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143rd Meeting

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University of Birmingham

The use of antibiotics in veterinary medicine and in animal husbandry, role in the appearance of antibiotic resistance in human pathogens is bothcontroversial and of global importance. Antibiotics have not only revolutionised human and veterinary medicine but have also played a prominent role in animal husbandry by their use as growth promoters. Some of the antibiotics typically used in animals include virginiamycin (analogue of synercid, a new streptogramin for human use), avoparcin(analogue of vancomycin, a glycopeptide), apramycin (analogue of gentamicin, an aminoglycoside) enrofloxacin (analogue of ciprofloxacin, afluoro4-quinolone) and tylosin (analogue of erythromycin, a macrolide).The lavish use of antibiotics has consequently resulted in the rapid emergence of antimicrobial resistance in both human and veterinary pathogens with resistance mechanisms having now been described for all known antibiotics currently available for human clinical use. Although the spread of clonally related resistant organisms poses a hazard in itself, if the resistance genes are chromosomally encoded in these organisms then they are contained within that bacterium. Protocols should, in this instance, be implemented that would eradicate the resistant organisms before they reach humans. Of equal or greater concern is the fact that in recent years a number of resistance genes have become associated with mobile DNA elements such as plasmids, conjugative transposons, and integrons. Association of resistance genes with plasmids assist in the rapid dissemination of the resistance genes normally within a bacterial genera and less frequently between bacterial genera, restricted predominantly by plasmid incompatibility or an inability to replicate in different bacterial genera. The association of resistance genes with mobile DNA is of greater concern as they assist in the rapid dissemination of antibiotic resistance genes not only within bacterial species but between different species and genera (horizontal transfer). This is primarily achieved because it effectively eliminates the need for plasmid compatibility to exist within different bacterial species. The ability of the mobile DNA elements to establish genetic connections between widely different species makes them likely to be principal players in the dissemination of a wide variety of antimicrobial resistance determinants. It is already evident that organisms isolated from humans or animals having prolonged exposure to antibiotics are resistant to those antibiotics. It has been suggested that there is a steady flow of antibiotic resistant organisms and/or antibiotic resistance genes between animals and humans and this may account for the rapid emergence of resistance to antibiotics within a short period of time from their introduction for human use. There is increasing molecular evidence to suggest that in animals and humans the resistant organisms are clonally related or that the mobile elements conferring resistance are from a common origin. This talk will concentrate on two examples of antibiotic resistance arising in animal bacteria, and suggested to have human health implications. Fluroquinolone resistance in campylobacter (chromosomally mediated) and vancomycin resistance in enterococci (transferable resistance).

**10.20 PREVALENCE OF ANTIBIOTIC RESISTANCE GENES IN ENVIRONMENTAL BACTERIA: NEW MONITORING STRATEGIES**

Kornelia Smalla and Holger Heuer
Federal Biological Research Centre for Agriculture and Forestry, Messeweg 11/12, D-38104 Braunschweig

The widespread use of antibiotics in human medicine, in animal therapy and animal husbandry has resulted in an increasing incidence of antibiotic resistance genes amongst bacterial populations. Many studies have shown that the rapid spread of antibiotic resistance genes is due to their localization on mobile genetic elements which are readily spread between bacteria. However, the majority of epidemiological studies has focused on the spread of antibiotic resistance in clinically relevant bacteria. The understanding of prevalence and circulation of antibiotic resistance genes in environmental bacteria and the role of selective pressure in different environments is in particular limited due to the fact that only a minor proportion of bacteria can be studied by standard cultivation techniques. Therefore, respective studies need to analyze the cultivated and non-cultured bacteria from different environmental habitats. Furthermore, appropriate sampling strategies are required to avoid biases due to sample heterogeneities and seasonal fluctuation. More and more molecular data on antibiotic resistance genes have become available since the eighties, allowing for the development of specific probes and primer systems. Primer systems and probes for different antibiotic resistance genes have been used to track their environmental dissemination (e.g. nptII, strA, strB, sat1-3). To overcome the well-known limitations of cultivation-based approaches, new monitoring strategies for antibiotic resistance genes have recently been developed. Both, exogenous isolation of antibiotic resistance genes in different bacterial hosts as well as PCR-amplification of antibiotic resistance genes from DNA directly extracted from environmental samples allows new insights. The talk will give an overview of potentials and limitations of different approaches to study the prevalence and diversity of antibiotic resistance genes in environmental bacteria.

**11.30 DRUG RESISTANCE IN MALARIA; THE CURRENT SITUATION**

D Arnot
University of Edinburgh

**12.10 NEW DRUG TARGETS IN BACTERIA**

I Chopra
University of Leeds

## MAIN SYMPOSIUM
## MICROBIAL SIGNALLING AND COMMUNICATION
*Tuesday 13 April 1999 & Wednesday 14 April 1999*

Full papers are published in the SGM Symposium Volume 57 - Microbial signalling and communication (Editors: R England, G Hobbs, N Bainton, D Roberts)*

**09.00 BACTERIAL CROSSTALK - COMMUNICATION BETWEEN BACTERIA, PLANT AND ANIMAL CELLS**
P Williams- University of Nottingham

**09.45 INTERCELLULAR SIGNALLING AND THE MULTIPLICATION OF PROKARYOTES: BACTERIAL CYTOKINES**
A S Kaprelyants - Russian Academy of Science, Moscow

**11.00 QUORUM SENSING IN GRAM-NEGATIVE BACTERIA: ACYLHOMOSERINE LACTONE SIGNALLING AND CELL-CELL COMMUNICATION**
E P Greenberg - University of Iowa, USA

**11.45 QUORUM SENSING IN A*REOMONAS* AND *YERSINIA***
S Swift -University of Nottingham

**14.00 SIGNALLING IN *PSEUDOMONAS AERUGINOSA***
B H Iglewski - East Carolina University, USA

**14.45 MULTIPLE ROLES FOR ENTEROCOCCAL SEX PHEROMONE PEPTIDES IN CONJUGATION, PLASMID MAINTENANCE AND PATHOGENESIS**
G M Dunny - University of Minnesota, USA

**16.00 PHEROMONE COMMUNICATION IN THE FISSION YEAST SCHIZOSACCHAROMYCES POMBE**
J Davey - University of Warwick

**16.45 INTERCELLULAR SIGNALLING FOR MULTICELLULAR MORPHOGENESIS**
D Kaiser
Stanford University, USA

**09.00 REGULATION OF CARBAPENEM ANTIBIOTIC AND EXOENZYME SYNTHESIS BY CHEMICAL SIGNALLING**
G P C Salmond - University of Cambridge

**09.45 AUTOREGULATORY FACTORS AND REGULATION OF ANTIBIOTIC PRODUCTION IN STREPTOMYCES**
Y Yamada - Osaka University, Japan

**11.00 HOST/PATHOGEN INTERACTIONS DURING INFECTION BY ENTEROPATHOGENIC *ESCHERICHIA COLI*: A ROLE FOR SIGNALLING**
G M Frankel - Imperial College, London

**11.45 SURVIVAL STRATEGY OF YERSINIA IN ITS HOST**
G R Cornelis -Universite Catholique de Louvain, Belgium

**14.00 SIGNALS AND INTERACTIONS BETWEEN PHYTOPATHOGENIC ZOOSPORES AND PLANT ROOTS**
N A R Gow - University of Aberdeen

**14.45 MATHEMATICAL MODELLING OF SIGNALLING IN *DICTYOSTELIUM DISCOIDEUM***
J A Sherratt - Heriot-Watt University

**16.00 HYPHAL INTERACTIONS**
G W Gooday - University of Aberdeen

**16.45 SIGNALLING IN DINOFLAGELLATES**
J Burkholder -North Carolina State University, USA

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**09.00 ANAEROBIC CONTROL OF ELECTRON AND CARBON FLOW PATHWAY GENES IN *E. COLI***

