide for infected patients treatments that neutralize or prevent attachment of a critical virulence factor such as Shiga toxin; (3) identify and genetically characterize virulence markers that would enable rapid detection of EHEC in foods; and (4) determine virulence indicators that are unique to EHEC strains which are human pathogens to enable differentiation from Shiga toxin-producing *E. coli* which are not harmful to humans. Considering the frequent occurrence of *Listeria monocytogenes* of a variety of serovars in foods and the predominance of only specific serovars associated with human listeriosis, further characterization of the pathogenic mechanisms and virulence factors of *L. monocytogenes* could enable regulatory agencies to develop more specific policies targeted to strains responsible for abortion and meningitis. Identification of the virulence plasmid of *Yersinia enterocolitica* has provided a scientific basis for differentiating the widely distributed avirulent strains of *Y. enterocolitica* from the rather uncommon isolates of pathogenic *Y. enterocolitica*.

1050 **Campylobacter: pathogenesis**

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Campylobacter jejuni, along with a number of closely related species (e.g. *C. coli*, *C. lari*), is the major cause of human enterocolitis and diarrhoea and poses an increasing clinical and economic problem. There are probably about 500,000 infections per year in England and Wales, of which about a tenth get reported. Recent estimates indicate the costs of *Campylobacter* disease run to several tens to hundreds of millions of pounds a year. *C. jejuni* causes a predominantly food- (particularly poultry-) borne illness to which humans seem particularly susceptible. In other animals, it often seems to act as a commensal. Despite considerable advances, it not clear what is the basic mechanism(s) by which it causes disease and whether isolates differ in this respect. It is also not clear what are the determinants of its persistence outside of the host and whether isolates differ in these also. These aspects need resolving since they both contribute to the disease-causing potential of specific strains. Putative virulence attributes for *Campylobacter* have been described, such as the flagella and motility, and some relatively poorly characterised adhesins. These include, lipooligosaccharide, several membrane proteins and possibly fimbriae. Other proposed virulence determinants are toxins, especially cytolethal distending toxin (CDT), possible capsular material, and the ability of the organism to invade and translocate across intestinal epithelial cells. It is also possible that different strains of *Campylobacter* use different pathogenic mechanisms, in a fashion analogous to the different pathovars of *E. coli*. Strains of *Campylobacter* clearly differ in their ability to cause disease, as evidenced by the broad spectrum of illness observed. Strains also differ in gut colonisation and tissue invasion ability in animal models such as SCID mice. Further, different strains of *Campylobacter* invade into, and translocate across, monolayers of the enterocyte-like cell line, Caco-2, with very different efficiencies, with their invasion ability not correlated with translocation. This suggests that stains differ considerably in their epithelial cell interactions. We have also shown that CDT is probably the single major toxigenic molecule produced by *C. jejuni* and that it may contribute to intestinal invasion in a SCID mouse model. Further work on the identification of the pathogen's survival and virulence determinants will be greatly facilitated by the recent completion of the genome sequence by the Sanger Centre. Indeed, the availability of the sequence has already significantly improved our abilities to identify protein homologues in proteomic approaches to identify survival and virulence determinants. Much more work, however, is needed to better define *Campylobacter* pathogenesis.

1120 **Mucosal cellular responses in the gi tract to Campylobacters**

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We and others have previously reported persistent post-dysenteric bowel dysfunction in 25% of patients, 3 months after bacterial enteritis in spite of normal routine histology. Our aim was to describe, in detail, the changes in mucosal cell populations during recovery from acute *Campylobacter* enteritis and to relate this to mucosal injury, recovery and functional disturbance. This is an area of relatively little research, due to the difficulties of follow-up of patients and a low proportion consenting to invasive procedures.

Patients with a positive *Campylobacter* stool culture, isolated by the PHLS, were approached to take part in this study. Rectal biopsies were taken at initial presentation, 6, 12 and 52 weeks and compared with a normal control group. Assessments of gut permeability, and stool lactoferrin and interleukins were made. Biopsy specimens were stained immuno-cytochemically for immune cells including lymphocyte populations, NK cells, mast cells and macrophage populations; nerve fibres, enteroendocrine cells, and mucosal neuro-peptides. Marked changes in all cell populations were observed lasting up to 12 months in some situations. *Campylobacter* phenotype studies were also undertaken on the original isolates.

In this communication an overview of the results of this novel prospective follow-up investigation will be presented. These results will be put into the context of current perspectives of acute bacterial mucosal damage and recovery. The marked cellular changes seen in the acute stage, move into a prolonged resolution phase with a characteristic pathology, this being difficult to recognise by conventional means. These changes present several possible pathways by which acute bacterial enteritis might evolve into prolonged post-dysenteric bowel dysfunction, whose symptoms may persist for many months or years.

1150 **Listeria monocytogenes: intracellular life cycle and host cell responses**

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Listeria monocytogenes is a gram-positive facultative intracellular bacterial pathogen that causes severe diseases in both humans and animals and which was shown to invade many different mammalian cell types *in vitro* and *in vivo*. Upon uptake by mammalian cells, *L. monocytogenes* rapidly lyses the phagosomal membrane and gets access to the host cell cytoplasm where extensive intracellular replication takes place. Intracellularly growing *L. monocytogenes* then induces the polymerisation of host cell actin filaments at one pole of the bacterium which allows intracellular movement and cell-to-cell spread without leaving the cytoplasm. Invasion of endothelial tissues may be crucial in a *L. monocytogenes* infection leading to meningitis and/or encephalitis. We used human brain microvascular endothelial cells as a model system to study the interaction of *L. monocytogenes* with endothelial cells and show that *L. monocytogenes* invades these endothelial cells in an InlB-dependent and wortmannin-insensitive manner. Heterologous plaque-assays with *L. monocytogenes*-infected P388D1 macrophages as vectors demonstrate efficient spreading of *L. monocytogenes* from macrophages into endothelial, fibroblast, hepatocyte, and epithelial cells. Upon infection of macrophages which are important host cells for *L. monocytogenes*, a rapid activation of the transcription of proinflammatory cytokine genes and a repression of the transcription of cytokine receptor genes is observed. Additionally, *L. monocytogenes* infection of macrophages results in a biphasic activation of the mammalian transcription factor NF- κ B which is triggered by listerial lipoteichoic acid and the expression of the listerial phospholipases PI-PLC and PC-PLC. In a recent approach to further analyse the interaction of *L. monocytogenes* derived proteins with mammalian proteins we used the yeast two-hybrid system to search for cellular binding partners for listerial virulence factors. The identification and characterization of a new ActA binding protein will be discussed.

1445 **Salmonella survival responses: new insights from proteomics**

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Survival of *Salmonella* spp. in food and in the host is a remarkable feat requiring the coordinated expression of diverse molecular components at appropriate times and locations. As it passes through the food chain, *Salmonella* encounters specific

stresses including fluctuations in pH, temperature and osmolarity as well as exposure to potentially deleterious agents such as digestive enzymes, chaotropes, reactive oxygen species and antimicrobial peptides. Until recently, however, systematic investigation of bacterial responses to such factors has been largely restricted to reporter gene fusion approaches. While such studies have been extraordinarily useful in identifying some of the components involved, they give little information on post-transcriptional events and generally cannot identify genes essential for cell viability that also are involved in adaptive responses. We are therefore using proteomics, the systematic analysis of the protein expression profiles of cells grown in different conditions, to define *Salmonella* responses to various stresses.

The talk will focus on the identification and regulation of overlapping sets of *Salmonella* proteins involved in stress adaptation. For example, in exponential phase cells, proteins such as phase-1 and phase-2 flagellins are down-regulated in response to low pH but not to oxidative stress whereas others, such as the periplasmic proteins glycerophosphoryl diester phosphodiesterase and D-galactose binding protein, are down-regulated in response to both conditions. All of these proteins are also repressed upon activation of the PhoPQ signal transduction system, consistent with its role in regulating responses to low pH. Still other proteins, such as a novel homologue of alkyl hydroperoxidase reductase, are repressed only in response to oxidative stress and are unaffected by low pH or the PhoPQ system. In keeping with these results, prior induction of acid tolerance causes increased resistance to hydrogen peroxide although the converse is not the case. The challenge now is to provide accurate biological explanations for the complex and sometimes enigmatic responses observed at the proteomic level. Ultimately, this information will provide an integrated understanding of the survival strategies used by this versatile pathogen.

1515 *Salmonella*: molecular epidemiology

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For the epidemiological investigations of *Salmonella* infections the policy of the Laboratory of Enteric pathogens is to use a hierarchical approach based in the first instance on phenotypic methods followed by molecular subtyping using a range of DNA-based techniques. Phenotypic methods include serotyping, phage typing and antibiogram analysis; DNA-based methods include plasmid typing and more recently, a range of techniques targeted at identifying chromosomal polymorphisms. The latter methods include DNA/DNA hybridisation-based methods such as ribotyping and insertion sequence (IS) 200 fingerprinting, targeted at specific genes or insertion sites, and pulsed-field gel electrophoresis (PFGE) following enzymatic digestion to provide a macrorestriction fingerprint of the whole genome. A range of PCR-based methods using non-stringently hybridising (e.g., RAPD) or linker-mediated stringently hybridising primers (e.g., AFLP) are also in use or are being evaluated. Molecular fingerprinting using PFGE has become increasingly used over the last decade and is now regarded as the "gold standard" for epidemiological studies which require DNA-based fingerprinting for enhanced surveillance. In developed countries PFGE has been particularly successful in outbreaks caused by rare or unusual serotypes such as *S. agona*, *S. anatum* and more recently *S. java* associated with imported coconut but less so for strains such as *S. enteritidis* PT4 or multiresistant *S. typhimurium* DT 104. In such strains the epidemiological picture has been dominated by the spread of a small number of closely related clones in food-producing animals and humans. Particularly for *S. typhimurium* DT 104 the identification of small areas of heterogeneity in the bacterial chromosome often associated with differences in antibiogram have been important in outbreak investigations. In contrast, in developing countries the identification of "epidemic" plasmids common to several serotypes or phage types and of integrons coding for resistance to several antimicrobial drugs have been important in elucidating the epidemiology of salmonella strains causing outbreaks in humans over wide geographical areas. In this presentation the applicability of current DNA-based molecular methods for outbreak investigations will be assessed and the applicability of new sequence-based methods will be evaluated.

1610 *Salmonella* SIPs, SOPs, SPIs and pathogenesis

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The nature and severity of salmonellosis is dependent on the combination of the infecting *Salmonella* serotype and target host species. Some *Salmonella* serotypes have a broad host range (e.g. *S. typhimurium*) and are typically associated with the enteric form of disease. Other serotypes typically have a narrow host range (e.g. *S. gallinarum*, chickens; *S. abortusovis*, sheep; *S. choleraesuis*, pigs and *S. dublin*, cattle) and are typically associated with systemic disease. The virulence factors influencing enteropathogenesis and serotype host specificity remain poorly characterised. The interactions of the above serotypes, (using strains of defined virulence for cattle), with bovine ileal mucosa were characterised in an ileal loop model that allows the quantification of invasive and enteropathogenic phenotypes. *S. dublin* and *S. gallinarum* were equally invasive for ileal mucosa with or without Peyer's patches, and were significantly more invasive than *S. choleraesuis* and *S. abortusovis*. This observation suggests that the magnitude and route of intestinal invasion is not mediating *Salmonella* serotype host specificity. The magnitude of the enteropathogenic response did not correlate with oral virulence. *S. typhimurium* and *S. gallinarum* were significantly more enteropathogenic than the other serotypes, indicating serotype specific factors influence entero-pathogenesis. Thus factors influencing *Salmonella* serotype/host specificity appear to act at a stage in pathogenesis beyond the intestinal mucosa and await clarification. Disruption of genes in *Salmonella* Pathogenicity Island (SPI) 1 blocked the secretion of *Salmonella* Invasion Proteins (Sips) and *Salmonella* Outer Proteins (Sops). Such mutants were significantly less invasive for intestinal mucosa *in vivo* and significantly less enteropathogenic. Disruption of *sopB* and *sopD* significantly reduced enteropathogenesis without influencing intestinal invasion and these two genes appear to act in concert. Surprisingly, disruption of *stm*, a gene cloned from *S. typhimurium* on the basis of its homology to cholera toxin did not influence enteropathogenesis. *SopB* was mapped to the 20 centisome of *S. typhimurium* and is flanked by 5 genes that are organised in a manner typical of a pathogenicity island, which we have termed SPI-5. Mutation of the other genes in SPI-5 also attenuated enteropathogenesis but not virulence for mice, suggesting SPI-5 is a key locus specifically influencing *Salmonella* enteropathogenesis.

1640 *Salmonella* carriage and the carrier state: persistence of *Salmonella* within avian splenic macrophages

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Salmonella enteritidis and *S. pullorum* have been shown to persist within the spleen and reproductive tissue of the chicken without causing disease in the host animal, but still may be transmitted from the host, notably via eggs. The cell types involved in carriage and the reasons for the absence of immune clearance are not known. Therefore a model of infection using *S. pullorum* containing a plasmid constitutively expressing Green Fluorescent Protein (GFP) was developed to allow detection of *S. pullorum* in cells and tissues. Initial experiments using flow cytometry (FACS) and immunofluorescence were unsuccessful due to the low numbers of persisting bacteria. Therefore on the basis that *Salmonella* typically localise within macrophages, persistence of *S. pullorum* within macrophages was determined by the adherent properties of macrophages. Cell homogenates were prepared from the spleens of infected birds and incubated in tissue culture plates for 4 hours to allow cell

adherence. Intracellular bacterial counts after incubation in media with gentamicin was determined for both the adherent and non-adherent cells portions. *Salmonella* were found to persist in the adherent cells, comprising mainly of macrophages and dendritic cells, for several months post infection, but were not found to persist in non-adherent cells. *Salmonella* expressing GFP could be visualised within splenic cells of macrophage-like morphology by fluorescence and confocal laser microscopy. This suggests that carriage of *Salmonella* in the chicken spleen is primarily within macrophages.

1655 *Salmonella* secreted proteins and the Enteropathogenic response

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The ability of enteropathogenic salmonellae to recruit inflammatory cells and induce secretory responses in the infected ileum is considered to be a main feature in *Salmonella*-induced enteritis. Interactions between the pathogen and intestinal epithelial cells result in a variety of cellular responses mediating inflammation and fluid secretion. It is apparent that proteins secreted by the Inv/Spa type III secretion system of *Salmonella* play a key role in the induction of these responses.

We demonstrated that at least three *Salmonella* protein effectors are involved in the induction of the inflammatory responses. The protein effectors SopB and SopD are both translocated into eukaryotic cells via a Sip-dependent pathway and mediate inflammation and fluid secretion in infected ileal mucosa in a concerted manner.

In this work we have identified the *Salmonella* protein effector SopA. This is involved in trans-epithelial migration of neutrophils and the induction of fluid secretion in intestinal mucosa.

Thus, SopA, SopB and SopD are all involved in the induction of the enteropathogenic responses during infection. This is an example of specific events in the pathogenicity of *Salmonella* being operated by multiple effectors with complimentary or synergistic effects.

This work was supported by the BBSRC.

0910 BSE/nvCJD: general overview on prion diseases

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Abstract not submitted

0940 Small round structured viruses

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Small round structured viruses, also known as Norwalk-like viruses (NLV), are the commonest cause of foodborne viral infections in the UK. Electron microscopy has been the mainstay for diagnosis with the development of alternative assays being severely hampered by our inability to culture these viruses in the laboratory. NLVs predominance as the most important foodborne viral infection is in part due to their ability to cause infection at any age, both as a result of their antigenic diversity and the short term immunity induced by these agents. NLVs also appear to be highly stable in certain environments.

There are two important means of transmission of NLVs to food. First, the contamination of bivalve molluscs that have sequestered virus from sea water during filter feeding which are then eaten raw. Second, the contamination of food during preparation by infected food handlers which is not then subject to further cooking. This latter route is numerically more important and, overall, NLVs are associated with about 6% of foodborne outbreaks in England and Wales.