Robert P. Gunsalus.
Department of Microbiology and Molecular Genetics, U.C.L.A., Los Angeles, USA

*Escherichia coli*, like many enteric, marine, and soil bacteria, can utilise a variety of alternative electron acceptors for cellular respiration and energy generation. The respiratory substrates include oxygen, nitrate, nitrite, IMAC, DM50 and fumarate. Depending on the availability of the various compounds, one or more of the appropriate oxidoreductase complexes are preferentially synthesized by the cell. Oxygen reduction occurs via either the cytochrome o oxidase (*cyoABCDE*) or the cytochrome d oxidase (*cydAB*) enzyme. Anaerobic nitrate respiration is accomplished by the Nar and Nap nitrate reductases (*narGHJI*, *napAB*), whereas nitrite is reduced by the Nrf (*nrfABCDE*) nitrite reductase. Several organic electron acceptors are reduced by the DMSO/TMAO reductase (*dmsABC*), an alternative TMAO reductase (*torA*), or the fumarate reductase (*frdABCD*).

How does *E. coli* detect the anaerobic state, and how does it then co-ordinate respiratory pathway gene expression to maximise energy generation? The aerobic/anaerobic transcriptional control is provided by two global control circuits consisting of the ArcA/ArcB two-component regulatory system and the Fnr regulatory protein. Whereas ArcB functions as the sensor-transmitter for anaerobiosis, ArcA is the response regulator that binds DNA to activate or repress gene expression upon phosphorylation by ArcB. Fnr also functions as either a repressor or an activator. Together, they co-ordinate expression of the respiratory and TCA cycle pathway genes.

The localization of the DNA binding sites for ArcA and Fnr has provided the basis for working models that explain how the *cyoABCDE* and *cydAB* operons are microaerobically transcribed. Analogous studies have addressed how ArcA and Fnr co-ordinate the expression of the TCA cycle genes and of the fermentation pathway genes. Discussions will focus on the global control of energy and carbon flow in *E. coli* as a model for understanding facultative cell growth in a variety of pathogenic and non-pathogenic microorganisms.

**09.45 WHEN PROTONS ATTACK: ACID STRESS RESPONSES OF *SALMONELLA* AND *E. COLI* O157:H7**

John W. Foster
Department of Microbiology and Immunology, University of South Alabama College of Medicine, Mobile, AL 36688, USA

Enteric microorganisms have developed several inducible mechanisms for surviving transient periods of extreme acid stress. *S. typhimurium* survives pH 3 stress through an acid tolerance response (ATR) induced in minimal medium by short exposures to mild acid stress. More than 50 acid shock proteins are induced during log phase acid adaptation. Eight ASPs are regulated by the major iron regulatory protein Fur in an unusual iron-independent manner. The two component regulator PhoP is an autoinduced ASP that controls the induction of three additional ASPs. The stress sigma factor $s^S$ is an ASP that regulates induction of 10 ASPs. Acid induction of $s^S$ is due to its decreased proteolytic turnover via the ClpXP protease in conjunction with the two component-type response regulator MviA(RssB in *E. coli*). Mutations in any of these three regulators leads to a defective ATR. Repair of pH stress-induced DNA damage appears to require the Ada protein ($O^6$-methylguanine methyltransferase) since an *ada* mutant is both acid and alkaline sensitive. In contrast to *S. typhimurium*, *E. coli* survives pH 2.5 acid stress via stationary phase acid resistance systems induced in complex media that include a glucose-repressed system protective at pH 2.5 without amino acid supplementation, a glutamate decarboxylase (GAD) system that requires glutamate, and an arginine decarboxylase system. GAD is the most effective acid resistance system. Stationary phase induction of GAD requires $s^S$ but acid induction is $s^S$ -independent. The two GAD isozymes present in *E. coli* are required for survival at pH 2. A role for acid resistance systems in pathogenesis is evident in that bovine fecal shedding of O157 lacking the GAD system is severely reduced.

**11.00 SIGNAL TRANSDUCTION PATHWAYS IN STRESS RESPONSES OF BUDDING AND FISSION YEAST**

Brian A. Morgan
Medical School, University of Newcastle-upon-Tyne, UK

Yeast cells constantly monitor and respond to changes in their environment. Adverse conditions lead to changes in the regulation of gene expression, resulting in increased levels of proteins that protect against the stress. This regulation utilises signal transduction pathways which communicate the presence of a particular stress to the nucleus. Recently the stress-induced signal transduction pathways and gene expression have been intensively studied in the budding yeast *Saccharomyces cerevisiae* and the fission yeast *Schizosaccharomyces pombe*. It is apparent that many aspects of stress control in these evolutionarily distant organisms are conserved, thus making these two yeast excellent model organisms for this type of study. For example, both organisms use two component signal transduction pathways and a similar MAP kinase pathway. In addition, the nuclear localisation of specific transcription factors are regulated by similar mechanisms. In this presentation an overview of the regulatory networks that control gene expression in *S. cerevisiae* and *S. pombe* will be given. With the availability of the complete genome sequence of *S. cerevisiae* and the imminent completion of the *S. pombe* Genome Project, studies in these two organisms promises a wealth of information regarding stress response control in fungi and eukaryotes.

**11.45 STRESS RESPONSE OF FOOD SPOILAGE FUNGI TO CLASSICAL AND NOVEL PRESERVATIVES**

Stanley Brul[1] , Peter Coote[1] , Frans M. Klis[2] and Peter W. Piper[13]

[1] Unilever Research Division p/a P.O. Box 114, 3130 AC Vlaardingen,
[2] Department of Molecular Cell Biology, BioCenter Amsterdam, University of Amsterdam 1098 SM Amsterdam, The Netherlands;
[3]Department of Biochemistry and Molecular Biology, University College London, London WC1E6BT, UK.
Fungal spoilage forms an increasing economic problem in the food industry. Chemical antifungals are becoming less attractive as food preservatives and hygiene agents due to the development of resistance and because of stricter legal regulations concerning the permitted concentrations. Finally, consumers tend to demand more 'naturally preserved' or preservative-free products.
Here we review first our understanding of the mechanisms of action and fungal stress response to classical antifungals such as weak organic acids. The available evidence suggests that the inhibitory action of weak organic acids such as sorbic acid is due to the induction of an energetically expensive protective mechanism that compensates for any reduction in pHi homeostasis but results in less available energy for normal growth. One aspect of this mechanism is represented by the membrane P-type ATPase that pumps excessive protons to the extracellular environment. Interestingly, recent studies by Piper and co-workers have additionally identified a membrane pump in yeast that under normal conditions is involved in the secretion of the anion of the weak organic acids sorbic acid, benzoic acid and acetic acid into the surrounding cellular medium. In order to prevent futile cycling through active acid efflux followed by passive inward diffusion at an extracellular pH of 4-5, a final cellular adaptation reaction may exist, which results in alterations of its plasma-membrane and/or cell wall structure such that inward diffusion of these antifungals is prevented or at least severely impaired. Indeed Loureiro-Dias recently discussed at the 19th ISSY in Braga, Portugal (September 1998), that preservative adapted cells are able to reduce the diffusion coefficient for benzoic acid such that presumably re-access of effluxed preservative back into the cell is significantly impaired.

Next, we evaluate the scientific basis underlying the application of novel, natural antifungals which are often based on wall weakening systems composed of lytic enzymes and membrane active biomolecules. These compounds often occur in edible plants and are thus attractive alternatives for the chemical antifungal agents. Unfortunately, fungi are also able to adapt themselves to the presence of these naturally occurring antimicrobial compounds. The stress response mechanisms all include the Pkc1-pathway. Cells with a non-functioning Pkc1-pathway can only survive in osmotically stabilised media and are sensitive to centrifugational forces. Preliminary experiments by de Nobel and Oomes have shown that indeed *mpk1Δ* and *pkc1Δ* are much more sensitive to wall lytic enzymes than wild type cells or *fks1Δ* cells which are deficient in ß-1,3-glucan. The stress response has as major output an increase in cellular chitin levels and some cell wall mannoproteins next to the induction of a specific glucan synthase.
The eventual aim of the reviewed work is to generate mathematical lag-time models in real foods that predict the microbiological stability of the food, and are based on a mechanistic understanding of the chain of events that leads to cell death, or an extension of lag-time of the initiation of outgrowth.

### 14.00 THE CHARACTERISATION OF STRESS TOLERANCE AND *RPOS* STATUS IN WILD TYPE *SALMONELLA TYPHIMURIUM* DT104 AND *S. ENTERITIDIS* PT4 ISOLATES

Tom Humphrey,
PHLS Food Microbiology Reseach Unit, Church Lane, Heavitree, Exeter EX2 5AD
*Salmonella* spp., in common with other foodborne pathogens, can respond to a wide variety of non-lethal changes to their environment. For example, alterations in either temperature and/or pH can markedly change the heat and acid tolerance of bacterial populations. Such changes, particularly when they result in an increase in tolerance to potentially hostile conditions, are known as stress responses and are frequently mediated by the synthesis of proteins known as "shock proteins". This is not always the case, however. For example, the culture of *Salmonella enteritidis* in a mildly acidic environment markedly increases subsequent acid tolerance. The bacterium possesses two response systems. The first, which is very rapid, does not require protein synthesis. The second system, which requires protein synthesis, takes longer and acid tolerance is not maximised until after 60 minutes. The former, rapid system is sufficient to protect cells in an environment with pH values of pH 2.5 or more. The second system becomes more important in more acidic environments.

It is important that account is taken of stress responses when assessments are made of the efficacy of treatments used in food processing, for example. The most usual site of contamination of egg contents with *S. enteritidis* is either the yolk membrane or the albumen surrounding it. In these sites, bacteria will be exposed to high pH. This results in the synthesis of heat shock proteins which increases the heat tolerance of the *Salmonella* cells. Thus, if assessments are to be made of the heat treatment of raw egg, which may contain *S. enteritidis*, it is important to use bacteria which have been pre-exposed to alkaline conditions. It is also becoming ever more apparent that within wild type populations of *Escherichia coli* O157 and *Salmonella* spp, mutations to *rpoS* are common. This will result in isolates with markedly different tolerance/resistance profiles. These bacteria will also differ in certain stress responses. When studies on the survival of such bacteria are undertaken it is important that isolates of known provenance and known *rpoS* status are used. These and other matters will be discussed.

A particular feature of the presentation will be a discussion of the behaviours of isolates of *S. enteritidis* phage type (PT) 4 and *S. typhimurium* definitive type (DT) 104 in environments that lead to the formation of very long filamentous cells. These are formed at 40C and at low $a_w$, for example. Filaments comprise many salmonella cells and are a consequence of incomplete septation. When cultures are warmed or re-hydrated, septation is rapidly completed and what can appear to be only 1 cfu, if counts are performed before the change, is in fact up to 40-50 individual cells. The public health implications of this will be discussed.

### 14.45 STRESS AND THE REGULATION AND EVOLUTION OF THE *SPV* VIRULENCE GENES OF *SALMONELLA TYPHIMURIUM*

Charles J. Dorman[1], David Marshall,[1] Orla Keane, [1] Ruth C. Massey, [1,2], Paul B. Rainey[2] and Brian J. Sheehan[1]
[1] Department of Microbiology, Moyne Institute of Preventive Medicine, Trinity College, Dublin 2, Ireland,
[2] Department of Plant Sciences, University of Oxford, South Parks Road, Oxford OX1 3RB, UK
The *spv* virulence genes of *S. typhimurium* are carried on a 90 kb plasmid and are activated at the level of transcription following entry of the culture into stationary phase. The genes are also activated strongly when the bacterium enters macrophages. Activation requires the RpoS sigma factor and the LysR-like transcription factor, SpvR. We have investigated the interaction of the SpvR protein with the *spvR* and *spvA* promoters in vivo using dimethyl sulphate (DMS) mediated DNA methylation analysis. SpvR binds to two operator sites upstream of *spvA* and site-directed mutagenesis has defined key residues in the DNA required for the protein-DNA interaction. A related operator site is found upstream of *spvR*. When this is altered to match more precisely the spvA sites, it binds SpvR better but becomes less efficient in transcription activation. Using electrophoretic mobility shift analysis and DNase I footprinting, we have found that the leucine-responsive regulatory protein, Lrp, binds to the *spvR* operator located promoter-proximal to *spvA*, and that Lrp is a repressor of this gene in bacteria growing in rich medium. Using the same techniques, we have found that the integration host factor binds at the *spvR* promoter and is a positive regulator of that gene. Thus, the *spv* genes belong simultaneously to several regulatory circuits and we believe that this assists in fine-tuning their expression in response to environmental stress. When bacteria are subjected to osmotic stress a deletion occurs upstream of *spvR* that fuses this gene at the level of transcription to *sprA*, a gene normally located 5 kb away. SprA encodes a member of the resolvase family of site-specific recombinases and an active sprA gene is required for the deletion to occur. The resulting fusion over-expresses the *spv* operon at a level 70-fold above wild-type values. Deletions were detected in a strain harbouring a *spvB-lacZ* fusion and were only detected in a lactose-containing medium. The mutants are significantly fitter than the ancestor strain in this growth medium. Deletion formation required osmotic stress but did not result in increased fitness in an osmotically-stressful medium. The deletion event required normal DNA gyrase activity, suggesting that the mechanism includes a role for osmotically-induced changes in DNA structure. Thus, in addition to possessing a sophisticated form of conventional transcription regulation, *spv* has the potential to undergo stress-induced evolution to a strongly-expressed derivative.

**16.00 IS RPOS A KEY ELEMENT IN THE SURVIVAL OF BACTERIA THROUGH FOOD PROCESSING?**
Christine E R Dodd
Div Food Sci, School Biological Sciences, Univ Nottingham, Sutton Bonington Campus, Loughborough, LE12 5RD UK
The alternative sigma factor RpoS identified in certain Gram negative bacteria is expressed maximally at the onset of stationary phase and in turn regulates a series of stationary phase genes, many of which convey protective mechanisms on the cell. This results in stationary phase cells demonstrating enhanced thermotolerance, osmotic and oxidative stress resistance and resistance to starvation. In *Salmonella* the virulence gene *spv* is also regulated by *rpoS* and hence stationary phase cells are potentially also more virulent. Control of RpoS expression is complex, however external stresses such as treatment by weak acids can result in the induction of functionally active levels. This suggests that sub-lethal environmental stresses such as are found in foods and food manufacture may induce RpoS, making the organism more resistant to a series of processing treatments and also potentially more virulent. Through the use of an *spvRA::luxCDABE* reporter, which indicates the levels of functional RpoS in the cell, we have examined *rpoS* expression in *Salmonella* species under a number of food related conditions and treatments. This reporter, and other *lux*-based reporters, have also allowed us to examine in detail the reason for enhanced resistance to heat and freeze thaw injury demonstrated by *Salmonella typhimurium* when in the presence of high levels of a competitive microflora. From these studies have arisen new ideas of how bacteria respond to stress conditions.