This decade has seen an explosion of research into these viruses fuelled by the initial sequencing of Norwalk virus (NV) and the observation of the spontaneous formation of virus-like particles by baculovirus expressed NV capsid protein. These have led to the development of molecular based approaches for the detection of NLV RNA and viral protein in clinical and environmental samples (including food and water). Having overcome many difficult technical problems the application of these assays are beginning to improve our understanding of the epidemiology and biology of this important group of viruses.

1010 *Mycobacterium paratuberculosis* and milk

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Mycobacterium paratuberculosis is the causative agent of Johne's disease in cattle, sheep, goats and other ruminants, and may also be implicated in the aetiology of Crohn's disease in humans, although evidence supporting a link between *M. paratuberculosis* and Crohn's disease remains controversial. The two diseases share similar clinical symptoms, and both are chronic inflammatory diseases of the gut, frequently affecting the young. If *M. paratuberculosis* is implicated in Crohn's disease then milk is a potential vehicle of transmission of the organism from animals to humans.

M. paratuberculosis is the slowest growing of the cultivable mycobacteria. It has a growth requirement for mycobactin J, an iron-chelating compound produced by other mycobacteria, and can require up to 18 weeks incubation at 37°C for primary isolation, although an established culture will grow within 4-8 weeks. No selective culture medium is available for *M. paratuberculosis* and, consequently, isolation of this organism from milk is reliant upon adequate chemical decontamination of the milk sample prior to culture in order to inactivate other non-acid-fast organisms present. *M. paratuberculosis* may infect milk as a result of intra-mammary and extra-mammary (faecal) contamination. Little information is available about the numbers of *M. paratuberculosis* likely to be present in milk from an infected animal. Results of laboratory pasteurisation studies employing artificially spiked milk indicate that if the organism is present in milk in sufficient numbers (>100 cfu/ml) then it may not be completely inactivated by heating milk at 72°C for 15 s (HTST pasteurisation). Evidence suggests that it is the natural tendency for *M. paratuberculosis* cells to exist in clumps that may aid the survival of some cells during heating. A milk survey is currently being carried out to determine the incidence of viable *M. paratuberculosis* in raw and pasteurised cows' milk from throughout the UK. Chemical decontamination prior to culture, and a novel immunomagnetic PCR assay developed at Queen's University, are being used to detect the presence of *M. paratuberculosis* in 50 ml samples of milk. It is hoped that this milk survey will establish whether there is any possibility that humans are being exposed to viable *M. paratuberculosis* by consuming commercially pasteurised milk.

1110 Low temperature survival of *Campylobacter jejuni* in the plateable and non-plateable states

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Survival of the food poisoning bacterium *Campylobacter jejuni* outside the natural host will influence the reservoir of infection and the potential for infectivity. Work from a number of laboratories has shown that both temperature and gas atmosphere affect the rate of conversion of spiral cells to non-viable coccoid cells and also the rate of loss of plating ability. However evidence for 'resuscitation' of cells that have become non-plateable either by *in vitro* incubation in/on microbiological media or by passage through animal hosts is contradictory. We have examined the survival and resuscitation of *C. jejuni*, strain 81116. During a short period (3.5d), the plating ability of 4 °C shocked cells was lost but cells could be resuscitated by culture in fresh broth. Entry into the non-plateable state correlated with an increase in calcofluor whitestaining. Further incubation resulted in loss of cytoplasmic integrity, indicated by increased propidium iodide staining.

The response of *C. jejuni* to cold shock appears to be passive in that cold-shocked cells showed little or no *de novo* synthesis of proteins as judged by 2-dimensional gel electrophoresis. We propose a model in which plateable spiral cells change to non-plateable, non-resuscitable coccoid cells by a number of distinguishable stages.

1125 Photoquant™ - a novel internal standardisation method for ATP bioluminescence assays

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The use of ATP bioluminescence for the rapid detection and quantification of contaminant micro-organisms or substances is routinely used, especially in the food and beverage industries. Since the method is enzyme-based particular environmental factors and chemicals can have an effect on the luciferase activity and stability. It is therefore important to calibrate or standardise the assay. The use of caged ATP, an inert ATP analogue, has been validated as an internal standard. Photoquant™ enables the release of a specific amount of ATP upon exposure to a flash of light.

The luciferase-ATP light emission was assessed in the presence of various potential inhibitors using Photoquant™ and Biotrace Clean-Trace™ devices. Light output was quenched in the presence of two QAC based cleansing solutions, a household cleaner and an alkaline foam cleanser. Whereas an acid foam cleanser caused quenching effects at high concentrations and enhancement at lower concentrations. Quenching effects due to alcohol and colour were also assessed during the validation process. Photoquant™ was more reliable than current ATP standard solutions as pipetting and dilution steps are avoided.

1400 *Escherichia coli* 0157 and other VTECs in the UK

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Abstract not submitted

1430 Pathogenesis of Verotoxin (VT) - producing *E. coli* (VTEC) infection

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The two major virulence features of VTEC are VT-production and specific bowel colonization mechanisms. Hemolytic uremic syndrome probably results from a specific receptor-mediated systemic action of the VTs on microvascular endothelial cells in the kidneys and other tissues. Following internalization by receptor-mediated endocytosis, and subsequent processing, the VTs cause cytopathology by inhibiting protein synthesis. Cytokines may be involved in potentiating toxin action through induction of the cellular receptor, Gb3. Diarrhea due to VTEC O157: H7 and other EHEC serotypes is associated with a characteristic attaching and effacing (AE) pathology in enterocytes, as seen with enteropathogenic *E. coli* (EPEC). A ca. 35-kb chromosomal pathogenicity island, the locus of enterocyte effacement (LEE), encodes all the virulence factors necessary and sufficient for the formation of the AE lesion. The LEEs of EPEC and EHEC comprise 41 closely related open reading frames (ORFS) which encode the structural, accessory, and effector molecules of a type III secretion system. The LEE genes are organized into 3 regions with the central region comprising the *eae* gene, which encodes the adhesin, intimin, and the *tir* gene, which encodes the translocated intimin receptor. Downstream of *eae* are the *esp* genes, which encode the secreted proteins EspA, EspB, and EspD, while upstream are many genes (*esc* and *sep*) that are thought to encode the type III secretion machinery that facilitates the transport of the effector molecules to their active sites. The secreted proteins are involved in signal transduction events that lead to actin polymerization and profound cytoskeletal changes that are the hallmark of the AE lesion. Esp A is a component of a filamentous structure ("translocon") which is believed to facilitate the translocation of EspB, Tir, and possibly other proteins from the bacterial cytoplasm into the host cell. The first gene (*Ler*), in the LEE 1 operon, activates transcription of the LEE 2, 3, and 4 operons which, in EHEC, are also activated by quorum sensing. The 92-kb plasmid of *E. coli* O157: H7 (pO157) has been fully sequenced. Encoded by its 100 ORFS are additional putative virulence factors including an enterohemolysin, catalase peroxidase, a serine protease, and a clostridial-type toxin, whose pathogenetic significance is unknown. The contribution of various host (demographics, lifestyle, immunity, and genetic factors) and parasite determinants (infectious dose, toxin types, accessory factors) to disease susceptibility and severity remains to be fully understood.

1540 *E. coli* O157: clinical experiences (the Central Scotland Outbreak)

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The Central Scotland Outbreak of *E. coli* O157 gastro-enteritis (November/December 1996) was centred around a butcher's premises supplying a large area of Central Scotland. Eventually, 2663 symptomatic individuals were investigated, of whom 1198 attended a special out-patient facility. 503 were confirmed as definite cases according to the Outbreak Control Team definition. There were 20 deaths during the outbreak, making it one of the worst ever recorded with respect to mortality.

Retrospective analysis of clinical features of disease and biochemical and haematological parameters during illness in a large cohort of sufferers has allowed important observations with regard to the prognostic value of these factors. The importance of the early levels of serum albumin and the peripheral neutrophil count has been demonstrated. The use of laboratory markers in the monitoring of the clinical status for the development of complications will be presented. A reasonable management plan for individual cases can be evolved and markers of complication development confirmed. A significant association between antibiotic use, particularly with quinolones and poor outcome / the development of complications was recognised. The potential danger of Antibiotic therapy during the illness will be discussed.

Twenty two cases during the outbreak developed clinical and laboratory evidence of Haemolytic Uraemic syndrome and an early decision was taken to treat these aggressively with the relatively untried modality of Therapeutic Plasma Exchange. There was an overall mortality of 31% in this group compared to a historical expected mortality of 87%. The implications for management of cases in the future will be discussed.

1610 Interactions of shiga toxin-producing *Escherichia coli* and StxS with the intestine and its environment

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Escherichia coli O157:H7 is the most common microbial cause of bloody diarrhea (hemorrhagic colitis or HC) in the United States, and a sequela of infection with this organism, the hemolytic uremic syndrome (HUS), can lead to acute renal failure and death. Although O157:H7 is the most frequently isolated disease-associated serotype of Shiga toxin-producing *E. coli* (STEC), other serotypes do cause HC and HUS. In this symposium, we will focus on four aspects of STEC pathogenicity. First, we will present findings that reveal that intimin is the primary adhesin of O157:H7 as assessed by HEp-2 cell adherence assays and by O157:H7 intestinal colonization studies in gnotobiotic pigs and neonatal calves. In corroboration of these observations, we will show that antibody to O157:H7 intimin or its C terminal domain reduces adherence of O157:H7 and related STEC to HEp-2 cells. Second, we will demonstrate that the relative virulence of STEC strains correlates with the type of Stx produced as evaluated in a streptomycin (*str*)-treated mouse oral infection model. Third, we will provide evidence that the most virulent of the STEC isolates in the *str*-treated mouse model are strains that produce Stx2d because this variant of Stx2 becomes more toxic (is activated) when exposed to mouse (or human) intestinal mucus. Fourth, we will describe results that support the premise that activation of Stx2d reflects cleavage of amino acids in the C terminus of the A2 portion of the toxin by intestinal elastase III. We will also speculate as to how such a structural alteration might lead to increased cytotoxic activity of Stx2d for target cells. We will conclude by offering a model as to how various combinations of virulence factors in different subsets of STEC can lead to pathogenicity in the human host.

1640 Mucosal and systemic immune responses to the lipopolysaccharide of *E. coli* O157

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E. coli O157 and other VTECs are now well recognized as being major causes of morbidity and mortality in the young and the elderly. Infections caused by these strains, viz. haemorrhagic colitis and haemolytic uraemic syndrome, appear to be more common in Scotland than the rest of the UK. Because of increased incidence at the extremes of age there is a suggestion that immunity may influence susceptibility to disease, and it is likely that the mucosal defences may be more important than systemic immunity.

We have embarked on a study to investigate these ideas by measuring humoral immunity to virulence determinants of VTECs in healthy controls and convalescent patients. Here we report on our studies of the immune responses to LPS. LPS is recognized as being involved in interactions with host cellular functions including the possibility of a synergistic interaction on the uptake and action of the verotoxin.

The LPS of *E. coli* O157 can be considered as possessing two major antigenic components, the serotype specific O-polysaccharide and the core oligosaccharide. Five different LPS cores have been identified in *E. coli* (R1-R4 and K12), and although similar each can be distinguished serologically because of the arrangement of the terminal sequence of hexoses and hexosamines. All VTECs appear to have R3 LPS cores.

Measurement of antibodies was determined by ELISA on microplates coated with polymyxin complexes of LPSs extracted from *E. coli* O157 LPS or the R3 R-mutant. Normal levels of serum antibodies were determined by testing ca. 900 samples of serum from local blood donors. Mucosal IgA antibodies were measured in saliva and whole gut lavage fluid (WGLF). Controls included a group of healthy volunteers (n=16) and a group of GI patients (who had been lavaged for routine clinical investigations but subsequently shown to be immunologically normal (n=53). Two other "control" groups were slaughterhouse workers (saliva and serum) and healthy hospital workers in Dhaka, Bangladesh (WGLF only). Differences in both IgG and IgA antibodies to the two antigens differed between groups. One of the most striking differences was the raised IgA levels to the R3 LPS in the Dhaka control population compared to healthy controls from Scotland. We suggest that protection may be conferred to VTECs and other enteropathogenic *E. coli* by a raised mucosal IgA response to the LPS, and this is conferred naturally in developing countries

We gratefully acknowledge the Scottish Office Department of Health for funding this project

1655 Human antibody response of patients infected with verocytotoxin-producing *Escherichia coli* to protein antigens encoded on the *LEE* locus

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Sera from fourteen patients, who were culture-positive for Verocytotoxin-producing *Escherichia coli* (VTEC) O157, and twenty control sera from healthy blood donors, were used to examine the antibody response to proteins involved in expressing the attaching and effacing phenotype. The purified recombinant proteins included a conserved region of intimin, EspA, EspB, and three domains of Tir. Ten of 14 patients, but none of the controls had antibodies to the conserved region of intimin. Seven of nine patients and one control serum contained antibodies to EspA, and two of the seven patients and three controls had antibodies to EspB. Five of eight patients' sera and two of the controls contained antibodies to the intimin binding region of Tir, while none of the sera contained antibodies to either of the other two domains of Tir. These results suggest that these virulence factors were expressed in vivo infection and highlight the potential of an intimin-based serodiagnosis test for detection of evidence of infection with Verocytotoxin-producing *Escherichia coli*.

0910 Acid tolerance in *Escherichia coli* O157:H7 and commensal *E. coli*

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E. coli O157 has become a major cause of food poisoning in the last ten years. It is clear that the infective dose of *E. coli* O157:H7 is very low and this has given rise to the view that this organism is unusually tolerant of the stresses that are used by the body as defence mechanisms. Acid tolerance, in particular, has been highlighted as an important physiological characteristic of this organism. Commensal flora and O157:H7 strains exhibit similar levels of acid tolerance. Three main types of tolerance may be distinguished: intrinsic tolerance exhibited by cells in rapid exponential phase growth, slow growth-induced tolerance (both RpoS-dependent and RpoS-independent) and acid habituation. *E. coli* O157:H7 exhibits significant strain to strain variation in each of these traits. The loss of viability of exponential phase cells at pH 3 in citrate-phosphate buffered medium is biphasic. After transfer to pH 3 there is a 4-log reduction in viability within 15-20 min. The surviving population exhibits considerable acid tolerance and persists for >90 min. These cells are sensitive to alcohol and hydrogen peroxide. Filtration and transfer to fresh medium (pH 7) results in rapid establishment of exponential growth and a population of cells that exhibits similar acid sensitivity to the parent culture. The data suggest that physiological variants within the culture play a major role in survival of commensal and O157:H7 strains.

0940 Epidemiology of VTEC in the food chain

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Verocytotoxin-producing *Escherichia coli* infections are often, but not exclusively, associated with the consumption of contaminated food, particularly unpasteurised milk and undercooked beef products. Other sources will be discussed.

In May 1993 a cluster of 7 cases of HC and HUS caused by VT+ *E. coli* O157 infection occurred in a small well defined geographical area of NW Sheffield. Initial epidemiological evidence suggested that these cases may have been linked to consumption of untreated milk from one farm. Using enrichment culture and a newly developed immunomagnetic separation technique (IMS) we isolated VT+ *E. coli* O157 from 10 of 105 milking cows, from one sample of farmyard slurry, and from one of ten milk samples taken from the individual milking jars used for the rectal swab-positive cattle. This was the first isolation of the organism from a suspected food source in the UK.

In surveillance studies at local abattoirs during 1995-1998 we isolated VT+ *E. coli* O157 from rectal faeces of 15.7% of cattle and 2.2% of sheep, but not from pigs or chickens. The monthly prevalence of VT+ *E. coli* O157 in cattle varied from 4.8% to 36.8% and was at its highest in spring and late summer. Seventeen of the 22 isolates from sheep were also made in summer. During the period of this study, clusters of human cases of VT+ *E. coli* O157 infections followed a very similar seasonal distribution to that of the prevalence of the organism in cattle and sheep. Surveillance of food samples in our area is ongoing to determine the prevalence of VT+ *E. coli* O157 in retail beef and lamb products. So far, VT+ *E. coli* O157 has been isolated from 5.9% of lamb products, from 1.5% of beef products and most frequently from lambburgers (7.2%). Work is continuing in an attempt to explain why *E. coli* O157 is apparently more prevalent in lamb products than in beef products in our area, despite a much higher prevalence of the organism in healthy cattle than in healthy sheep.

1010 VTEC: The animal reservoir

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The ecology of verotoxin- (or shigatoxin)-producing *E. coli* (VTEC or STEC) is complex and largely unknown. A great diversity of VTECs exist but only in the case of *E. coli* O157:H7, a common human foodborne pathogen, has sufficient research been done to allow generalizations about the ecology. Yet, scattered evidence does indicate that many, perhaps most, VTEC share the following characteristics with *E. coli* O157:H7: lack of host specificity such that indistinguishable or very similar isolates can be found in a variety of species; an apparent predilection toward ruminants in which some VTECs (including *E. coli* O157:H7) are ubiquitous at the herd level; transient residence in the gastrointestinal flora of individual animals; temporal clustering at the population level such that most total shedding of particular VTECs are confined to sharp bursts of fecal shedding in a high percentage of animals separated by much longer periods of very low prevalence; a much higher prevalence in young animals in comparison to older ones; a higher prevalence in animals with floral disturbance such as that caused by transit, feed changes or antimicrobial dosing; a higher prevalence during warm months; typically asymptomatic transient gastrointestinal carriage (with notable exceptions, including *E. coli* O157:H7 in humans). Molecular epidemiological studies of *E. coli* O157:H7 have demonstrated that subtypes of the organism can persist on cattle farms for years, thus supporting a conclusion that cattle farms represent a reservoir. Yet on such farms, common subtypes are often found in environmental niches and in other species of animals; thus it is not completely clear that cattle themselves are the reservoir. New subtypes are periodically observed on particular farms, and indistinguishable subtypes can be found on farms that are separated by hundreds of kilometers even in the absence of any obvious animal movements between them. The number of subtypes found on a farm does not appear to be qualitatively correlated with cattle movements (e.g., purchases) into the farm. Commercial feeds are sometimes contaminated with *E. coli* O157:H7, and it seems likely that feeds represent an important route of dissemination for this agent and other VTEC. Mixed feeds collected from feeding troughs (i.e., exposed to cattle) are commonly positive for *E. coli* O157:H7, as are cattle watering troughs. This contamination could be incidental, but the higher prevalence of *E. coli* O157:H7 in cattle (and humans) in summer is consistent with environmental replication, which readily occurs in mixed feeds (with > 25% moisture) and water trough sediments at summer temperatures. *E. coli* O157:H7 has been found to persist for at least 4 months in water trough sediments, which leads to the radical hypothesis that the organism could be sustained in an environmental niche rather than an animal reservoir. Traditional means of controlling infectious agents, such as eradication or test and removal of carrier animals, do not appear to be feasible for VTECs.

Nevertheless, certain farm management practices--especially those related to maintenance and multiplication of the agent in feed and water--may provide practical means to substantially reduce the prevalence of these agents in cattle on farms and in those arriving at slaughter plants. The common seasonal pattern of *E. coli* O157:H7 prevalence in cattle and *E. coli* O157:H7-associated disease in humans, which has been repeatedly found in the US and the UK, does suggest that the risk of disease in humans could be impacted by lowering the prevalence in cattle populations.

1115 Laboratory surveillance of vero cytotoxin-producing *E. coli* O157 (O157 vtec) in England and Wales, 1995-1998: comparison of outbreak strains by genotypic and phenotypic methods

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Annual isolations of confirmed O157 VTEC from human sources in England and Wales increased between 1995 and 1998 compared with the period 1992-1994. The largest annual total was 1087 in 1997. The majority (76%) of strains were VT2 and 23.3% were VT1+VT2. The predominant phage type (PT) continued to be PT2 but numbers declined from 54% of the total in 1995 to 31% in 1998. This was accompanied by rises in the isolation of PT8 and PT21/28 to reach 17.5% and 15.5% respectively in 1998. About 12% of total isolates were associated with 68 general outbreaks of infection, of which about 80% were attributed to strains of PT2, PT8 or PT21/28. In some cases strains of the same PT from different outbreaks could be distinguished by their carriage of VT1 and/or VT2 genes or by antibiotic resistance. Generally DNA-based methods such as VT gene subtyping and pulsed field gel electrophoresis (PFGE) were essential for differentiation of outbreak strains of the same phage type. Thus in 11 outbreaks caused by PT8 strains - two strains were different phenotypically whereas all 11 strains were distinct by PFGE of *Xba*I restriction fragments. This approach was important in distinguishing cases of sporadic infection from those that were part of outbreaks and provided further evidence for the association of food vehicles or animals with human disease.

1130 Induction of toxin synthesis and release in clinical isolates of Shiga-toxigenic *Escherichia coli* (STEC) O157:H7 in response to antibiotics

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In order to study the regulation of type 2 Shiga toxin production by STEC, we constructed a *lacZ* transcriptional fusion spanning the A and B subunit genes in the *stx2* operon of a recent isolate of *Escherichia coli* O157:H7. The phenotype of the reporter strain led us to suspect that the toxin was synthesised predominantly during induction of the converting phage that encodes the *stx2* genes. This view was supported by a substantial up-regulation of reporter gene expression when strains were exposed to antimicrobial agents that induced an SOS response (e.g. mitomycin C and 4-quinolones), as observed in agar diffusion /X-gal assays. Subsequent determinations of cell-free and cell-associated β -galactosidase activities in broth cultures showed that induction followed classical λ phage burst kinetics. Similar induction experiments in wild-type strains, including recent isolates from STEC outbreaks, confirmed that the titres of plaque-forming phage and biologically active toxin correlated closely. The combined observations of a substantial ($\sim 10^4$ -fold) drop in colony forming units, cell lysis and phage production in these induction experiments indicate that toxin production occurs primarily in cells committed to phage-mediated lysis. These findings have important implications for the pathogenesis of STEC disease and the spread of *stx* genes.

1145 Identification of the role of Fis in the regulation of virulence in enteropathogenic *Escherichia coli*

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The regulation of virulence gene expression in enteropathogenic *Escherichia coli* (EPEC) is dependent upon a number of factors including temperature, osmolarity, pH, H₂CO₃⁻, Ca²⁺ and NH₄⁺ ions. The regulatory elements involved in the perception of these signals are still poorly understood. In addition, EPEC contains a transcriptional activator PerA, encoded by the EAF virulence plasmid, which is required for activation of many of the virulence genes including those encoding the bundle forming pilus (BFP), intimin, a type III secretion apparatus and the secreted proteins EspA, EspB, EspD and Tir. BFP expression occurs for a short period during early exponential phase. To identify the mechanism responsible for the growth-phase dependent expression of BFP, we deleted the Fis gene (encoding a nucleoid-associated protein Fis), which is expressed during early exponential phase, from the prototype EPEC strain E2348/69. We observed that the mutant failed to induce the accretion of filamentous actin normally associated with attaching and effacing (A/E) lesion formation, although it retained the ability to colonize HEp-2 cells in a localized adherence pattern. Analysis of several virulence factors indicated that BFP and intimin expression were not diminished. However, expression of all the type III secreted proteins required for A/E lesion formation was greatly reduced in the fis mutant. The FAS test reaction and protein secretion were restored by induction of fis from an IPTG-inducible promoter, providing, for the first time, evidence of a role for Fis in virulence regulation.

1200 *Bacillus* species of clinical origin demonstrate adhesion and invasion properties in Hep-2, Vero and Caco-2 cell lines

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Selected cultures of *Bacillus* species isolated from clinical specimens were assessed for adhesion and invasion properties in various mammalian cells.

All the isolates demonstrated high levels of adhesion to Hep-2, Vero and Caco-2 cells (> 68 %) whilst showing varying degrees of invasion (ranging from 0.1 % to 32 %).

The kinetics of adhesion of the various *Bacillus* species were also investigated both microscopically and via the adhesion/invasion assay using the Hep-2 cell-line.

A comparison of the adhesion/invasion properties with other putative virulence factors of these *Bacillus* species was then undertaken.

All the isolates were tested for lecithinase activity, production of catalase and the ability to induce haemolysis in 7 % horse erythrocytes. The presence of toxins was assessed by the BCET-RPLA immunoassay and by measurement with the tetrazolium salt MTT (cytotoxicity in mammalian cells).

Although all the samples demonstrated various virulence properties, our results showed that no relationship existed between adhesion and invasion properties and other putative virulence factors.

Currently *Bacillus* species (except *B. anthracis* and *B. cereus*) are considered to be 'inconsequential' in the domestic and hospital environment. However, as our studies revealed that many *Bacillus* species expressed virulence factors, they should therefore be considered as potential pathogens.

1400 *Bacillus cereus* and other bacillus spp

PER EINAR GRANUM

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Bacillus cereus and closely related organisms are becoming more important causes of food poisoning in the industrialised world. This might well be due to the extensive use of *B. thuringiensis* in many parts of the world. This species is from a food poisoning point of view the same as *B. cereus* and is producing the same virulence factors. Members of the *B. cereus* group produce one emetic toxin (causing an intoxication) and three different enterotoxins (causing infections). The emetic toxin is a ringformed structure of three repeats of four amino- and/or oxy-acids: [D-O-Leu-D-Ala-L-O-Val-L-Val]₃. This ring structure has a molecular mass of 1.2 kDa, and is chemically closely related to the potassium ionophore valinomycin. Two of the three characterised enterotoxins have been shown to be involved in food poisoning. They both consist of three different proteins acting together. Each of the enterotoxin complexes are transcribed from one operon. The genome of different strains may contain one or both these operons. One of the enterotoxin complexes (HBL) is haemolytic whilst the other is not (NHE). Both the enterotoxin complexes are positively regulated by *plcR*, a gene first described to regulate the *plcA* (phospholipase C). The third enterotoxin is a single component protein, but has not been shown to be involved in food poisoning. Other *Bacillus* spp. have been involved in food poisoning, but none of their virulence factors have been characterised. However, cytotoxic strains have been isolated, and it has been claimed that proteins crossreacting with components of HBL and NHE are produced by *Bacillus* species not belonging to the *B. cereus* group.

1430 Staphylococcal enterotoxin structure (superantigen)

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Staphylococcal enterotoxins (SEs) are a large group of true exotoxins that are both acid and protease resistant. They are major causes of food poisoning characterized by vomiting and diarrhea 2 to 8 hours after ingestion of preformed toxin. SE serotypes thus far described include A to K, excluding F which is now known as toxic shock syndrome toxin-1. The SEs fall into 2 major subgroups typified by the SEB/C subgroup and the SEA/D/E subgroup. The three dimensional structures of SEs A, B, C, and D have been determined with high resolution. All of these toxins have a similar three dimensional structure characterized by an A domain B grasp protein fold and a B domain O/B protein fold. All 4 of these toxins, as well as those whose structures have not been determined, have 18 amino acid residues in the same position spatially and scattered throughout the molecules. Because of this property and similarity among the toxins, the three dimensional structures of the whole SE family can be predicted with high accuracy. The location of host cell receptor interactions with SEs have been determined for SEB/SEC by cocrystallization experiments. Finally, some SEs bind zinc atoms with variable effects on toxicity.

1530 Identification of a novel exotoxin-like gene cluster in *Staphylococcus aureus*

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Staphylococci are a predominant cause of food-borne illnesses. Of the pyrogenic exotoxins secreted by *Staphylococcus aureus*, the staphylococcal enterotoxins (SEs) are thought to be the main causative agents of food poisoning and, together with toxic shock syndrome toxin 1 (TSST-1), these proteins are responsible for toxic shock syndrome. At present, ten SEs have been isolated from *S. aureus* although it is widely believed that more remain to be discovered, as the known enterotoxins do not account for all cases of food poisoning.

In a previous study, an alkaline phosphatase fusion strategy was used to screen for *S. aureus* DNA encoding extracellular proteins. This screening methodology identified a novel gene which shared some similarity with the known staphylococcal enterotoxin genes. The novel gene has been isolated on a 5.2kb *EcoRI* fragment and DNA sequencing has revealed a cluster of four complete and one partial open reading frames which we have designated *set1-5* (staphylococcal exotoxin). All five genes encode novel exotoxin-like secreted proteins which possess the streptococcal/staphylococcal exotoxin consensus signature 2 and a region similar to the streptococcal/staphylococcal consensus sequence 1. Phylogenetic comparison of the SETs to the enterotoxins and TSST-1 indicates that these novel proteins form a distinct family of exotoxins that lie between the enterotoxins and the distantly related TSST-1.

The proteins encoded by this newly discovered exotoxin-like gene cluster may be important pathogenicity determinants in staphylococcal diseases.

1545 Isolation and identification of a family of protease-like exotoxins from *Staphylococcus aureus*

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Staphylococcus aureus is a major human pathogen that is implicated in diverse diseases affecting many of the tissues of the body. Whilst a large number of putative virulence factors have been identified and characterized only a small subset of these exotoxins have been implicated in disease pathology and it is widely believed that many more remain to be discovered. As a result of our efforts to purify novel exotoxins we have identified two cationic proteins with apparent molecular masses of 28kDa and 25kDa. The genes for these proteins encode for the histidine consensus sequence found in the V8 family of serine proteases and a region that is similar to the serine consensus sequence of this family of proteases. These consensus sequences are also found in the staphylococcal exfoliative toxins. We have designated the novel 25kDa and 28kDa proteins, protease-like exotoxin (PLE) 25 and PLE28. Cloning and expression of *ple25* and *ple28* and functional analysis of the recombinant proteins using a limited number of substrates suggests that they do not have proteolytic activity. The lack of proteolytic activity may be due to the fact that the PLEs do not have a fully conserved V8 protease serine active site. However the exfoliative toxins possess fully conserved histidine and serine V8 protease. PLE28 and PLE25 stimulate cytokine production by human peripheral blood mononuclear cells, an activity shared by the exfoliative toxins. Thus our results suggest that this new family of protease-like exotoxins may share similar biological activities to the exfoliative toxins. Preliminary molecular analysis of the regions upstream and downstream of the *ple28* and *ple25* genes has revealed that these genes form part of a cluster of at least four *ple* genes.

1600 Botulism in the 1990s

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The toxins produced by *Clostridium botulinum* (serotypes A-G) are the most potent known and ingestion of which often results in the often fatal syndrome "botulism", characterised by widespread flaccid paralysis. The toxins act by preventing neurotransmitter release at the neuromuscular junction, cleaving three proteins involved in the release process: vesicle-associated membrane protein (VAMP), synaptosomal associated protein (SNAP-25) and syntaxin. The neurotoxins appear to fall into a distinct class of endoproteases characterised by their high specificity and strict substrate requirements.

An overview of the incidence of botulism outbreaks in Europe will be given and the mechanism of action of these neurotoxins will be discussed. A range of novel diagnostic assays, based on the mode of action of the neurotoxins, will be presented that can be applied in outbreak situations and risk analysis studies.

1630 Fungi in food and mycotoxins

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We have always had an ambiguous attitude to the presence of moulds in and on foods. Species of *Penicillium* have been used for many years in the production of mould ripened cheeses and meat products such as fermented sausages. Species of *Aspergillus* have been used in the production of koji and a species of *Fusarium* is the basis for the manufacture of mycoprotein which is incorporated into a number of commercially successful food products.

However, species of these same genera also produce toxin secondary metabolites. The toxicology of aflatoxin, ochratoxin, patulin, deoxynivalenol, zearalenone and the more recently discovered fumonisins will be reviewed. An historical perspective of ergotism in Europe, alimentary toxic aleukia in Russia, yellow rice disease in Japan, chronic nephropathy in the Balkans and oesophageal carcinoma in parts of southern Africa will be discussed.