**16.45 FISSION YEAST STRESS RESPONSE: SUM1 AND THE INTERACTION WITH THE TRANSLATIONAL MACHINERY**
T Humphrey
MRC Harwell, Oxford
The Stress Activated Protein Kinase (SAPK) pathway can be activated by multiple environmental stresses and proinflammatory cytokines to induce highly specific transcriptional response programmes. This pathway is highly conserved, and in fission yeast, the SAPK pathway is activated by similar environmental insults, where in addition to activating a transcriptional response, the stress response pathway also functions to advance cells into mitosis (1).
A novel gene, *sum1+* was isolated as a high copy plasmid suppressor of a class of checkpoint mutants in fission yeast (2). Overexpression of *sum1+* can also inhibit the normal cell cycle response to osmotic stress. Sum1 is a highly conserved WD-repeat protein, and structural homologs of Sum1 have been identified in a wide range of eukaryotes including the human TGF-b-receptor interacting protein, TRIP-1, and the translation initiation factor 3 subunit eIF3-p39, encoded by the *TIF34* gene in *Saccharomyces cerevisiae*. TIF34 is required for translation initiation, and plays a central role in generating/ maintaining the translation initiation complex eIF3 (3). Preliminary data indicate that *sum1+* is required for translation initiation in fission yeast. These findings suggest that overexpression of *sum1+* may inhibit the normal cell cycle response to stress by modulating translation initiation. We are therefore using fission yeast genetics to examine the role of translation initiation in stress response.
1. Wilkinson, M.G and Millar, J. B.A. SAPKs and transcription factors do the nucleocytoplasmic tango. *Genes Development* 12:1391-1397, 1998.
2. Humphrey T, Enoch T. Sum1, a highly conserved WD-repeat protein, suppresses S-M checkpoint mutants and inhibits the osmotic stress cell cycle response in fission yeast. *Genetics* 148:1731-42, 1998.
3. Naranda T, Kainuma M, MacMillan SE, Hershey JW The 39-kilodalton subunit of eukaryotic translation initiation factor 3 is essential for the complex's integrity and for cell viability in *Saccharomyces cerevisiae. Mol Cell Biol* 17: 145-53, 1997.
**17.00 DEMONSTRATION OF FREE RADICAL PRODUCTION IN RESPONSE TO SUB-LETHAL INJURY**
T. G. Aldsworth, C.E.R. Dodd and G.S.A.B. Stewart.
Division of Food Sciences (Microbiology), School of Biological Sciences, The University of Nottingham, Sutton Bonington Campus, Loughborough, Leics, LE12 5RD
It has recently been proposed that rapidly dividing and aerobically metabolising bacterial cells, when subjected to a relatively

mild shock (such as heat, ethanol or osmotic shock), will undergo a 'suicide response'. This 'suicide response' is predicted to be the result of an uncoupling of growth from metabolism as a result of stress. Since intracellular superoxide production and dismutation are very finely balanced, any disturbance to this balance is likely to have serious consequences for a bacterial cell. Thus uncoupling of growth from metabolism in exponential phase cells due to stress is anticipated to lead to a lethal burst of free radical production. The aim of the experiments reported here was to determine whether there is indeed a burst of free radical production in exponential phase bacterial cells when they are subjected to stress.

There are a number of different means to detect/ quantify intracellular free radicals which include: the chemiluminescent agent, lucigenin; the chromophore, MTT; and the use of spin trapping agents to determine electron spin resonance spectra. Exponential phase cultures of various bacterial strains, including *Escherichia coli*, *Streptococcus mutans*, *Mycobacterium smegmatis* and *Mycobacterium aurum*, were subjected to mild stress whilst radical production was monitored using the methods noted. It was found that exponential phase bacterial cells did indeed experience a significant increase in radical concentration when subjected to stress as compared with the control cultures. These data agree with the predictions of the 'suicide response' hypothesis.

### 17.15 THE SURVIVAL AND ADHESION CHARACTERISTICS OF A NATURALLY-ATTENUATED *ESCHERICHIA COLI* O157:H7 STRAIN

A. L. Cookson and M. J. Woodward.
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Verotoxigenic *Escherichia coli* (VTEC), especially serotype O157, are a continuing concern in the food industry as contaminants of red meat. Cattle are considered the major reservoir of VTEC. Human infection may caused by very small numbers of VTEC and often leads to life-threatening illnesses such as haemorrhagic colitis (HC) and haemolytic uraemic syndrome (HUS).This study focused on a particular O157:H7 strain, A84, isolated from cattle during routine surveillance. It was confirmed as having both verotoxins (VT1 and VT2), the locus for enterocyte effacement (LEE) and did not ferment sorbitol and hence was defined as a classical VTEC strain. In an initial study where gnotobiotic calves were dosed with $10^9$ CFU of A84, this strain could still be isolated 24 days post inoculation, but attaching and effacing lesions were not observed. Further genetic analysis of A84 revealed an *rpo*S gene containing a 96bp deletion that conferred an acid sensitive phenotype on this strain. There was no difference in the levels of adherence to Hep-2 tissue culture cells, or expression of intimin (*eae*A) in Western blots by A84, compared with other O157:H7 strains (cattle and human isolates). The formation by A84 of attaching and effacing (A/E) lesions on phalloidin-stained Hep-2 tissue culture cells was also observed. These data indicate that despite the lesion in the *rpo*S regulon of A84 and its sensitivity to inorganic acids - its ability to adhere to tissue culture cells and produce A/E lesions *in vitro*, was unaffected and it was still able to persist in an animal model. The possibility that a high mutation rate in *rpo*S during stationary phase may confer an adaptive advantage in non-dividing cells and allow VTEC O157:H7 strains to persist whilst others cannot will be discussed.

## POSTERS:

### C1 THE EXTRA-CYTOPLASMIC STRESS RESPONSE (ESR) IN *SALMONELLA TYPHIMURIUM.*

Sue Humphreys, Andrew Stevenson and Mark Roberts. Veterinary Microbiology Research Group, Department of Veterinary Pathology, University of Glasgow, Bearsden, Glasgow, G61 1QH

In *E. coli*, extracytoplasmic stress is partially controlled by the alternative sigma factor, RpoE (sE). In response to environmental stress or alteration in the protein content of the cell envelope, sE upregulates the expression of a number of genes including *htrA*. HtrA expression is also regulated by the two-component regulatory system CpxA/CpxR. It has been shown that *htrA* is required for intramacrophage survival and virulence in *S. typhimurium*. To investigate if sE -regulated genes other than *htrA* are involved in salmonella virulence we inactivated the *rpoE* gene of *S. typhimurium* SL1344 by allelic exchange and compared the phenotype of the mutant (GVB311) in vitro and in vivo with its parent and an isogenic *htrA* mutant (BRD915). Unlike *E. coli*, sE is not required for growth and survival of *S. typhimurium* at high temperatures. However, GVB311 did display a defect in its ability to utilise carbon sources other than glucose. GVB311 was more sensitive to hydrogen peroxide, superoxide and antimicrobial peptides (such as cecropin P1, BPI and polymyxin B) than SL1344 and BRD915. Although able to invade both macrophage and epithelial cell lines normally, the *rpoE* mutant was defective in its ability to survive and proliferate in both cell lines. Western analysis using a HtrA specific antibody indicates that HtrA is not expressed in the rpoE mutant. In order to understand further the complex regulation of *htrA* we are currently investigating conditions in which HtrA expression can be induced in the *rpoE* mutant which would indicate CpxR controlled expression.

### C2 INFLUENCE OF ENVIRONMENTAL STRESSES ON BIOCIDE SUSCEPTIBILITY OF *E. COLI* O157:H7

K E Middleton [1], M W Whitehead [1], D J Hill [1], J. T. Holah [2] & H Gibson [1]
[1] University of Wolverhampton, [2] Campden & Chorleywood Food Research Association

Bacteria on food contact surfaces experience a range of environmental stresses including extremes of temperature and pH, osmotic shock and desiccation, variation in nutrient availability and exposure to chemicals. Previous studies have shown that pre-exposure to such environmental stresses can induce a cross-protection response in bacteria leading to subsequent resistance to other stresses. In addition, other studies have shown that surface-attached bacteria are more resistant to biocides than those in suspension. The aim of this research is to investigate the relationship between pre-exposure to environmental

stresses, relevant to the food processing environment, on subsequent disinfectant resistance, using a non-toxigenic mutant of *E. coli* O157:H7 as a model organism.
Results are presented that show the effect of pre-exposure to both organic and inorganic acids and commercial detergents on the susceptibility of *E. coli* O157:H7 to quaternary ammonium compounds. Cells were pre-exposed to acetic, citric, lactic, malic, nitric, phosphoric, tartaric and hydrochloric acids at pH 4.5, and both non-ionic and anionic surfactants at their working concentrations, for 4 hours in liquid culture. The techniques used to determine the effect of these environmental stresses on *E. coli* O157:H7 were suspension tests, calculating the total viable counts, and surface-attachment studies using swabbing and direct epifluorescent microscopy.

The results confirmed that exposure to both organic and inorganic acids and detergents has an effect on both growth and viability of *E. coli* O157:H7. Results are also presented to show the subsequent effect of these pre-treatments on the biocide susceptibility of *E. coli* O157:H7 cells in suspension and when surface-attached.

### C3 A MUTATION IN *S. TYPHIMURIUM* LT2 *RPOS* RESULTS IN AN INCREASED RPOS INDUCTION TIME AND REDUCED LEVELS OF PROTEIN EFFICIENCY IN STATIONARY PHASE

Suzanne J. Jordan,1 Christine E. R. Dodd,1 and Gordon S. A. B. Stewart.2
1University of Nottingham, School of Biological Sciences, Food Sciences Division Sutton Bonington Campus, Loughborough, Leics., LE12 5RD, 2School of Pharmaceutical Sciences, University of Nottingham, University Park, Nottingham, NG7 2RD

In order to survive in relatively harsh micro-environments created by food, many bacteria employ a range of defence and virulence mechanisms, some of which are transcribed with the aid of the sigma factor, RpoS. Recent evidence suggests that a rare start codon in *S. typhimurium* LT2 *rpoS*, may influence cell survival upon exposure to sublethal stress e.g acid shock.

The current experimental work compared RpoS expression of *S. typhimurium* LT2 with two other environmental isolates of *Salmonella enterica* spp. RpoS expression was measured during growth via the use of the bioluminescent reporter plasmid pSB367 (*spv::luxCDABE*). In the LT2 strain, the induction time was significantly increased, and the efficiency of protein expression in stationary phase was reduced. Thus use of this strain to study *rpoS* and genes under its control may not reflect the situation in environmental isolates.

### C4 THE STRESS RESPONSE OF *BACILLUS SUBTILIS* DURING SPORULATION

S Movahedi and W. Waites,
School of Biological Sciences, Sutton Bonington Campus, University of Nottingham, Sutton Bonington, Leics LE12 5RD

The heat shock response was investigated in the Gram positive bacterium *Bacillus subtilis* using SASP- and SASP+ strains. The heat resistance of spores grown at 30C was enhanced by pretreatment at 48C for 30 min, 60 min into sporulation. Pretreatment of sporulating cells of the SASP- mutant of *B. subtilis* increased resistance to heating at 95C by five-fold whereas heating the SASP+ wild type spores at 95C resulted in a two-fold increase in D values. Concurrently to this acquired thermotolerance, two dimensional gel electrophoresis indicated that the cells induced the generation and/or overexpression of 11 heat specific shock proteins (estimated molecular weights ranging from 11 kDa to 87 kDa) in heat shocked cells compared with unstressed cells after the temperature upshift. The longer the time between exponential growth and heat pre-treatment, the fewer differences were observed on corresponding protein profiles. The greatest number of new proteins produced as a result of heat shock were observed between 30-60 min after heat shock. Alkaline phosphatase, dipicolinic acid (DPA) and TEM studies were performed on sporulating cells of *B. subtilis* cells in order to correlate the time of synthesis of stress proteins to stage in sporulation. The period of maximum increase in resistance as a result of heat shock and maximum new proteins appearing on two-dimensional gels corresponds to stage I-II of sporulation, before alkaline phosphatase, SASP and DPA production.

### C5 RECOVERY OF *CAMPYLOBACTER JEJUNI* CELLS AFTER LOW TEMPERATURE INCUBATION

R J T Thomas, M P Gallagher and F B Ward
University of Edinburgh

Abstract not received

## C6 THE EFFECT OF SALT AND pH STRESS ON THE BIODEGRADATION OF ANILINE

K. Bromley-Challenor, F. O'Neill and J. S. Knapp
Department of Microbiology, University of Leeds, Thoresby Place, Leeds LS2 9NL, UK

Aniline is a common raw material used in the manufacture of pesticides, drugs and dyestuffs. Consequently, unreacted aniline is found in industrial wastewater. Aniline is generally considered to be readily biodegradable under relatively low salinity (0.85% NaCl (w/v)) and neutral pH. However, prior to treatment, industrial wastewaters are often highly saline and low in pH; without dilution high salinity may limit the rate and extent of aniline degradation. This project is examining the possibility of operating reactors at high salinity and low pH and the study is looking at the rates and extent of biodegradation under these conditions.

Through enrichment, bacterial consortia capable of aniline degradation were isolated under various conditions (0.2, 4, 7% NaCl (w/v); pH 5 and pH 7). The original inoculum was derived from various environmental sources. Preliminary work included growth characteristics of stable cultures (yield, mean generation times, aniline die-away analysis and ammonia production). The effect of transient changes in salinity and pH has also been investigated. In most cases growth was slower at higher salinity, whereas a decrease in pH did not adversely affect growth. In all cases aniline was completely removed. Consequently, it may be possible to treat aniline-containing wastewater at high salinity and low pH rather than traditionally diluting and neutralising the wastewater.

**14.00 REACTIVITY OF EXPRESSED HEPATITIS B SURFACE ANTIGEN VARIANTS IN 7 COMMERCIALLY AVAILABLE DIAGNOSTIC ASSAYS**
J Ireland, J Kean, B O Donnell, A A Basuni, L A Wallace, W F Carman
University of Glasgow
Abstract not received

**14.15 HBV DNA QUANTIFICATION BY REAL-TIME PCR ON THE 'LIGHT CYCLER'**
N Saunder and R Begej
HRL, CHPL, London
Abstract not received

**14.30 CYTOMEGALOVIRUS DIAGNOSIS BY NESTED PCR**
C McCaughey, D E Wyatt, P V Coyle and H J O'Neill
Regional Virus Laboratory, Royal Hospitals Trust, Belfast
Abstract not received

**14.45 THE PERSISTENCE OF DRUG-RESISTANT VARIANTS OF HIV-1 IN THE BRAIN FOLLOWING COMBINATION THERAPY**
P M Strappe, J E Bell and P Simmonds
University of Edinburgh
Abstract not received

**15.00 LABORATORY AUDIT OF SEROLOGICAL DIAGNOSIS OF RESPIRATORY CHLAMYDIAL INFECTIONS**
M Sudhanva, R McCartney, E Abraham and W F Carman
Regional Virus Laboratory, Gartnavel General Hospistal, Glasgow
Abstract not received