POSTERS:

M1 Selection of invasive *S. typhimurium* using rabbit ileal epithelia *in vitro*

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To study the invasiveness of *S. typhimurium* for gut enterocytes *in situ* we have used rabbit ileal tissue in organ culture. Here we describe the adaptation and validation of this system for the enrichment of invasive organisms present in low numbers in the initial culture.

A mixture of *S. typhimurium* LT7 (a carbenicillin-sensitive hypoinvasive strain) spiked with *S. typhimurium* TNP-5 (a carbenicillin-resistant invasive strain) in the ratio of 500:1 was prepared and allowed to interact with the mucosal surface of

rabbit ileum for 2 hours. Numbers and identities of external and internalised organisms were then quantified by viable counts. The ratio of internalised TNP-5 to LT7 at the end of the first organ culture experiment was used as the inoculum ratio for a second organ culture. This cycle was carried out three times.

No significant change in the ratio of external organisms was observed. By three passes the ratio of internalised *S. typhimurium* TNP-5 to *S. typhimurium* LT7 had significantly increased thus demonstrating that this technique could be used to enrich invasive organisms from initial cultures where they are present in very low numbers. This may be useful in identifying determinants of invasiveness.

M2 Pulsed electric field inactivation of food-borne bacterial enteropathogens

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There is an increasing consumer demand for minimally processed, fresh food produce. As a result, there is growing interest in non-thermal processes that do not adversely affect the nutritional and organoleptic value of foods. Consequently, the potential use of non-thermal, pulsed-power approaches such as pulsed electric field (PEF) treatment, are gaining in popularity as alternative or complimentary processes.

Experiments have been carried out to investigate the effect of pulsed electric fields on the inactivation of microbial populations suspended in liquids using non-flowing and continuous flowing test chambers. Electric fields of ~30 kV/cm with a pulse duration of 500 ns, were generated from aco-axial cable Blumlein PFN. These were applied to the test liquids using parallel plate, circular electrodes.

A range of Gram positive and Gram negative bacterial pathogens were studied. These included *Salmonella enteritidis*, *Escherichia coli*, *Listeria monocytogenes*, *Bacillus cereus*, and *Staphylococcus aureus*. In general, the results showed that the greater the number of pulses applied, the larger the measured reduction in bacterial population. All the treated test organisms were reduced by ~ 3 log orders after 3,000 pulses using the static treatment chamber. Test organisms were reduced by between 5 and 8 log orders after 15, 000 pulses using the continuous flow system.

M3 Cytotoxic properties of twenty-two bacillus species from the clinical environment characterised using four different cell line systems

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The cytotoxicity of numerous members of the genus *Bacillus* obtained from the clinical environment was measured using an improved tetrazolium salt 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl (MTT) assay.

Bacterial cultures grown in brain heart infusion broth for 10 -18 h, as reported by other research groups, may give rise to erroneous results due to the possible accumulation of toxic by-products in the growth media.

Optimum assay conditions for the expression of cytotoxic properties were identified using filtrates from *Bacillus* test cultures grown over a range of incubation temperatures (5 h - 18 h) and challenged using Hep-2, Vero, Caco-2 and MRC-5 cell lines.

After 18 h growth, all the *Bacillus* isolates demonstrated high cytotoxicity (> 60 %) in all cell lines. However, after a 5 h incubation, only specific samples repeatedly resulted in a similar level of cytotoxicity.

As the cytotoxins of a number of *Bacillus* species are proteinaceous by nature, thermal (100 °C for 10 min) and enzymatic (0.1 % trypsin) treatment should reduce the cytotoxic effect of the culture supernatant. Our findings demonstrated a significant reduction in this toxicity due to these treatments.

A relationship between the presence of cytotoxin in culture supernatants and other putative virulence factors *i.e.* lecithinase, haemolysis and catalase was also investigated.

M4 Surface structure and putative capsule of *Campylobacter jejuni*

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A knowledge of the surface structure of *Campylobacter jejuni* is important for a complete understanding of both the molecular basis of any serotyping scheme and as an important pathogenicity determinant. Several workers have previously demonstrated the presence of classical enterobacterial lipopolysaccharide (LPS). We have formerly published work suggesting that we can not find this classical LPS in any of our strains. We showed that no LPS was being expressed by any of 32 Penner serotyping type strains examined and that the antigen being detected was very heat-stable, resistant to proteolytic digestion and was easily eluted from the cell surface. We suggested that heat-stable serotyping antigens might be based on a capsular antigen.

Since then we have further examined the nature of the antigen and shown by immunogold labelling, deletion mutagenesis of a possible gene coding for a capsule and specific staining of a high molecular weight bacterial component a capsule may exist. The chemical analysis of a purified putative capsule has also been carried out.

M5 *Campylobacter* in raw meat and poultry at retail sale

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The incidence of campylobacter contamination and the type distribution of *C. jejuni* and *C. coli* strains isolated from bovine, ovine and porcine liver and fresh chicken portions from retail outlets was investigated. Sampling and primary microbiological examination were undertaken during February and March 1998 by Preston, Cardiff and Carmarthen PHL's. *Campylobacter* species were recovered from 489 (54.7%) of 894 samples examined. Isolation rates varied between sources, with chicken being the most frequently contaminated, followed by lamb then pork. *C. jejuni* was the predominant species in chicken (94%), lamb (83-85%), beef (70%) and pork (52-68%). *C. coli* accounted for 31-43% of pork isolates. *C. fetus* was identified in 23 % of beef samples.

Isolates were serotyped and phage typed by the Campylobacter Reference Unit and the data compared with those obtained from typing human isolates. The widest serotype distribution was seen among *C. jejuni* from chickens (24 HS serotypes), compared to 14 types from lamb, 5 from beef and 4 from pork. Types HS 35, HS 44 and HS 50 were common to all sources and associated with human enteritis, but within the predominant serotypes there was variation in phage type distribution between sources. Serotype HS 11, common only in chicken, was also frequently associated with human infection.

The application of the same typing techniques to the examination of isolates from food samples and human infection in this study has facilitated assessment of the relative importance of meat and poultry as sources of campylobacter enteritis in humans.

M6 A role for the BipA GTPase in host cell invasion by *Salmonella enteritidis*

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Previous proteomic studies have uncovered a novel GTPase termed BipA that is up-regulated when *Salmonella typhimurium* is exposed to the host defence protein BPI. Subsequent experiments with a homologue in an enteropathogenic strain of *Escherichia coli* (EPEC) indicate that BipA is involved in a range of virulence-associated processes, including cell motility and EPEC-induced accumulation of actin in infected host cells. Like EPEC, *Salmonella* spp. are also able to induce remodelling of the host cytoskeleton. In contrast to EPEC, however, such rearrangements frequently result in *Salmonella* internalisation (invasion) into host cells, a process that is strongly correlated with pathogenesis. In view of these similarities and differences, we have examined the role of BipA GTPase in host cell invasion by *Salmonella*.

A *bipA::kan* null mutant of *S. enteritidis* was constructed and tested for *in vitro* invasion of host cells. Invasion of HeLa cells by the null mutant was decreased by 43% relative to the parent strain. Moreover, invasion of marrow-derived macrophages by the null mutant was decreased by 66%; similar results were obtained using RAW macrophage-like cells. The mutant was also tested in the one-day chick model of infection. While the wild-type strain was readily detectable at 6 hours post-inoculation in the liver (6.97×10^3 c.f.u. per liver), the null mutant was not detected. A similar trend was observed with the spleen samples although in this case both strains were first detected at 24 hours post-inoculation. At later time points the difference between the wild-type and null mutant were less marked but remained observable. Taken together these results suggest that the BipA GTPase plays a role in host cell invasion by *S. enteritidis*.

M7 Detection of cholera-like toxin from *C. jejuni* strain NCTC 11351

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Campylobacter jejuni is a major food poisoning organism with 43,147 reported cases in England and Wales for 1998. Reported cases are on the increase in contrast to other food poisoning organisms. There are conflicting reports concerning the range of toxins present in campylobacter. These discrepancies could be partly due to strain to strain variation. One such toxin termed 'cholera-like' has been described by some workers as 60-70kDa. However there is no cholera toxin gene sequence identical to that found in the genomic sequence of *C. jejuni* NCTC11168. In contrast we have a strain of *C. jejuni* NCTC 11351 in which we can detect cholera-like toxins.

In strain *C. jejuni* NCTC 11351 cytotoxicity was observed towards N2A and CHO mammalian cell lines from samples of spent BHI broth media and cell suspensions from blood agar plates, indicating cytolethal effect and neurotoxicity. Separation of cell components using ion-exchange chromatography and outer membrane protein extract has demonstrated the presence of a toxin(s), which cross-reacts with cholera anti-toxin polyclonal antibody. The molecular weight of the cross-reacting bands was 90 kDa from ion-exchange and in contrast 70-75 and 50-55kDa from OMPs. It is therefore plausible that this strain contains a cholera toxin-like gene.

M8 A multinational study to standardise the use of pulsed-field gel electrophoresis for salmonella epidemiology

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Salmonella is one of the most commonly isolated pathogens from cases of food-poisoning in humans in England and Wales. Serotyping and phage-typing are among the initial methods for identification and subtyping of salmonella bacteria and have been largely standardised across Europe. International trade in food animals and food products enables the spread of infectious serotypes from country to country such that they have become widely distributed throughout the European Union (EU). Enter-net is an international surveillance network for human gastrointestinal infections involving all EU countries plus Switzerland and Norway. This recently formed network is an extension of Salm-net which was originally established in 1994 with a view to harmonising salmonella phage-typing and the development of an international database. However, the use of molecular typing for subdivision within serotypes and phage types is becoming increasingly important for the more precise identification of salmonellas. The use of a standardised method for DNA fingerprinting will allow different countries in the EU to rapidly compare subtypes of salmonella organisms responsible for international food-related outbreaks. We aim to standardise pulsed-field gel electrophoresis (PFGE) in selected laboratories within the Enter-net group using a panel of serotypes and phage types of epidemiological importance. Due to wide variation in the presentation of data from PFGE we have proposed a scheme for the nomenclature of PFGE profiles. This will assist in the provision of a sound basis for the molecular identification of salmonella organisms involved in outbreak situations.

M9 Effect of environmental and nutritional factors on growth and toxin formation by diarrhoeagenic and emetic strains of *Bacillus cereus*

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Bacillus cereus is recognised as a spoilage organism by the food industry; the combination of heat resistant spores and the capacity for psychrotrophic growth creates specific problems in milk, dairy products and other pre-cooked refrigerated food products. A potentially more serious problem is that *B. cereus* is associated with two types of food poisoning syndromes; the emetic syndrome has been responsible for the death of a young, athletic teenager from fulminant liver failure. However, little is known about the factors that influence toxin formation by *B. cereus*. The effects of environmental and nutritional factors on growth and toxigenesis have therefore been investigated under controlled conditions in batch and continuous cultures. Toxicity was detected using an improved cell cytotoxicity assay in which the tetrazolium salt MTT was used to assess the metabolic status of the CHO cell line.

Growth of *B. cereus* 1230-88 was maintained over a wide range of conditions. Enterotoxin formulation was not affected by growth rate (T_D 2.3-13.8h), but was influenced by the nature of the nitrogen source, at extremes of pH (5,9), and was not formed under anaerobic conditions in the defined medium used. Toxin formation in this strain was not enhanced by starch and there was no evidence for glucose repression of toxigenesis as previously reported.

The emetic strain of *B. cereus* (NCTC 11143) was also able to sustain growth over a wide range of conditions. With the exception of medium in which the carbon and nitrogen sources were starch and ammonium salts respectively, this strain produced toxin under all growth conditions examined.

Bacillus cereus is capable of growth over a wide range of environmental and nutritional conditions. When growth occurs it is generally accompanied by toxin formation. Therefore the presence of this food-borne pathogen in the food chain compromises both the shelf life and safety of the food products concerned.

M10 Assay of *Bacillus cereus* emetic toxin production under different environmental conditions

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Bacillus cereus is known to produce diarrhoeal and emetic food poisoning syndromes. The emetic toxin is potentially more dangerous than the diarrhoeal enterotoxins as it is known to cause rhabdomyelitis, renal damage and fulminant liver failure in extreme cases. Assay of the emetic toxin has been laborious and subjective in the past, relying on visual examination of vacuolation in Hep-2 cell culture. This led us to develop a semi-automatic metabolic staining assay for the toxin (Finlay et al. 1999. Appl. Env. Micro. 65). This assay has been used to study fundamental requirements for emetic toxin production, such as oxygen concentration, water activity and sporulation. Previous researchers have found that high levels of emetic toxin production in milk require vigorous shaking. This appeared to suggest that oxygenation of the culture medium in vitro, is a major factor for high toxin production in 10% skim milk medium. To investigate this total counts, spore counts and toxin production by 7 known *B. cereus* emetic strains were examined under static and shaken aerobic, anaerobic and microaerophilic conditions. Strains grew rapidly in static anaerobic conditions to numbers comparable to those seen with static aerobic and micro-aerophilic growth but without producing emetic toxin; sporulation levels were, however much lower in the anaerobic conditions.

Toxin production under microaerophilic conditions was considerably increased when the culture was shaken. Mean toxin titre values for shaken microaerophilic growth were nearly 80% of those observed in shaken aerobic growth. These findings suggest that high oxygen concentration is not a major factor for production of the emetic toxin.

M11 Do cells of campylobacter jejuni adapt to acid and alkaline shock?

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The infective dose of different enteric pathogens has been reported to be related to the relative abilities of these pathogens to resist acid killing during transit through the stomach (median pH of c. 2.0 under fasting conditions). Bacteria like *Shigella flexneri*, which can survive a pH of 2.5 for several hours, have RpoS-dependent mechanisms of acid resistance. Unadapted cells of *Salmonella typhimurium* survive treatment at pH 4.0 for several hours, but acid tolerance at low pH is increased by adaptation at an intermediate pH. We have investigated whether cells of *Campylobacter jejuni*, previously shown to have a lower growth limit of c pH 5.0, can show an adaptive response to pH. The minimal pH for growth was tested for both broth and plate cultures and shown to be 5.5-5.6 with phosphate as the buffering system. However in contrast to previous work with *S. typhimurium*, incubation at an intermediate pH resulted in no increased acid resistance when cells were challenged subsequently to pH 3.8, conditions lethal for normal cells. Immediately after acid shock at pH 3.8, cells showed a decrease of several log counts in plating ability but this was regained within 2h before decreasing again. The lack of adaptive response to acid shock was confirmed by 2 dimensional gel electrophoresis of cellular proteins. By contrast an adaptive response to alkaline pH shock was clearly demonstrated. Survival of *C. jejuni* within the stomach may be principally due to enhanced survival on solid particulate surfaces rather than pH adaptation.

M12 Adaptive stress responses of Campylobacter jejuni

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Knowledge of *Campylobacter* adaptive responses to stress is severely limited though adaptive responses to heat-shock and alkaline Ph have been demonstrated. The ability of *Campylobacter jejuni* to adapt to stresses will affect survival potential in the environment and infectivity within the host and so merits further study.

We have examined the stress responses of *C. jejuni* 81116 to a number of chemical agents utilised within the dairy and food industries including trisodium phosphate, hypochlorite, chlorine dioxide and hydrogen peroxide. Sublethal concentrations of hydrogen peroxide (0.1 mM) provided protection against normally lethal concentrations (0.5 mM); similarly prior exposure to 14 mM trisodium phosphate significantly increased survival on subsequent challenge with 20 mM (lethal to unadapted cells). For hydrogen peroxide the level of adaptation was similar in logarithmic and stationary phase cells but the stationary response to trisodium phosphate was less marked than the response in exponential phase. The adaptive responses to sublethal concentrations of hydrogen peroxide were measured after various periods of exposure. The initial response was a sharp decrease in labelling of proteins followed by recovery to the pre-treatment levels. By contrast no adaptive responses were observed for hypochlorite, chlorine dioxide or cold shock. The relation of these results to stress survival, stress regulation and protective enzymes will be discussed.

M13 A molecular genetic study of some pathogenicity factors associated with food-borne bacillus spp.THARARAT CHAITHONG^{1,2}, NEIL J. ROWAN¹ & IAIN S. HUNTER²

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Bacillus spp. are becoming increasingly prevalent as food-borne pathogens, especially in the immuno-compromised and the young. Enterotoxins and endotoxins have been reported, depending on the species and conditions. Using *B. cereus* as a model, we have used the polymerase chain reaction (PCR) to amplify the *bceT* and *hblC* genes: both associated with pathogenicity. Historically, detection of the *bceT* gene product (by immunological means) has been notoriously difficult.

Culture collection stains and clinical isolates of *B. cereus* have been screened by PCR for the presence of *hblC* and *bceT* - for which the results were compared with the immunological assay. We have also used Southern blots to characterise the genetic architecture around *bceT* in these strains.

The study has been extended to isolates of other *Bacillus pp*: *B. licheniformis*, *B. pumilus*, *B. circulans*, *B. sphaericus* and *B. subtilis*. Our data indicate that a surprising majority has the genetic potential to elaborate production of toxin. It may be that the environmental conditions in which these species normally exist do not usually predicate expression of toxin. However, the potential to do so poses a threat.

Lastly, we have expressed a fragment of *bceT* in *E. coli* as a tool to prepare an antibody to this gene product so that we may follow its expression under various environmental conditions.

M14 Please refer to M8**M15 Non-flagellated Listeria monocytogenes are compromised in initial attachment to stainless steel surfaces**

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The attachment of a flagellated wild type strain of *Listeria monocytogenes* to stainless steel surfaces was compared with that of a mutant of the same organism (containing *flaA* mutation) which was unable to produce flagella at any temperature. At 22°C the attachment of the wild type, which had flagella but was held under conditions which ensured it was non-motile, was 10 fold higher than the mutant in the first four hours. At 37°C when the wild type strain was non-flagellated, attachment was the same as for the mutant. This study has shown that the presence of the flagella, even in the absence of motility, facilitates the early stage of attachment to stainless steel by *L. monocytogenes*.

M16 Identification and analysis of stationary phase and starvation-survival components of Staphylococcus aureus

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Previously, we have shown that carbon starvation induces a starvation-survival state, whereby cells become smaller and more resistant to environmental assault.

By screening a Tn917-LTV1 transposon insertion library, several mutants were isolated which showed marked increase in their sensitivity to carbon starvation. DNA sequence analysis revealed that one of the mutants carries an insertion in a gene homologous to tellurite resistance protein (TelA).

Reporter gene fusion studies showed that *telA* expression is induced at the onset of stationary phase under carbon limitation. Exoprotein analysis of *telA* showed a marked reduction in the amount of secreted proteins equivalent to *agr* defective mutant. Western blotting using specific antisera and activity assays demonstrated a dramatic decrease in alpha-hemolysin and V8 serine-protease production.

The possible of *telA* and other components in the co-regulation of toxin production, starvation-survival and other stationary phase-associated phenomena will be discussed.

M17 Development of a spectrophotometric assay for the detection of mannuronan C5 epimerase from *Ps. aeruginosa*

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Ps. aeruginosa FRD462 produces poly M alginate as shown by NMR analysis. The alginate was deacetylated to allow it to act as a substrate for the epimerase AlgG. The epimerase was expressed as an AlgG-glutathione-S-transferase fusion allowing purification by affinity chromatography. The action of epimerase on poly M alginate was detected by use of NMR to show conversion of mannuronate to guluronate. To detect this epimerase activity spectrophotometrically a coupled assay has been developed by linking the above reaction to a guluronate specific lyase, the degradation products of which can be detected using thiobarbituric acid which forms a chromogen. Details of the assay will be presented.

M18 Characterisation of a putative ABC compatible solute transporter in the food-borne pathogen *L. monocytogenes*

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Listeria monocytogenes is a Gram-positive food-borne pathogen that has the ability to grow at refrigeration temperatures and withstand high environmental osmolarity. Osmotolerance is often conferred by transporting compatible solutes (osmolytes with minimal impact of enzymatic processes) into the cell. Here we present a molecular characterisation of a putative compatible solute transporter in this pathogen. An operon has been identified which encodes a homologue of the *opuC* operon in *B. subtilis*. This operon encodes a ABC transporter responsible for transporting several compatible solutes into the cell in response to elevated external osmolarity. We have mutated this locus in *L. monocytogenes* and examined its role in osmotolerance. In addition the sequence has revealed a putative SigB-dependent promoter upstream from the first gene of the operon. SigB is an stress-inducible sigma factor, recently shown to be involved in osmotolerance in *L. monocytogenes*. We have investigated the SigB-dependent regulation of *opuC* using *lux*-based transcriptional fusions to the *opuC* promoter region.

M19 Genetic recombination induced by African swine fever virus

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Replication and recombination of plasmids occur in Vero cells infected with African swine fever virus (ASFV) transfected with these DNAs. Replication and recombination seem to be related processes (Rodríguez *et al.*, Virology, 188: 67-76, 1992), but are still far to be fully understood. The achievement of an *in vitro* recombination system would thus be a good tool to develop experiments for the study of recombination mechanism and related activities. An *in vitro* system obtained from ASFV infected cells permeabilized with lysolecithin that efficiently replicates endogenous and exogenous DNA (Caeiro *et al.*, Virology, 179: 87-94, 1990) was used in recombinant studies although with a low efficiency. In order to improve its efficiency it became necessary to know the period(s) of infection crucial for recombination activities. In this respect it was specially important to find out the earlier time after infection when recombination of transfected DNAs occurs as well as the time after infection when transfected DNAs are no more able to be recombined. For this purpose: i. infected Vero cells were transfected with plasmids at different times after infection (0-12 h p.i.) and ii. these transfected cells were latter collected for DNA extraction at different times after infection (6-21 h p. i.). The results of these kinetics suggest that it will be necessary to mix up cell extracts obtained at different periods of infection - early (4 h p. i.) and late (12 h p. i.) extracts - in order to obtain adequate recombination of exogenous DNA.

M20 *Yersinia pseudotuberculosis* and *Yersinia enterocolitica* translocate yopE into target cells via a yopB dependent mechanism

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Upon contact with target cells pathogenic *Yersinia* translocates Yop effector proteins into the cytosol of the eukaryotic cell via a type III secretion system. Upon translocation of YopE the actin microfilament structure of the target cell is disrupted and this can be visualised as a characteristic rounding up of the cell. It has earlier been shown that this translocation process is dependent on the YopB and YopD proteins. However, the involvement of YopB in the translocation process was recently questioned by Lee and Schneewind, (1999, Mol Microbiol. 31, 1619). We have reexamined the involvement of YopB in the translocation process and present here data that solves this controversy.

0920 Bacterial bc₁ complex

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In the bc₁ complex, the extrinsic domain (head) of the iron sulfur protein (ISP) subunit is found in different positions in different crystal forms. Six different positions in “native” complexes have been identified, plus others in complexes co-crystallized with inhibitors. Because the distance to one or other reaction partner is too great for rapid electron transfer in any single static structure, we suggested that the ISP head must move between catalytic interfaces on cyt b and cyt c₁ subunits during catalysis; this would minimally require rotation through ~57° about a virtual axis. Analysis of electron densities in the structures suggests that the ISP head is relatively weakly bound by the protein (K_{ass} ~1). Mechanistic and kinetic studies show that formation of the enzyme-bi-substrate complex (E.QH₂.ISP_{ox}) involves ligand formation between the two substrates, which likely contributes ~7.5 kJ/mol to stabilization; tight complexes between ISP_{red} and UHDBT or stigmatellin are also well characterized. Diffusion over the distance seen between reaction partners (~22 Å) is likely rapid (~25 ns for the 1-D diffusion appropriate for a tethered “substrate”). The picture that emerges from this analysis is of a stochastic process in which the ISP head diffuses relatively freely between catalytic interfaces, and is constrained by interactions involved in formation of ES-complexes. Although this movement is not linked to mechanical transduction, the mechanism offers new insights into the forces associated with the large change in configuration. The movement of the ISP also offers a new paradigm for electron transfer in biological complexes; movement of substrate in a restricted volume can overcome the constraints of the Marcus energy-gap law on electron-transfer distance, without the entropic sacrifice involved in simple free diffusion.

1000 F1 FO ATP synthase

1100 The bacterial flagellum a propeller with a rotary motor

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Bacterial flagella are readily recognised as microbial molecular machines, as they use the electrochemical ion gradient that exists across the cytoplasmic membrane to produce protein rotation and bacterial swimming. Bacterial movement, driven by these machines, was recognised in the 1600s by Leeuwenhoek, yet still today the molecular details of how flagellar motors work are still not fully understood... Considerable transcription & translation of some 50 genes are required to produce the flagella and the sensory system that allows bacteria to make motile “tactic” responses to stimuli. This paper will review what we have learned about the key proteins which generate flagellar rotation in the pond-dwelling bacterium *Rhodobacter sphaeroides*. This bacterium swims rapidly by proton-motive-force-driven rotation of a single flagellum, it modulates its motility to respond to a wide range of tactic stimuli in the ponds in which it dwells. We have characterised flagellar proteins in this bacterium including the MotAB proteins which are involved in proton entry, FliF comprising the basal disc which is rotated within the membrane and FliG & M which are required for rotation and for modulating rotation in a tactic response to stimuli. We will describe the features of the flagellar motor of this bacterium and will compare this to what is known from the work of other groups on *E. coli* flagella, and marine *Vibrio* flagella which are driven by sodium ion gradients. We will review what is known about the likely mechanisms of flagellar rotation and tactic switching and discuss where future advances are needed to fully understand this type of molecular machine.

1145 Making measurements on molecular motors

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Single molecular motors are tens of nanometres across, far too small to be seen in a light microscope. To observe their workings it is necessary to attach a visible handle. A wide variety of handles have been used. Glass microneedles, bacterial cells, micron-sized glass or polystyrene beads and fluorescent actin filaments have been attached in various combinations to myosin molecules, kinesin molecules, actin filaments, microtubules, the bacterial flagellar motor and the rotating gamma subunit of the F1-ATPase. Several experimental arrangements will be described, along with the techniques used for detecting the motion of the handle produced by the motor and for applying external forces to the motor. Using quadrant photodetectors in combination with optical trapping or the bending of glass microneedles, displacements of a few nanometres and forces of a few piconewtons have been measured on timescales down to hundredths of seconds. At the other end of the scale, the technique of electrorotation has been used to rotate tethered bacteria at speeds up to 1,000 Hz. Some of the conclusions that have been drawn from these experiments will be discussed, along with challenges for the near future.

1225 Actin-based motility in *Listeria*

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Listeria monocytogenes harnesses the actin polymerization-based motor of its host cell in order to move intracellularly and thus spread from cell to cell. Moving bacteria are associated with a comet tail of short, cross-linked actin filaments which is stationary with respect to the cytoplasm and whose depolymerization is governed by the surrounding environment. "Artificial bacteria"—synthetic particles coated with a single bacterial protein (ActA) are capable of growing actin tails and moving at rates similar to the movement of living *L. monocytogenes*. The likelihood of initiating directional movement depends on particle size, shape and ActA density. However, once movement begins, velocity is independent of these factors. Studying these particles has given us new insights into the constraints of actin-based motility, an important process both for pathogens and for the movement of the host cells themselves.

1400 DNA HELICASES: 'INCHING FORWARD'

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Linear motor proteins couple the energy released from nucleoside triphosphate (NTP) hydrolysis to translocate along a polymeric lattice. DNA helicases can be defined as motor proteins since they use the energy from NTP hydrolysis to translocate unidirectionally along the DNA lattice unwinding the double helix in the process.

Recently we reported the structure of the PcrA DNA helicase from *B. stearrowthermophilus* complexed with a tailed DNA substrate. The enzyme was trapped in two different conformations providing different snap-shots of its catalytic mechanism. In one conformation the enzyme was complexed with ADPNP (a non-hydrolysable analogue of ATP) and Mg^{2+} thus representing a 'substrate' complex whereas in the second structure the enzyme was complexed with a sulphate ion bound in the active site precisely at the position where the phosphate released after ATP hydrolysis is bound thus mimicking a 'product' complex. These structures reveal the molecular details of DNA unwinding and translocation suggesting an 'inchworm' type of translocation mechanism that might be of general applicability to other helicases.

1440 The EcoKI type I DNA restriction enzyme as a molecular machine

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Molecular machines may be defined as molecules that can perform multiple functions on other molecules depending on their environment. Type I DNA restriction and modification (RM) systems may be regarded as "smart" molecular machines as they have the ability to detect the methylation status of their DNA target sequence and respond with alternative activities, including extensive translocation of DNA. These enzymes protect the bacterial cell from viral infection by cleaving foreign DNA which lacks N6-adenine methylation within a target sequence and maintaining the methylation of the targets on the host chromosome. They comprise three types of subunit, S (sequence recognition), M (modification /methylation) and R (restriction/cleavage) and can function solely as a M₂S₁ methylase or as a R₂M₂S₁ bifunctional methylase/nuclease. The subunits contain domains related to those found in other smaller methylases, nucleases and helicases. The restriction reaction relies upon extensive DNA translocation driven by ATP hydrolysis prior to endonucleolytic DNA cleavage. Cleavage occurs between two, widely-separated, target sites. This is consistent with the translocation process causing the collision of two enzymes on the DNA. Current experiments to dissect this translocation process and relate it to enzyme structure will be discussed.

1520 EcoR124I restriction endonuclease as a molecular motor

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Type I restriction enzymes are unusual amongst restriction enzymes in that they translocate their DNA substrate prior to DNA cleavage. This motion of DNA, through the enzyme complex, is driven by ATP and the endonucleases are powerful ATPases. Therefore, their potential as a molecular motor resides in this ability to move DNA, but is hampered by DNA cleavage.

These enzymes are composed of three subunits - HsdR, HsdM and HsdS - with DNA recognition residing with the HsdS subunit, which together with HsdM can produce a DNA methyltransferase consisting of a M₂S₁ complex. HsdR is absolutely required for DNA cleavage and ATPase activity.

EcoR124I has been shown to control the restriction (cleavage) activity through the last step in the subunit assembly pathway. The purified endonuclease was found to be a mixture of R₂M₂S₁ and R₁M₂S₁ complexes. The R₁-complex cannot cleave DNA, but is an ATPase. Since we can purify and assemble the R₁-complex in vitro from methyltransferase plus HsdR, the potential exists to develop a molecular motor which does not cleave the DNA substrate. Other methods also exist for production of the R₁-complex alone, which will be discussed. Therefore, we will discuss the translocation activity of the endonuclease and the R₁-complex and speculate about possible novel uses for such a molecular motor.

1620 RNA polymerase

E.P. GEIDUSCHEK

University of California, USA

Abstract not submitted

1640 Ruv proteins and holliday junction resolution

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Recent work has led to the structure determination of the E.coli protein RuvA and its complex with its DNA substrate, the Holliday junction. RuvA functions in concert with two other proteins, RuvB and RuvC, to repair DNA damage by homologous recombination. RuvA binds a fourway crossover point (Holliday junction) in the DNA such that the four duplex arms of the junction sit in orthogonal grooves upon its surface, arranged about a central pin structure which helps to "unzip" the incoming parent duplexe before the single strands are re-annealed with new partners to form outgoing daughter duplexes. The Society for General Microbiology - 144th Meeting - Leeds - 7-10 September 1999

driving force behind this “zipping” motion is generated by RuvB, a hexameric, ATP-dependent helicase with a ring-like structure, which has been targeted to the crossover by RuvA and acts as a molecular motor to power the strand exchange. RuvC protein is then responsible for cutting symmetrical nicks in the DNA at the junction point and facilitating the resolution of the system into repaired DNA duplexes.