**15.15 AN ENZYME LINKED OLIGONUCLEOTIDE PCR ASSAY FOR THE DETECTION OF HEPATITIS G VIRUS. ITS APPLICATION IN THE STUDY OF SUBJECTS AT RISK OF PARENTAL INFECTION**
J Connell[1], G Kaminski[1], C Keating[1] and J Riordan[2]
[1] University College Dublin, Dublin
[2] Blood Transfusion Service Board, Dublin
Abstract not received

**16.00 HUMANISED ANTI-VARICELLA-ZOSTER VIRUS ANTIBODY FRAGMENTS SUITABLE FOR TOPICAL THERAPY**
P.D. Drew, M. Moss, C. Grose[1], P. Cash, W.J. Harris and A.J.R. Porter
Dept. Molecular and Cell Biology, University of Aberdeen, Institute of Medical Sciences, Foresterhill, Aberdeen AB25 2ZD, Scotland, UK and [1]Dept. Microbiology, University of Iowa College of Medicine, Iowa City, Iowa 52242, USA
The herpesvirus varicella-zoster virus (VZV) causes two clinically distinct diseases (i) chickenpox and (ii) shingles. Chickenpox, the primary infection, is normally a mild, highly contagious disease of childhood, though in the growing immunocompromised population it can cause severe symptoms. During primary infection, VZV establishes and maintains latency in neural tissues. In some individuals reactivation occurs resulting in shingles, which generally affects older individuals and is a common and potentially serious complication of immunosuppressive therapies. Monoclonal antibody 206 binds to VZV coat glycoprotein H, neutralising VZV *in vitro* in the absence of complement and inhibiting cell to cell spread of VZV in cultured cells. This murine antibody has been humanised and shown to retain *in vitro* neutralising activity, and cross-reactivity with numerous clinical isolates. For shingles treatment, topical delivery of this antibody would be preferable for penetration to neural tissues, and to this end we have produced antibody fragments that are less than a third of the size of the parental humanised mAb 206. These antibody fragments retain the binding properties of the whole antibody, as demonstrated by immunofluorescence and cell monolayer ELISA. Large quantities of antibody fragments can be made inexpensively in bacteria, and in parallel studies we have shown antibody fragments are amenable to genetic manipulation to improve their stability in adverse environments. Therefore antibody fragments are ideal candidate therapeutic molecules for topical treatment of disease, where application may require formulation in oils and creams.

**16.15 THREE DISTINCT EPIDEMIOLOGICAL PATTERNS OF SRSV INFECTION**
A D Hale[1], K L Mattick[2], D C Lewis[2], X Jiang[3], R P Eglin[2], M K Estes[4], D W G Brown[1]
[1] ERVL, CPHL, London
[2] PHL, Leeds

[3] Centre for Pediatric Research, Eastern Virginia Medical School, Norfolk, USA
4 Baylor College of Medicine, Houston, Texas, USA
Abstract not received

**16.30 METAL ION CATALYSIS OF RNA CLEAVAGE BY THE INFLUENZA VIRUS ENDONUCLEASE**

L Doan, B Handa, N A Roberts and K Klumpp
Roche Discovery, Welwyn
Abstract not received

**16.45 IMPLICATIONS OF NOSOCOMIAL TRANSMISSION OF HBV TO TWO PATIENTS BY AN UNKNOWN ROUTE**

D Lobidel[1], M Smellie[3], D Goldberg[2], D Walker[3] and W F Carman[1]
[1] University of Glasgow
[2] Scottish Centre for Infection and Environmental Health
[3] Ayrshire and Arran Health
Abstract not received

**17.00 SEMINAL FLUID AND BLOOD HIV-1 RNA; RESPONSE TO ANTIRETROVIRAL THERAPY AND THE EMERGENCE OF DRUG RESISTANCE**

B Choudhury, Taylor J,. Workman, D Pillay and P.Cane
Antiviral Susceptibility Reference Unit, University of Birmingham Medical School. Edgbaston, Birmingham B15 2TT
An HIV positive individual initially failing treatment on a regimen of AZT and 3TC, provided matched sequential samples of blood and seminal fluid over a period of 40 weeks for viral load and genotypic analysis. During this period the patient received treatment with a combination of RTV, SQV and D4T.

A nucleic acid sequence based amplification (NASBA) technique was used to quantify HIV-1 RNA in both blood and seminal fluid. The emergence of genotypic drug resistance across both compartments was studied by sequencing multiple clones derived from the reverse transcriptase and protease regions.

During the study, the blood viral load varied between 130,000cp/ml and 650cp/ml, while the seminal fluid viral load ranged from <800cp/ml* and 1,000cp/ml. Drug resistance associated mutations were observed in both blood and seminal
fluid; at codons 41, 67, 70, 210, 215 (conferring resistance to AZT) and 184 (conferring resistance to 3TC) in the reverse transcriptase, and at codons 84 and 90 (conferring resistance to RTV and SQV respectively) in the protease. (* NASBA lower detection limit for seminal fluid).

**17.15 ANALYSIS OF HEPATITIS B VIRUS RESISTANCE TO LAMIVUDINE IN CHRONICALLY INFECTED PATIENTS**

PM Cook[1], D Radcliffe[1], D Mutimer[2], D Pillay[1], PA Cane[1]
[1] PHLS Antiviral Susceptibility Reference Unit,
[2] Department of Medicine, University of Birmingham Medical School, Birmingham B15 2TT
Lamivudine, a nucleoside analogue, is a strong inhibitor of hepatitis B virus (HBV) replication, and is currently in Phase III clinical
trials with HBV infected patients. However, resistance of HBV to lamivudine has previously been reported, and is associated with mutations in the polymerase YMDD motif at codon 550, of either YIDD or YVDD. Viral populations with a YVDD mutation also had a second mutation of L to M at position 526.

Three patients, chronically infected with HBV, whose viral loads were increasing while on lamivudine therapy, had virus from their blood investigated by PCR and sequencing of multiple clones containing a polymerase gene fragment.

Mutations associated with lamivudine resistance were found in all three patients, but the HBV populations were not homogeneous. Patients 1 and 2 had a mixture of YVDD and YIDD type polymerases, while patient3 had the YIDD type only. A sub-population of virus from patient 1 had the YVDD motif but did not have the mutation at 526. Also, in 15% of the clones, which contained a YIDD motif in the HBV polymerase, from patient 1, and 7-8% of the clones from the other two patients, there was an additional M at position 526. All clones in patients 1 and 3 had a further resistance associated mutation of V to L at codon 519.

In conclusion, although a particular mutated species of HBV may predominate in patients failing lamivudine treatment, other species are detectable by analysis of multiple clones. These may be of significance in cross-resistance to other antiviral drugs.

**09.00 LICENSED INHIBITORS OF INFLUENZA - TIME FOR A CHANGE?**

K Nicholson
Leicester Royal Infirmary
Abstract not received

**09.45 INFLUENZA NEURAMINIDASE INHIBITORS**

F G Hayden
University of Virginia, USA
Influenza neuraminidase plays an essential role in replication by allowing release of virus from infected cells and facilitating spread within the respiratory tract. The active ensyme site is highly conserved across influenza A and B viruses. Zanamivir (GG167) and GS4071 are potent, selective influenza neuraminidase inhibitors *in vitro* but have different pharmacokinetic

properties. Both topical zanamivir and oral GS4104, the ethyl ester prodrug of GS4071, are active in experimental animal and human infections. Twice daily inhaled zanamivir treatment ameliorates symptoms and, in high risk persons, reduces complications. Twice daily dosing of oral GS4104 is effective in treating acute influenza in adults and also reduces the risk of complications . Once daily dosing was 82% effective in preventing influenza illness. Studies of these drugs for prevention of influenza in nursing homes and for treatment of influenza in high risk patients and children are in progress. Intranasal delivery of trivalent, cold-adapted, live-attenuated influenza vaccine is highly protective in young children. Protection was shown last season against the A/Sydney drift variant, for which the injected vaccine provided minimal protection, and also in working adults. In summary, neuraminidase inhibitors and the intranasal cold-adapted vaccine provide new means of confronting the continuing threat of influenza infections.

**11.00 HCV HELICASE; TARGETS FOR NOVEL INHIBITORS**

N Yao
Schering-Plough Research Institute, USA

Abstract not received

**11.45 HBV TREATMENT WITH NUCLEOSIDE ANALOGUES AND EMERGENCE OF DRUG RESISTANCE**

D Pillay
University of Birmingham

Abstract not received

**14.00 HIV DRUG RESISTANCE AND RESISTANCE MONITORING**

B Larder
Virco UK Ltd, Cambridge

Abstract not received

**14.45 HIV TREATMENT, WHERE DO WE GO FROM HERE**

B Gazzard
Chelsea & Westminster Hospital, London

Abstract not received

**16.00 NEW DEVELOPMENTS IN ANTI-HERPES AGENTS**

E de Clerq
The Rega Institute for Medical Research, Belgium

Abstract not received

**09.20 THE BIOTECHNOLOGY YOUNG ENTREPRENEURS SCHEME - A PROBLEM BASED APPROACH TO BUSINESS AWARENESS**
J Peberdy
University of Birmingham
Abstract not received

**10.00 DOING IT YOURSELF: ENRICHMENT CULTURE AS A WAY OF KNOWING/LEARNING**
E Leadbetter
Marine Biological Laboratory, Woods Hole, New England, USA
Abstract not received

**11.10 A TWO-PART TALK**
**LEARNING ABOUT BACTERIAL PATHOGENS VIA A FICTIONAL BIO-WARFARE SITUATION**
**UNDERGRADUATE PROJECTS ON PUS FOR THE BLIND**
R E Sockett
University of Nottingham
Abstract not received

**11.50 STUDENT CENTRED LEARNING PROJECTS IN MICROBIOLOGY**
J Verran
Manchester Metropolitan University
Abstract not received

**14.00 UNDERGRADUATE PROJECTS DESIGNING PROBLEM-BASED LEARNING TUTORIALS FOR MICROBIOLOGISTS**
P Handley
University of Manchester
Abstract not received

**14.30 INDUSTRIALLY-RELEVANT TEACHING OF MICROBIOLOGY TO PHARMACISTS**
J Holland
University of Nottingham
Abstract not received

### 09.00 DIVERSITY AND ADAPTATIONS OF DEEP-SEA HYDROTHERMAL VENT PROKARYOTES

Daniel Prieur
CNRS/UMR 6539, IUEM/UBO, Brest, France

Before the discovery of deep-sea hydrothermal vents in 1977, the deep sea was perceived as a cold, dark, high pressure and nutrient-poor environment inhabited by psychrophilic, oligotrophic and barotolerant-to-barophilic microbial communities. By contrast, deep-sea vents areas are warm-to-hot and inhabited by animal communities whose density may reach 50 kg m-2. Invertebrates living in these warm biotopes are frequently in endosymbiotic relationships with autotrophic sulfur-oxidizing, in some cases methane-oxidizing, bacteria. In the hot areas of the ecosystem, temperature often reach 350 C, and precipitation produces mineral structures (black smokers) that contain thermophilic organisms. Most of the strains isolated and described were strict anaerobes (fermentative heterotrophs, sulphate reducers and Methanogens) belonging to the domain Archaea. Also, Thermus and Bacillus strains have recently been isolated from certain porous mineral structures that occur frequently at the Mid-Atlantic Ridge. With the exception of the recently isolated *Pyrolobus fumarii*, no novel genus specific to the deep-sea vents has been reported, but the dep-sea isolates are novel species (e.g. *Pyrodictium abyssi*, *Archaeoglobus profundus, Pyrococcus abyssi*, *Thermococcus profundus*, *Methanococcus jannaschii*) of genera previously described in coastal geothermal environments. Several species had even been found previously in coastal areas (e.g. *Staphylothermus marinus*, *Methanopyrus kandleri*) and probably have a ubiquitous distribution. Several deep-sea hyperthermophiles isolated at atmospheric pressure have been exposed to in situ pressures, which showed that the growth rate of most was increased by pressure, and that their optimal and maximal temperatures for growth increased by 3-4 C. Moreover, a novel strain of Thermococcales has been isolated which grows faster under hydrostatic pressure, but expresses a protein resembling to a stress protein, when cultivated at atmospheric pressure. Rather recently, plasmids have been found in deep-sea Thermococcales (anaerobic heterotrophic Archaea), and the plasmid pGT5 from *Pyrococcus abyssi* strain GE5, fully described. This plasmid, represent the first genetic element suitable for the construction of a cloning vector for Thermococcales, studies of which are presently limited by the lack of genetic tools. If we also consider that in addition to autotrophic endosymbionts and black smoker hyperthermophiles, free-living and surface bound mesophiles also exist in these environments, it is obvious that deep-sea vent microbial communities are highly diverse metabolically, physiologically and taxonomically. Because they are exposed to particularly extreme conditions, they show a great, but largely unexplored, potential for biotechnological applications. Unfortunately, progress remains slow, because access to these deep-sea sites is difficult, expensive, and limited to a small number of investigators. An increase in research efforts, accompanied by improved accessibility of micro-organism collections in both academic institutions and industry, on the basis of cooperation agreements, is probably the key to the future.

### 09.45 ARCHAEA: THE THIRD DOMAIN COMES OF AGE

D A Cowan
Unversity College London

Since the evolution of the concept of a third Domain of life over 20 years ago, the Archaea have been principally viewed as ‘oddities’, a collection of primitive micro-organisms largely restricted to the extreme environments of temperature (hyperthermophiles), salinity (extreme halophiles) and complete anoxia (methanogens). The apparent restriction of Archaeal distribution to these ‘exclusive’ habitats is countered by the evident diversity of anaerobic niches and by a growing awareness of the scope and volume of the thermophilic subterranean biosphere. Nevertheless, the apparent absence of Archaea from some of the more obvious niches (e.g., aerobic mesophilic habitats) has been the subject of some speculation. The recent application of molecular phylogenetic techniques has opened a new phase of Archaeal research. The discovery of non-methanogenic Archaea in mesophilic marine and terrestrial habitats suggests a much wider environmental distribution than previously considered, and highlights the need for novel isolation strategies to access these ‘unculturables’.

With a growing awareness of the distribution of the Archaea comes an increasing understanding of the metabolic diversity of these organisms. The deeply branched position of many Archaea on the phylogenetic tree makes these organisms a particular focus for investigation of primitive metabolic features. Such approaches have also led to the Archaea having a unique position in studies (and speculation) on the origins of life on Earth (and elsewhere).

The propensity of some Archaea to exist at, if not define, the outer boundaries of life has stimulated a number of research areas. Most notable are the uses of Archaeal gene products and metabolic systems as models for understanding fundamental biochemical processes. The development of our understanding of the mechanisms of protein hyperthermostability is used as an example.

## 11.00 HALOBACTERIA: ECOPHYSIOLOGY, DIVERSITY AND APPLICATIONS

William D. Grant
Dept Microbiology and Immunology, University of Leicester, Leicester LE1 7RH, UK

The red-pigmented extremely halophilic archaea (the halobacteria) occur ubiquitously in nature where the salt concentration is high, i.e. in salt lakes, soda lakes and salterns. The most striking feature of the halobacteria is their absolute requirement for high concentrations of NaCl. Although some strains may grow at salt concentrations as low as 1.5 M, most of the strains grow best at concentrations of 3.5 - 4.5 M NaCl and grow well in saturated NaCl (5.2 M). Halobacteria are the most halophilic organisms known, and form the dominant microbial population when hypersaline waters approach saturation, frequently imparting a red or pink coloration to the brines. To compensate for the high salt concentrations in the environment, the organisms accumulate mainly KCl, up to 5 M and may be growth limited by the amount of KCl in media.