POSTERS:

PBM1 *Escherichia coli* SecA: intramolecular communication between the primary domains regulates ATP hydrolysis and is essential for protein translocation

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The SecA ATPase is the major component of the bacterial preprotein translocase. It interacts with all the components of the reaction, SecY, preprotein, SecE, lipids and ATP (1,2). It is also a motor-like protein that converts ATP into mechanical work (1). SecA undergoes cycles of membrane insertion-deinsertion with ATP binding and hydrolysis (3,4), that result in the sequential movement of the translocating chain, through the translocase (5). Experiments in our laboratory have shown that SecA is a large and elongated dimer that consists of two globular protomers, distinctly separated from each other (6). In order to understand the mechanism of action of SecA, we dissected it in two distinct structural domains (7). These were purified as independent polypeptides and were characterized functionally and physically. These domains represent the N-terminal ATPase domain (N-domain; aa 1-610) and the membrane inserting C-domain (aa 610-901) which interacts with SecY (3, 8). We now show that these two primary domains can both physically and functionally reconstitute SecA and that they have distinct functional roles. A model for the domain organization of dimeric SecA will be presented. Biocomputing analysis revealed regions at the C-domain, within the SecY binding region, which are highly conserved in the SecA protein family. These residues have been mutated and we show that they are essential for SecA translocation function and SecY-dependent activation of ATP hydrolysis. We conclude that the C-domain of SecA is absolutely essential for both translocation and translocation ATPase activity through its SecY-binding activity. Our data provide also an explanation of how interaction of the SecA C-domain with SecY, activates SecA ATP hydrolysis at the N-domain and promotes SecA membrane cycling during translocation.

PBM2 A comparative analysis of a nickel resistance gene family in enteric bacteria

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A family of homologous genes conferring resistance to nickel was identified in a collection of enteric bacteria isolated in the Lake District from domestic sewage effluent. The archetypal gene of this family, *nrf*, is carried on the conjugative plasmid pFBA30 in the *Enterobacter cloacae* strain FBA30. Most of the other genes in the family are carried on the chromosomes of enteric bacteria such as *Enterobacter*, *Escherichia* and *Klebsiella*. All of these genes demonstrate strong homology with *nrf* when chromosomal DNA samples from these strains are probed in Southern hybridisations, and comparison of restriction endonuclease sites within and flanking these genes permits the classification of these genes into groups, reflecting their divergent evolution. Nickel resistance is expressed under aerobic and anaerobic conditions, and these strains are also resistant to other metals. It is considered that the resistance profiles of these strains have been selected for by the presence of nickel (and other metals) present in human faeces.

PBM3 pFBA28: A small, conjugative nickel resistance plasmid from *Enterobacter cloacae*

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The *Enterobacter cloacae* strain FBA28 carries two homologous genes for nickel resistance: one is located on its chromosome, and the other is sited on a small nickel resistance plasmid, here designated pFBA28-1. The plasmid specifies nickel resistance through possession of a member of the *nrf* family of resistance genes, which shares homology with its relative on the chromosome. The plasmid has been cloned and is being sequenced.

The plasmid is only 5.5 kilobases (kb) in size. In spite of its small coding capacity, pFBA28 is able to transfer between enteric strains of bacteria by conjugation. The plasmid has a narrow host range and is confined to enteric gram negative species. Transfer is most efficient on plates, rather than under broth mating conditions.

pFBA28 DNA also demonstrates unusual properties affecting its mobility on agarose gels. The significance of this is discussed.

PBM4 The *glnKamtB* operon: a conserved gene pair in prokaryotes suggesting a novel interaction for P_{II}-like signal transduction proteins

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One notable conclusion from studies of bacterial genome sequences is that genomes are plastic and gene order is not highly conserved. Conservation of gene order in distantly related organisms is found only in operons encoding essential cellular apparatus, e.g. ribosomal and cell division proteins, or in operons in which the gene products physically interact. We describe here a conserved gene pair, *glnKamtB*, that has not been identified in other studies. The AmtB family is ubiquitous in nature and representatives are found in eubacteria, archaebacteria, fungi, plants, protists and lower animals. Whilst there is debate as to whether AmtB serves as an ammonium transporter or an ammonia facilitator protein in bacteria, it is certainly involved in movement of some form of ammonium across the cytoplasmic membrane. GlnK is a member of the P_{II} family of signal transduction proteins that are almost invariably involved in sensing the nitrogen status of the cell and transducing this signal by protein-protein interaction to enzymes concerned with nitrogen metabolism. The *glnK* and *amtB* genes are not essential for growth and we therefore propose that GlnK and AmtB interact physically either to modulate AmtB activity or to provide an additional nitrogen-sensing pathway.

PBM5 Ammonium transport in *Escherichia coli*: the role of the AmtB protein

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The recent discovery of a new family of ammonium transporters in yeast and plants has given new impetus into research into bacterial ammonium transport. The *amtB* gene of *Escherichia coli* encodes a member of this family of membrane-bound transport proteins. This gene forms an operon with *glnK*, which encodes a P_{II}-like signal transduction protein, and their expression is induced during growth in nitrogen limiting conditions. We have investigated the roles of the *amtB* and *glnK* gene products in ammonium transport in *E. coli*, using the ammonium analogue ¹⁴C-methylammonium. We also report the effects of in-frame deletions of *amtB* and *glnK* on the ability of *E. coli* to grow under a variety of nitrogen limiting conditions.

The AmtB protein contains 428 amino-acids, the majority of which are hydrophobic. Using a combination of hydrophobicity plots and topology prediction programs, we have generated an *in silico* topological model for the structure of the AmtB which will serve as a starting model for experimental topology determination. Due to the high sequence similarity of AmtB homologues from a diverse range of organisms, this model should enable structural predictions to be made across the whole of the ammonium transporter family.

PBM6 Regulation of the aerobic C₄-dicarboxylate transport (*dctA*) gene of *Escherichia coli*

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The gene (*dctA*) encoding the aerobic C₄-dicarboxylic transporter (DctA) of *Escherichia coli* has been mapped to 79 minutes and is homologous to the *Sinorhizobium meliloti* and *Rhizobium leguminosarum* H⁻:C₄-dicarboxylate symporter, DctA. Regulation studies employing a *dctA-lacZ* transcriptional fusion showed that *dctA* is catabolite activated in a CRP-dependent manner, and is repressed anaerobically by ArcA. Furthermore, *dctA* is weakly induced by the DcuS-DcuR system in response to C₄-dicarboxylates. Interestingly, in a *dctA* mutant, expression of the *dctA-lacZ* fusion is independent of C₄-dicarboxylates suggesting a role for DctA in regulating its own synthesis. In addition, the *dctA* mutant exhibited the ability to utilise the C₄-dicarboxylate, succinate, indicating an independent transporter capable of succinate uptake. Northern blotting analysis revealed a single, monocistronic *dctA* transcript and confirmed regulation of *dctA* by catabolite repression and CRP. Reverse-transcriptase mediated primer extension indicated a single transcriptional start site centred 81 bp downstream of a strongly predicted CRP-binding site.

PBM7 Accumulation of intracellular reserves in *Micromonospora echinospora*

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Members of the genus *Micromonospora* exhibit complex developmental cycles, differentiating both morphologically and physiologically. The physiology of the genus is poorly understood when compared with other actinomycete genera such as *Streptomyces*. It is believed that a greater understanding of *Micromonospora* physiology will lead to increased industrial potential of the genus. This particular study has focused on the accumulation of intracellular reserves in *Micromonospora echinospora*, in a commonly used actinomycete culture medium, Yeast Extract-Malt Extract agar (YEME). YEME agar permitted full differentiation of *M. echinospora*, with spores appearing after 120 hours. Interestingly biomass accretion was not found to be associated with glucose consumption. Instead, glucose was utilised by the culture during a second phase of accretion coinciding with extensive sporulation and accumulation of the polysaccharide glycogen. Glycogen was accumulated in two phases, initially during early exponential growth and again with the onset of sporogenesis. The second phase of glycogen accumulation coincided with the depletion of ninhydrin-positive amino compounds, suggesting glycogen synthesis occurred in response to nitrogen limitation. Glycogen constituted a maximum level of 15 % (w/w) during the stationary phase of growth. In contrast, trehalose accumulated within the mycelium during exponential growth, accounting for 5 % of dry cell weight at the onset of sporulation and remaining constant at this level during sporogenesis. This data suggests that these carbohydrates constitute an important storage reserve in *M. echinospora*.

PBM8 The tol-dependent translocation of *E. coli* colicins

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E colicins are protein antibiotics produced by strains of *Escherichia coli* and closely related bacteria that bind to the BtuB receptor, which is an essential component of the high affinity vitamin B12 transport system. Killing of *E. coli* cells by E colicins requires three stages, receptor-binding, translocation and cytotoxicity. The central (R) domain is responsible for receptor-binding, whilst the N-terminal (T) domain mediates translocation. The DNase (colicins E2, E7, E8 and E9) and RNase (colicins E3, E4 and E6) type E colicins have been termed the enzymatic E colicins. The process by which the cytotoxic domains of the enzymatic E colicins are delivered to their site of toxicity in the cytoplasm of *E. coli* cells by the tol-dependent translocation system is almost unique in bacteria.

Here we investigate the protein-protein interactions between the T domain of colicin E9 with TolB, an essential protein of the tol-dependent translocation, using the yeast two hybrid system. We also report our progress in the determination of the crystal structure of TolB.

PBM9 Using *E. coli* colicins to deliver polypeptides into the cytoplasm of *E. coli* cells

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Colicins are a diverse group of plasmid-encoded antibacterial proteins, produced by many strains of *Escherichia coli*. Members of the *E. coli* colicin family bind to the BtuB receptor and exhibit one of three types of cytotoxic action; a membrane-depolarising (E1), a DNase (E2, E7, E8 and E9) or an RNase (E3, E5 and E6). The process by which the enzymatic DNase or RNase domains are translocated into the cytoplasm of target *E. coli* cells, crossing both the outer and inner membranes, is little understood and could have important implications in the design of novel polypeptide antibiotics.

We have utilised a transposon mediated pentapeptide insertion system to screen for permissive sites within the ColE9 DNase domain that do not affect the biological activity of the colicin. This has enabled us to identify regions of the DNase domain that have the potential to translocate foreign polypeptides into *E.coli* cells. We are now investigating the insertion of other, larger polypeptides at these permissive sites.

PBM10 The influence of growth rate on autolytic activity in *Streptomyces coelicolor*

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Autolysins are potentially lethal enzymes capable of hydrolyzing the peptidoglycan layer of bacteria. They are characterized by their ability to cleave specific bonds and monomers of peptidoglycan. These enzymes have been well studied in *Bacillus subtilis*, where they appear to be involved in cell wall turnover, cell separation and sporulation.

Our work has focused on autolytic activity in *Streptomyces coelicolor*, and in particular the activity of N-acetyl-glucosaminidase and N-acetyl-muramidase, during batch and chemostat fermentations. In batch cultures, muramidase activity could be detected throughout growth, reaching a maximum activity at the end of the active growth phase. In contrast, glucosaminidase activity was only detected after the major biomass accretion phase. In chemostat cultures, at slow growth rates ($D=0.05h^{-1}$) only glucosaminidase activity could be detected. Whereas, at a faster growth rate ($D=0.2h^{-1}$) only muramidase activity was found. The presence of muramidase activity during growth and at elevated growth rates would suggest a role for this enzyme in cell wall turnover. In contrast, it would appear that glucosaminidase is associated with stationary phase/slow growing cells, indicating that it may play a part in nutrient recycling in starved cultures.

PBM11 Mercury resistance of thermophilic *Bacillus* species

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Compost has long been known to be a rich source of thermophilic microorganisms and has often been associated with concentration of heavy metal contaminants. Thermophilic mercury resistant bacteria were selected by growth at 62°C on Luria agar containing HgCl₂. Sequence analysis of 16S rRNA genes of two isolates showed closest matches with *Bacillus pallidus* (organism 4) and *Bacillus thermosphaericus* (organism 2). Minimum inhibitory concentration (MIC) values for HgCl₂ were found to be 25µg/ml and 80µg/ml for organisms 2 and 4 respectively, compared to an MIC of 10µg/ml for *B.pallidus* H12 DSM 3670, which was used as a mercury sensitive control. The standard mercury-resistant *Bacillus* strain, *B.cereus* RC607, had an MIC of 50µg/ml.

Growth of organisms 2 and 4 in media containing HgCl₂ resulted in the formation of a black precipitate, thought to be HgS from Energy Dispersive Analysis by X-ray (EDAX) analysis of the precipitate. Growth in media containing ²⁰³HgCl₂ at 45°C and 62°C showed a progressive removal of radioactive mercury from the media. This cannot be explained by HgS precipitation as any ²⁰³HgS formed would remain within the cultures medium. This progressive removal of ²⁰³Hg from the media appears to be by reduction of ²⁰³Hg²⁺ to ²⁰³Hg⁰ by mercuric reductase, one of the proteins encoded by bacterial *mer* operons.

PBM12 Transport functions and protein interactions in mercuric ion resistance

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Inorganic mercury compounds are highly toxic to bacteria, many of which encode specific resistance mechanisms to deal with mercury. These resistances are often on plasmids or transposons, among the best-studied of which is the *mer* determinant of transposon Tn501. The *mer* genes encode transport proteins (periplasmic MerP and the inner-membrane protein, MerT) for the specific uptake of mercuric ions, and the cytoplasmic enzyme, mercuric reductase. The net result is the uptake and reduction of Hg(II) and the production of non-toxic, volatile Hg(0).

The transfer of Hg(II) from the periplasmic protein, MerP, to the inner membrane transport proteins, MerT, and the subsequent transfer to mercuric reductase, has been dissected by directed and random mutagenesis of these proteins. Analysis of the effect of mutations on mercuric ion resistance, uptake and volatilisation have shown that charged residues in MerT may be responsible for solvent access to essential cysteines in MerT and that MerT interacts directly with MerP to transfer Hg(II); these data also provide evidence that mercuric reductase interacts with the cytoplasmic face of MerT. This work is beginning to dissect the protein-protein interactions occurring in the uptake and subsequent reduction of Hg(II).

PBM13 The Na⁺-dependent malonate transporter MadLM from *Malonomonas rubra*

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The *madL* and *madM* genes, located in the malonate fermentation gene cluster of *Malonomonas rubra*, encode for a secondary active Na⁺-dependent malonate uptake system.

Expression of both genes was shown to be imperative to render an active malonate transporter. Transport studies with *Escherichia coli* cells synthesizing MadLM indicated that the uptake of malonate is solely driven by the chemical gradient of Na⁺ ions. Furthermore, the kinetics of malonate uptake at different pH values implied that Hmalonate⁻ is the transported malonate species. It is suggested that the MadLM complex is an electroneutral Na⁺/Hmalonate⁻ symporter.

PBM14 Periplasmic binding protein dependent ABC transporters are bi-directional!

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The established paradigm of ABC transporters is that they are uni-directional, responsible for either the uptake or the efflux of solutes. However, the initial characterisation of the general amino acid permease (Aap) of *Rhizobium leguminosarum* indicated a bi-directional phenotype. If excess homologous or heterologous solute is added to cells that have been preloaded with a labelled amino acid, an efflux of labelled amino acid is observed. The rate of efflux is dependent on the copy number of *aap* in the strain. Therefore, either the Aap is directly responsible for the exchange of solutes or it is regulating a separate efflux system. It is known from Southern blot analysis that *Salmonella typhimurium* does not contain a transporter homologous to the Aap. The Aap of *R. leguminosarum* was therefore expressed in this strain. In addition to facilitating the uptake of α -aminoisobutyric acid, a solute of the Aap not otherwise transported by *S. typhimurium*, the Aap allowed the exchange of this solute. When the better characterised His transporter of *S. typhimurium* was examined, it was also found to exhibit a bi-directional phenotype. The specificity of solutes able to induce efflux of labelled solute via the Aap or His transporters matched the uptake specificity of the transporter.

PBM15 Characterization of a 50 kDa protein exported to the growth medium by the methanotrophic bacterium

***Methylococcus capsulatus* (bath)**

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Upon growth of *Methylococcus capsulatus* (Bath) a protein with apparent molecular mass 50 kDa was detected in the growth medium. Antibodies raised against the 66 kDa outer membrane protein, MopE, of the bacterium, recognized the secreted 50 kDa protein, called Δ MopE. Appearance of the exported protein seems to depend on the growth conditions, and growth phase.

Using a glycoprotein immunoblot reaction and silverstaining against glycoproteins, the results strongly indicated that both the 50 kDa protein and the 66 kDa protein are glycosylated. From cloning and sequencing, the encoded amino acid sequence indicated a molecular mass approximately 37 kDa for Δ MopE, and glycosylation is assumed to account for the observed difference in mass, when comparing with that estimated from SDS-PAGE

From isoelectric titrating the pI was calculated to be 4.28, close to the predicted value from the amino-acid sequence, using EMBL WWW protein database. The behavior of the protein on a chromatofocusing column, was according to this isoelectric point.

The 50 kDa protein was purified to apparent homogeneity using a Q-Sepharose column, followed by gel filtration through a Hiload Superdex® 75 column, coupled to a FPLC system.

Native Δ MopE was resistant to trypsin, and the protein did not exhibit protease activity.

PBM16 Characterisation of an outer membrane protein of unknown function from *Brucella abortus*

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Brucellae are facultative intracellular Gram-negative bacteria that infect humans and animals, causing brucellosis, a disease that occurs worldwide.

The *Brucella abortus* outer membrane protein OMP1 (molecular weight of 89 kDa, 782 amino-acid long) is still of unknown function. Homologous proteins to OMP1 has been found in several Gram negative bacteria where they appear to be highly immunogenic and seem to be essential to the bacteria survival. The function of these proteins in their respective bacteria is still unknown.

Previously, we suggested, on the basis of primary structure theoretical analysis, that OMP1 is a two domain protein reminiscent of recently published FepA structure. FepA is a high affinity receptor of ferrichrome and is implicated in its transport.

omp1, the gene encoding OMP1, is located about 30 bp upstream a cluster of genes encoding proteins involved in synthesis of lipid A. It can be hypothesised that OMP1 is involved in lipid A or LPS transport, from the periplasmic space to the bacterial outer membrane.

To assess whether omp1 and the nearby lpxD, fabZ and lpxA genes form an operon, we performed Northern blotting and RT PCR experiments. Results will be discussed in the poster.

PBM17 Sugar diffusion through *Brucella melitensis* porins

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Brucella sp. are Gram negative bacteria causing brucellosis, a widespread zoonosis occurring in numerous mammals species, including humans. Two closely related proteins (85% sequence identity) from the *Brucella* outer membrane, Omp2b and Omp2a, are known as the *Brucella* porins because they show some of the typical biochemical properties of *E. coli* porins, although there is no detectable sequence similarity between Omp2b / Omp2a and any other porin. Large amounts of pure Omp2b and Omp2a from *Brucella melitensis* were obtained after production in *E.coli* inclusion bodies and subsequent renaturation of the porin trimers.

Pore-forming activity of purified recombinant *B. melitensis* porins was assessed in planar lipid bilayers and in a liposome swelling assay. *B. melitensis* Omp2b and Omp2a show respectively an average single-channel conductance of 424 pS (S.E. = 5,10; n=738) and 563 pS (S.E. = 7,67; n=577). This difference is highly significant (p<0,0001). In the liposome swelling assay, the relative rate of sugar permeation for recombinant purified Omp2b are virtually identical to previous data concerning native porin extracted from *B. melitensis* outer membrane, indicating that purified recombinant Omp2b recovered most of the native protein properties. Omp2a was found to allow disaccharide diffusion at a much higher rate than Omp2b, which suggests a larger pore for Omp2a.

PBM18 Heterologous expression of LcrV and LcrG : proteins critical for the type III secretion system of *Yersinia pestis*

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LcrV and LcrG are key proteins in the type III secretion system of *Yersinia*. LcrV is a secreted protein, required for the translocation of Yop effectors into the mammalian cell. It is also regulatory, and enhances Yop expression and secretion under inducing conditions. This regulation is mediated intracellularly through a competitive interaction with LcrG. LcrG is believed to be an intracellular "gate", blocking type III secretion under non-inductive conditions.

The LcrV and LcrG interaction may be facilitated by coiled-coil domains. These are important for several protein interactions in type III secretion systems. Indeed predicted coiled coil domains are present in both LcrV and LcrG. Moreover a deletion within this region in LcrV functionally inactivates the protein.

The aim of this work is to probe the importance of such domains in the interaction of LcrV with LcrG. Initial studies have investigated affinity-tagged and unfused protein expression systems for LcrV and LcrG. LcrV proteins mutant at Leu residues shown to be essential for coiled-coil interactions have also been designed.

Using these recombinant proteins it may therefore be possible to determine amino acids important in the functional interaction of LcrV with LcrG within *Yersinia* type III secretion.

PBM19 Identification and characterisation of SirR, a transcriptional regulator in *Staphylococcus aureus*

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Staphylococcus aureus is a major human pathogen responsible for diseases such as bacteraemia, septicaemia, endocarditis and toxic shock syndrome. The ability to sequester iron is essential for the growth of all bacteria, except *Lactobacilli*, and is also implicated in establishing an infection. In Gram negative bacteria, the role of Fur (ferric uptake regulator) has been extensively characterised, and more recently homologues of Fur have been found in Gram positives. DtxR is a Fur-like protein in *Corynebacterium diphtheriae*, and a homologue of this, called *sirR*, has been found in *S. epidermidis*. The organisation of the *sirR* locus in *S. aureus* is the same as that of *S. epidermidis*, where *sirR* is divergently transcribed from genes encoding an ABC-type transporter. *LacZ* fusions have been constructed in *S. aureus* to both *sirR* and the ABC-type operon. The expression of these genes has been studied in both complex and a chemically defined minimal medium. In addition, the production of proteins by *S. aureus* in different media, with the addition of various metal ions has been investigated. A homologue of *sirR* in *B. subtilis* is involved in the regulation of manganese and cadmium transport, and we are currently investigating whether *sirR* fulfils a similar role in *S. aureus*.

PBM20 Identification and characterisation of novel autolysins in *Bacillus subtilis*

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Bacillus subtilis has multiple autolysins and that are capable of hydrolysing the peptidoglycan structure of the cell wall. Several autolysins of *B. subtilis* have already been characterized and shown to have roles in cell separation, motility, cell lysis and cell wall turnover. The complex compensatory nature of the autolysins means that it is important to identify and characterize the total complement of these enzymes to determine their individual and combined roles in cell growth and differentiation.

The advent of the total *B. subtilis* genome has enabled, through sequence homologies, the identification of a number of putative autolysins. We have insertionally inactivated 12 of these. Reporter gene fusion analysis has allowed the expression of the genes during growth to be determined. By the creation of multiple mutants combining mutations in the novel genes with the well characterized major autolysins, *lytC* and *lytD*, and the sigma factor σ^D the role of the enzymes has begun to be determined. Phenotypic analysis of the multiple mutants has identified five putative autolysins involved in cell division and motility. Two of the putative novel autolysins have been overexpressed and purified. This is enabling the hydrolytic bond specificity and biochemical properties of the enzymes to be determined.

Our current understanding of the number and nature of the members of the autolysin complement of *B. subtilis* will be presented.

PBM21 Characterisation of the starvation survival response in *Listeria monocytogenes*

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Listeria monocytogenes is a well known food-borne pathogen notable for high mortality rates after human infection (10-50%). Though widely studied for survival in varied foodstuffs, little effort has been made to investigate the basic mechanism of starvation survival. Here, we have developed a chemically defined medium in which the response of *L. monocytogenes* to starvation for glucose, amino acids or multiple nutrients could be studied. Under glucose or multiple-nutrient limitation, 90 to 99% of the population lost viability within 3 days, whilst the surviving cells stayed viable for over 50 days. Amino acid limitation did not elicit the creation of a starvation-survival phenotype, as viable cells became undetectable within 5 days. The development of a starvation survival phenotype was found to be independent of cell density in multiple nutrient starved cells at 25°C. The survival of long-term starved cells was independent of cell wall biosynthesis, whilst the cell survival was entirely dependent upon protein biosynthesis up to 8 hours after starvation, but became independent by 24 hours. The starvation survival phenotype of the wild type *L. monocytogenes* and a *sigB* mutant encoding the alternative sigma factor σ^B has been studied for glucose starvation survival kinetics, and starvation-induced stress resistance.

PBM22 Increased lipophilicity correlates with increasing antibacterial properties of phenothiazines

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Methylene blue (MB) and its two methylated derivatives monomethyl methylene blue (MMB) and dimethyl methylene blue (DMMB) have LogP values of -0.10, +0.7 and +1.0 respectively. Furthermore the derivatives show higher inherent photosensitising efficacies (11% increase for MMB and 20% for DMMB when compared to MB) and all the test compounds exhibited photobactericidal activity on illumination at a light dose of 6.3 J cm⁻². The antibacterial properties mirror the increasing lipophilicity when used against vancomycin resistant *Enterococcus spp.*, methicillin resistant *Staphylococcus aureus* and a range of non-resistant strains. This increase in inherent toxicity is thought to be due to more efficient targeting and in the case of photoactivation also due to methylation inhibiting cellular reduction of the phenothiazinium chromophore to the inactive leuco form.

PBM23 Re-routing of an exported protein of *E. coli* to the SRP pathway by signal sequence mutationsHENDRIK ADAMS, JOEN LUIRINK¹, PIER A. SCOTTI¹ & JAN TOMMASSEN²¹Dept of Molecular Cell Biology and Institute of Biomembranes, Padualaan 8, 3584 CH Utrecht, Utrecht University, The Netherlands, ²Dept of Microbiology, Biocentrum Amsterdam, The Netherlands

In our laboratory, outer membrane protein PhoE is used as a model protein to study protein export in *E. coli*. This protein is synthesized with an N-terminal signal sequence that targets the precursor to the Sec machinery. The normal targeting pathway of PhoE occurs via interaction with SecB and SecA. Introduction of specific missense mutations at position -10 in the signal sequence of PhoE did hardly or not affect the translocation across the inner membrane in a wild-type strain. However the translocation of these mutant precursors was strongly impaired in a *prlA4* mutant strain, which was originally selected as a suppressor of signal sequence mutations. In cross-linking experiments we observed that these mutated precursors had dramatically increased affinity for P48 (one of the constituents of the SRP homologue in *E. coli*) and that they are re-routed to the SecYEG translocon via the SRP pathway *in vitro*. Hence, it appears that the SRP targeting pathway is affected by the *prlA4* mutation.

PBM24 Use of *Staphylococcus aureus* mutants to overexpress and characterise an ABC transporter involved in antibiotic efflux

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Staphylococcal resistance to 14- and 15- membered ring macrolides and inducible resistance to type B streptogramins (MS^R) is conferred by *msrA* which encodes a 488-aa protein (MsrA). This hydrophilic protein is a member of the ABC transporter superfamily and consists of two fused ATP binding regions with no transmembrane domains. Isolated originally from a *Staphylococcus epidermidis* plasmid, MsrA functions in previously antibiotic sensitive *Staphylococcus aureus*. Thus MsrA may confer specificity on chromosomally encoded transmembrane complexes in staphylococci. *Staphylococcus aureus* mutant backgrounds were generated in which MsrA was non-functional. Membrane fractions of these mutants grown in the presence of erythromycin indicate lack of an 82kDa protein present in similar preparations from strains grown in the absence of erythromycin and always present in the wildtype. We aim to characterise this protein and test the idea that this is the missing component of the transporter. In addition we have found that these mutants overexpressed MsrA when grown to early log phase with erythromycin, induction. This represents 5% of the total membrane protein. Furthermore, MsrA can be solubilised using detergents from this staphylococcal background in contrast to the situation in *Escherichia coli* where it is overexpressed as insoluble inclusion bodies.

PBM25 Identification and characterisation of a putative rhodobacter sphaeroides gene that encodes a product resembling escherichia coli cytochrome B561 and R.sphaeroides cytochrome b562

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Rhodobacter sphaeroides belongs to the purple, non-sulphur group of bacteria and, like other members of this group, possesses considerable versatility in its growth capabilities. Depending on environmental conditions, R.sphaeroides can grow aerobically, anaerobically in the light (photoheterotrophically using a variety of organic compounds), fermentatively and it can perform anaerobic respiration using a variety of electron acceptors such as trimethylamine-N-oxide (TMAO), dimethyl sulfoxide (DMSO) or nitrate. The components of the photosynthetic and respiratory electron transport chains in cells grown under different growth conditions have been extensively studied. Amongst these studies was the finding that R.sphaeroides possesses a cytochrome b562. However, the role and function of this cytochrome remains unclear, since inactivation of the encoding gene resulted in no observable difference in growth properties or respiratory activities compared with the wild type (Yun et al. (1994).

Here we report on the identification, characterisation and inactivation of an additional putative cytochrome b562-like gene in R.sphaeroides. The gene product shares similarities (including the possession of conserved histidine residues) with the R.sphaeroides cytochrome b562 gene, the E.coli cytochrome b561 gene (for which a function has also remained elusive), and a recently identified E.coli gene encoding a cytochrome b561 homologue.

PBM26 An antiphagocytic role for the *Pseudomonas aeruginosa* type III secretion/translocation system?

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P. aeruginosa is a common opportunistic pathogen known to cause disease in immunocompromised individuals, for example patients with burnwounds, leukemia or cystic fibrosis. *P. aeruginosa* is known to secrete a number of virulence factors. Recently, *P. aeruginosa* was demonstrated to possess a type III secretion/translocation system. Interestingly, the type III system of *P. aeruginosa* and the pathogenic *Yersinia* exhibit an extensive homology and it has been demonstrated that individual components of the secretion/translocation system are interchangeable between the two species. For *Yersinia* it has been demonstrated that the translocated products YopE and YopH, act in concert to block uptake by macrophages. For *P. aeruginosa* it has already been demonstrated that the YopE homologue in *P. aeruginosa*, ExoS, has an antiphagocytic effect *in vitro* when expressed in *Yersinia*. In our work we have investigated the interaction between *P. aeruginosa* and macrophages/ HeLa cells using an immunofluorescence staining technique. Our preliminary results demonstrate that *P. aeruginosa* can resist phagocytosis also with a non-functional type III system indicating that other antiphagocytic factors are expressed by the bacteria.

PBM27 Cloning and characterisation of the *Candida albicans* sec61 gene

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The Sec61 protein (Sec61p) is the core component of the ER translocation apparatus and it has been proved to be essential for the insertion of both integral membrane and secretory polypeptides across the ER membrane in both yeast and mammalian cells. Sequence comparisons of Sec61p from different organisms reveal a high percentage of similarity, suggesting a high degree of functional conservation. With the aim of gaining further information on this essential element of the translocation machinery we have cloned the *SEC61* gene from the pathogenic yeast *Candida albicans* using a λ GEM12 genomic library. Several positive clones were obtained after the screening of the library with a 280 bp DNA probe synthesised by PCR. The nucleotide sequence of one of the selected clones predicts an open reading frame of 479 amino acids (52.6 kDa), 67.2 % identical to that of the *Saccharomyces cerevisiae* Sec61p. As it has been described for its *S. cerevisiae* counterpart, the hydrophathy profile analysis of the *C. albicans* Sec61p suggests that it is an integral membrane protein with several transmembrane domains. Heterologous complementation studies indicate that the *C. albicans* gene is not able to complement the phenotype of the *sec61-2* thermosensitive mutation in *S. cerevisiae*. Further characterization of the gene will be presented. (This work was partially financed by grant PB95-0755 DGES and by a F.P.U. predoctoral fellowship to J.M.R.).

PBM28 Biomethylation of antimony by *Cryptococcus humicolus*

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The yeast *Cryptococcus humicolus* was shown to biomethylate inorganic antimony, with the generation of trimethylantimony. This gas was detected by GC-AAS in the headspace of cultures under anaerobic conditions following aerobic growth; no other volatile species of antimony were detected. Hydride generation (HG) from liquid culture supernatants of this organism produced mono-, di- and trimethylantimony species, at 14, 66, and 20% relative amounts respectively. In contrast, for *Scopulariopsis brevicaulis* - the only other fungus known to biomethylate antimony - trimethylantimony alone was detected in culture headspace gases and after HG of culture media. Both organisms are known biomethylators of inorganic arsenic, with the generation of mono-, di- and trimethylarsine by *C. humicolus*, whereas only the last two species have been detected for *S. brevicaulis*. The enzymes involved in the arsenic biomethylation pathway are the most likely catalysts of antimony biomethylation. Detection of trimethylantimony suggests that the fungi are able to take antimony (III) through the entire analogous biochemical pathway during aerobic growth. The presence of mono- and dimethylantimony species in culture media of *C. humicolus* but not of *S. brevicaulis*, suggests that these species are intermediates in the antimony methylation pathway rather than products arising through oxidation of trimethylantimony.

The model of *E. coli* as magnified two million times with especial reference to molecules crossing membranes and some molecular machines

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In four consecutive poster sessions at meetings of the Society, this model of *E. coli* set out upon table tops has steadily evolved with an increasing number of aspects of structure and metabolism being brought together. For the fifth session attention has been paid to the nature and growth of the cell membranes and the principal types of molecular crossing mechanism have now been added. Magnification at 2×10^6 is sufficient for only very limited resolution of representations of the smaller molecules but enables import and export processes to be shown together in a comparative way. Emphasis is further placed on integration of activity of a few of the some 1850 species of protein accepted present in *E. coli*, and several examples of molecular machinery, either factual or hypothetical, are set out and presented for discussion. The sum total of the molecular species so far modelled and arranged together in various interactions gives some indication of the basis of life in terms of proteins, DNA, nutrients and energy to the non-microbiologist as well as to the microbiologist. The model, which is due for completion in April 2000, is intended to the scientist and the non-scientist.

1405 Lacticin 3147: a bacteriocin with applications in food and medicine

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Lacticin 3147 is a broad-spectrum, two-component bacteriocin produced by *Lactococcus lactis* DPC3147. This bacteriocin acts at the cytoplasmic membrane of sensitive cells by forming pores which leak potassium and phosphate ions. The genetic determinants for lacticin 3147 production and immunity are encoded on a 60.2 kb conjugative plasmid, pMRC01, the sequence of which has been fully elucidated. A 13 kb region, containing two divergently arranged gene clusters, has been cloned into *L. lactis* MG1363. Strains harbouring the resulting plasmid have the ability to both produce lacticin 3147 and are also immune to the bacteriocin. Lacticin 3147 has been incorporated in foods as an additive in a lacticin-enriched powder, and also a number of specialised lacticin-producing starters have been created. Transconjugants producing lacticin 3147 have been used as starter cultures in the manufacture of cheddar cheese where it was observed that the bacteriocin can control the developing microflora in the cheese during ripening. Incorporation of these lacticin-producing starters in cottage cheese to inhibit *Listeria* showed a 3-log reduction in numbers of deliberately inoculated *L. monocytogenes* Scott A over a five day period in cheese stored at 4 °C. Lacticin 3147 has also been used in the medical and veterinary field. A prophylactic role for the bacteriocin in prevention of mastitis in cattle has recently been developed where lacticin is incorporated into an intramammary teat seal product. Also, the effectiveness of lacticin in inhibiting human pathogens such as methicillin resistant *Staphylococcus aureus*, vancomycin resistant *Enterococcus faecalis* and penicillin resistant *Pneumococcus* has been demonstrated.

1420 Genetic analysis of amphotericin biosynthesis

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Amphotericin B is a medically important anti-fungal antibiotic that is produced by *Streptomyces nodosus*. Amphotericin B is the most effective drug for the treatment of serious systemic fungal infections in humans. However, the compound has many severe side effects especially nephrotoxicity. The polyketide synthase genes involved in amphotericin biosynthesis are being analysed as the first step towards engineering the biosynthesis of analogues with improved anti-fungal specificity and reduced toxicity.

A cosmid library of *S. nodosus* genomic DNA was constructed. Several clones were isolated that hybridised to a DNA probe derived from the erythromycin biosynthetic gene cluster. Restriction mapping techniques were used to assemble a set of overlapping clones that represent a 150 kilobase region of the chromosome. Sequence analysis of one end of this cluster revealed a series of polyketide synthase modules capable of catalysing cycles nine, ten and eleven in amphotericin biosynthesis. This analysis also revealed an ABC transporter, a LysR type transcriptional activator and a GDP-mannose dehydratase involved in synthesis of the mycosamine sugar moiety of amphotericin B.

Methods for disruption and replacement of *S. nodosus* genes were investigated as a means to engineer synthesis of novel amphotericins.

1430 The crucial role of a ferrous iron uptake system in iron acquisition and virulence in the human pathogen, *Helicobacter pylori*

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The genome sequence of the gastric pathogen, *Helicobacter pylori*, indicates that the organism possesses multiple iron-scavenging systems which probably enable its survival in the iron-limited environment of the human host. This study was undertaken to address the proposed roles of all the major predicted Fe acquisition genes in *H. pylori* and to delineate the mechanistic details of Fe assimilation. Mutants in *feoB* (HP0687), *tonB* (HP1341), *fecD* (HP0889), *fecE* (HP0888), *fecA* (HP0807), *fecA* (HP0686) and *fecA* (HP1400) were generated by insertional inactivation with a selectable antibiotic marker. Atomic absorption spectrophotometry revealed considerably lower intracellular Fe levels in the *feoB* mutant compared to the wild type strain. This mutant displayed decreased uptake rates of both ⁵⁵FeCl₂ and ⁵⁵ferric citrate. In contrast, the other mutants exhibited no transport deficient phenotype. Complementation of the *feoB* mutant restored both ⁵⁵FeCl₂ and ⁵⁵ferric citrate uptake. The Fe²⁺-trapping agent, ferrozine, inhibits Fe transport whether it is supplied in the reduced or oxidised form. These data indicate that FeoB plays a crucial role in the transport of both the reduced and oxidised species of Fe. Several lines of evidence implicate extracellular ferric reduction in the Fe uptake pathway. The kinetic parameters of Fe²⁺ transport suggest the presence of two uptake systems with different affinities for this substrate. Furthermore, we demonstrate that FeoB is responsible for high-affinity uptake but not for low-affinity uptake. The FeoB transporter is highly specific for Fe²⁺ and the sensitivity of transport to vanadate, FCCP and DCCD provides evidence for ATP hydrolysis being the driving force for high affinity Fe²⁺ transport. Finally, we have demonstrated that the expression of FeoB is a prerequisite for the successful establishment of infection in a mouse model, thus indicating its importance as a virulence determinant.

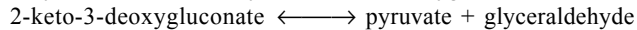
1450 A novel aldolase from the hyperthermophilic archaeon *Sulfolobus solfataricus*

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Sulfolobus solfataricus is a member of the hyperthermophilic Archaea with a growth optimum at 80°C and pH 3 [1]. A striking feature of this organism is that it metabolises glucose by a modified Entner-Doudoroff pathway that utilises non-phosphorylated intermediates [2]. The pathway involves an NAD(P)⁺ dependent dehydrogenation to gluconate that is dehydrated to form 2-keto-3-deoxygluconate [3]. This then undergoes an aldol cleavage to yield pyruvate and glyceraldehyde. At no point is there a requirement for ATP [2].

This study focuses on the enzyme 2-keto-3-deoxygluconate (KDG) aldolase which catalyses:



The enzyme has been purified from *Sulfolobus solfataricus* and subjected to N-terminal sequencing. The sequence data were then used to create a DNA probe that enabled a complete gene sequence to be obtained (AC AJ224174) from an λ EMBL genomic library. Amino acid sequence alignments suggest that this enzyme is a novel member of a sub-group of (α/β)₈ proteins that includes *N*-acetylneuraminase lyases (NAL) and dihydrodipicolinate synthases (DHDPS) [4].

KDG-aldolase has been shown to exist as a homotetramer, as have NAL and DHDPS [5,6]. We have demonstrated the enzyme to be a type I aldolase, forming a Schiff-base intermediate between pyruvate and an active site lysine residue. This lysine is strictly conserved across the superfamily. [4].

We propose that the KDG-aldolase reaction proceeds via a similar mechanism to that of NAL and DHDPS, and has considerable potential as a thermostable enzyme for the stereoselective synthesis of carbon-carbon bonds in biotransformations.

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1505 A specialised two-component system links cell-cell signalling to pathogenicity gene expression in *Xanthomonas campestris*

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The *rpf* (regulation of pathogenicity factors) gene cluster is required for the synthesis of extracellular enzymes and polysaccharide (EPS), and hence pathogenicity in *Xanthomonas campestris* pathovar *campestris* (*Xcc*). Two members of this gene cluster (*rpfF* and *rpfB*) are involved in a novel type of regulation mediated by a small diffusible molecule (DSF). The *Xcc* system is novel in that DSF is not an *N*-acyl homoserine lactone, which are widely distributed in Gram-negative bacteria, and RpfB and RpfF are not related to LuxI/R proteins implicated in the synthesis of *N*-acyl homoserine lactones. To identify further genes involved in the DSF signalling system, a mutant population of *Xcc* was screened, using a newly developed DSF bioassay, to select mutants with alterations in DSF level. Two DSF-hyper producing strains were identified. Paradoxically both of these strains also produced reduced levels of extracellular enzymes and EPS. Both mutants carried insertions in the gene, *rpfC*. RpfC belongs to a sub-class of two component regulators in which the sensor and response regulator domains are fused. Immediately upstream of *rpfC* in the same operon, are *rpfH* and *rpfG*, which also encode elements of a two-component system. We speculate that RpfC, RpfH and RpfG act in concert to couple the sensing of DSF and other environmental factors to the synthesis of pathogenicity factors such as extracellular enzymes and EPS. We are currently testing this hypothesis through the creation of mutations in *rpfG*, *rpfH* and *rpfC*, in both wild type and *rpfF* mutant backgrounds.

1550 Identification and characterisation of *Campylobacter jejuni* genes involved in host cell invasion

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The mechanism by which *C. jejuni* causes disease is as yet unclear. There is clinical evidence to suggest that host cell invasion is an important part of the infection process. In order to identify bacterial factors required for this invasiveness we used a gentamicin invasion assay to screen a cosmid library of *C. jejuni* strain 81116 for invading clones. The library was prepared in the *E.coli* host HB101 which was non-invasive. A total of forty invasive clones were identified using assays with or without centrifugation. Six clones were invasive in both assays. Two of the highest invading clones (pBT9737 and pBT9724) were selected for further investigation. A random subcloning and sequencing approach with pBT9737 has identified a large number of genes, some of which may be involved in pathogenesis, however no homology to known invasins has resulted. A number of homologies to genes involved in flagellar biosynthesis and motility were identified. Electron microscopy revealed the presence of type-1-like fimbriae on the surface of all the invasive clones. Mannose sensitive haemagglutination (MSHA) was used to screen the whole cosmid library and only 8% of clones expressed fimbriae, half of which were invasive. Similar fimbriae are expressed by HB101 only following several rounds of serial passage in static broth. These results suggest the presence of a regulator within the cosmid insert which induces fimbrial expression in HB101.

1605 Quorum sensing in *Yersinia pestis*

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Quorum sensing is the ability of bacteria to regulate gene expression in a cell density-dependent manner and is mediated by signal molecules such as the N-acylhomoserine lactones (AHLs). Two regulatory genes, homologues of the *luxR* and *luxI* genes of *Photobacterium fischeri*, encoding a transcriptional activator and an AHL synthase respectively, are involved in quorum sensing in a variety of Gram-negative bacteria. Such systems have been shown to be involved in the regulation of virulence in several species. In this study, we sought to determine whether *Yersinia pestis*, the causative agent of bubonic plague, utilises quorum sensing to regulate virulence. Two quorum sensing systems were identified and regulatory loci, designated *ypeRI* and *yepRI*, were sequenced. The AHLs produced by *YpeI* and *YepI* were separated by thin layer chromatography and tentatively identified. Mutants of *Y. pestis* GB defective in *ypeR'*, *yepR'*, or *yepI'* were obtained by insertional inactivation and each mutation was confirmed by the polymerase chain reaction. Phenotypic studies were performed to compare each of the three *Y. pestis* GB mutants with the wild type *Y. pestis* GB.

1620 The distribution of ammonia oxidizing bacteria in activated sludge flocs

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Activated sludge floc was considered as a random agglomeration of bacteria. We wish to determine whether this hypothesis is correct or not. If this hypothesis true the ammonia oxidizing bacteria (AOB) micro-colonies will be randomly distributed with respect size and location in the same way that currants are randomly distributed in a fruitcake. To test this hypothesis we have examined the distribution of ammonia oxidizing bacteria (AOB) micro-colonies in activated sludge flocs using fluorescent in situ hybridization (FISH) in conjunction with confocal scanning laser microscopy (CSLM). AOB from the β proteobacteria have been targeted using probes at the whole group (Nso1225) and the genus *Nitrosospira* (Nsv443) and the genus *Nitrosomonas* (Nsm156). The genus *Nitrosospira* predominated in the plant. Flocs from a full-scale plug flow plant with mechanical and diffuse aeration were examined. The frequencies and size variation of AOB micro-colonies were examined with respect to both depth and x-y cross-section area. The size and frequency of occurrence of the AOB microcolonies increased towards the centre of the floc. The increased microcolony size at the centre must reflect either faster and/or more prolonged growth in these AOB. In this work, it was shown that AOB micro-colonies are not randomly distributed. We deduce that, in the activated sludge plant examined, the flocs maintained a definite 3 dimensional structure for a sufficiently long time for AOB growth to be favoured in a specific zone. Thus the floc could be regarded as a small biofilm. In addition, the results suggest that activated sludge may also be engineered at the floc level and gives microbiological support to the work on simultaneous nitrification denitrification.

1635 Inhibition of echovirus entry into rhabdomyosarcoma cells by antiserum to cd59: a common cell-specific entry mechanism for echoviruses?

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The formation of a multi-protein receptor complex may be required for the infection of rhabdomyosarcoma cells (RD) with echoviruses (Ward *et al.*, 1998). This complex contains at least decay-accelerating factor (DAF), the receptor for many echoviruses, and MHC class I. We now show that antiserum to human CD59 blocks echovirus infection of RD cells. Infection by Poliovirus and Cocksackie B2/B3 viruses was not affected. Results indicate that CD59 is not an attachment molecule for echoviruses, but may play a role the latter stages of infection. One-step growth curve analysis and cold-synchronised eclipse products indicate a possible role of CD59 in uncoating of echovirus 7 (EV7).

Cells use membrane lipid organisation into microdomains of sphingolipid and cholesterol rich sub-domains as a mechanism to co-ordinate signal transduction and membrane trafficking (Simons, 1997). DAF and MHC class I have been localised to these sub-domains (Wu *et al.*, 1997 and Stang *et al.*, 1997). In combination with our data indicating a role of beta-2-microglobulin (part of the MHCI complex) in echovirus entry into RD cells, we predict that echoviruses may enter and infect RD cells via a common mechanism involving cholesterol rich lipid microdomains and antiserum to CD59 inhibits this process. This data indicates a common entry mechanism for all echoviruses in RD cells which is distinct from that used by Poliovirus or Cocksackie B2/B3 viruses.

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