Halobacteria currently comprise at least 12 genera (as defined by 16S rRNA gene sequence analysis), representatives of most genera being confined to neutral hypersaline environments such as marine salterns, but there are some types that are specifically adapted to the alkaline conditions found in extremely alkaline (>pH 10) and saline soda lakes. Neutral salt lakes and solar salterns may contain 107-108 cells ml-1 as judged by microscopic examination although the recovery of colony-forming units is usually two or more orders of magnitude lower. Saline soda lakes support comparable blooms of alkaliphilic types. Halobacteria can also be isolatedfrom crude solar salt and from proteinaceous products heavily salted with such salt (such as salted fish and salted hides), a consequence of the organisms becoming entrapped in a viable state for protracted periods within halite (NaCl) crystals, where they are to be found within fluid inclusions. Ancient halite deposits were once salt lakes that presumably supported dense populations of halobacteria (or halobacterial ancestors). These ancient deposits (often exploited as salt mines ) also contain populations of halobacteria, some of which seem to be within halite crystals and it is not clear what the origins of these organisms are - either they are the remnants of the original populations that flourished when the deposits were once salt lakes, or they are recent contaminants. Using molecular methods we have attempted to prove that halobacteria from ancient evaporites are derived from the original populations.

All of the halobacteria recorded so far are organotrophic and there is no evidence for autotrophy, despite the capacity for photophosphorylation possessed by those strains that contain light -driven chromophores. Halobacteria have yet to make a significant impact in biotechnology, although the light-driven, ion-translocating, retinal -based pigment rhodopsin is the subject of more patents than any other archaeal feature. There has been additional interest in halobacteria as producers of hydroxy alkanoates. Genetic systems including shuttle vectors are now available for detailed genetic manipulation and whole genome sequencing projects are well advanced.

## 12.05 INITIAL CHARACTERISATION OF NOVEL HALOPHILIC ARCHAEA, ABLE TO METABOLISE AROMATIC SUBSTRATES

D.J. Fairley[1,2], C.C.R. Allen[2], P. Morgan[4], D.R. Boyd[3], M.J. Larkin[1,2]
[1] School of Biology & Biochemistry, The Queen's University of Belfast
[2] Queen's University Environmental Science & Technology Research (QUESTOR) Centre, The Queen's University of Belfast
[3] School of Chemistry,The Queen's University of Belfast
[4] ICI Technology Ltd

Many diverse organisms posses aromatic oxygenases, and related enzymes, which are involved in the biodegradation of aromatic compounds. Numerous bacterial genera, and various eukaryotes are known to catabolise compounds of this type, by a range of well-characterised pathways. However, by comparison, very little is known about biodegradation pathways in the domain Archaea.

Recent work (Fu & Oriel, 1998) has confirmed that aromatic oxygenases are indeed present within the major group of aerobic Archaea - the extreme halophiles (order Halobacteriales). These organisms are particularly interesting because of their unique physiology and biochemistry, and because they may have industrial applications.

We have attempted to isolate novel extreme halophiles, with a view to studying biodegradation processes in hypersaline environments. We are also interested in establishing whether it will be feasible to exploit these organisms for the biotreatment of highly saline industrial effluents.

Enrichment cultures, using a range of substrates, have yielded several novel halophiles, able to grow in defined media containing >20% dissolved salts, using an aromatic substrate (Na benzoate) as the sole source of carbon and energy. The initial characterisation of these isolates is reported here.

*Funding bodies: Department of Education (Northern Ireland); CAST Award / ICI*

## 14.00 ARCHAEAL ENZYMES: A CONTINUUM OF STRUCTURAL AND FUNCTIONAL ADAPTATIONS FROM 0-100$^0$C

Michael J. Danson, David W. Hough & Garry L. Taylor
Centre for Extremophile Research, Dept Biology and Biochemistry, University of Bath, Bath, BA2 7AY

The Archaea include micro-organisms growing in some of the most extreme environments on earth. Consequently, their cellular components are remarkably stable entities, and this lecture will focus on our current understanding of the structure, function and stability of enzymes at extreme temperatures.

The strategies used by these proteins to achieve stability and activity at both low and high temperatures have been investigated in our laboratory by the determination and analysis of protein 3D-structures from organisms spanning the biological range of temperatures. The enzyme, citrate synthase, is currently our best-studied system, where we have determined high-resolution structures of a series of homologues from organisms growing optimally at 10, 37, 55, 80 and 100$^0$C. Structural trends have been identified and these potential adaptations have been correlated with protein inter-atomic forces and how these are thought to change with temperature. Possible stabilising features have then been tested by site-directed mutagenesis.

In addition to stability, cold-activity and thermo-activity will also be considered. What are the implications of temperature-induced changes in pKa values of ionisable amino acids involved in catalysis? In hyperthermophilic enzymes, does structural rigidity, necessary for stability, compromise catalytic activity, which depends on flexibility?

Finally, from the data available, are there likely to be general rules emerging on thermostability and thermoactivity? What are the prospects of introducing these features into proteins of our choice - should we engineer or evolve?

## 14.45 EXPRESSION AND CHARACTERISATION OF GLUCOSE DEHYDROGENASE FROM THE THERMOPHILIC ARCHAEON *SULFOLOBUS SOLFATARICUS*

David W. HOUGH, Ursula GERIKE, Narinder.I, HEYER and Michael J. DANSON
Centre for Extremophile Research, Dept Biology & Biochemistry, University of Bath, Bath, BA2 7AY, UK.

*Sulfolobus solfataricus* is a sulphur-oxidizing Archaeon and grows optimally at 85°C, pH 3. It metabolises glucose via the non-phosphorylated Entner-Doudoroff pathway, the first enzyme of which is glucose dehydrogenase (GDH):

$$\text{Glucose} + \text{NAD(P)}^+ + \text{H}_2\text{O} \rightarrow \text{Gluconate} + \text{NAD(P)H} + \text{H}^+$$

This reaction has been exploited to generate molecular hydrogen by coupling the GDH from *Thermoplasma acidophilum* (60°C) and a hyperthermophilic hydrogenase obtained from *Pyrococcus furiosus* (100°C). The process involves the breakdown of biomass to glucose and its subsequent conversion to gluconate by GDH. The resulting NADPH is then oxidised to molecular hydrogen by the hydrogenase, thus generating a renewable and non-polluting fuel source. However, in order to increase the efficiency of this conversion a more thermostable GDH is required and this project aims to produce the enzyme from *S. solfataricus* for this purpose.

Glucose dehydrogenase from *S. solfataricus* resembles the *T. acidophilum* enzyme in its molecular characteristics. It is a tetramer of polypeptide chain Mr ~ 40,000 and is catalytically active with the cofactors $NAD^+$ and $NADP^+$ and various sugars, including D-glucose and D-xylose. The corresponding gene has been cloned from a *S. solfataricus* genomic library, sequenced and expressed in *Escherichia coli*. The kinetic characteristics and thermal stability of the recombinant enzyme will be compared with the properties of the *T. acidophilum* enzyme

## 15.05 A NOVEL ALDOLASE FROM THE HYPERTHERMOPHILIC ARCHAEON *SULFOLOBUS SOLFATARICUS*: GENE CLONING AND EXPRESSION, AND CHARACTERISATION OF THE RECOMBINANTENZYME

Catriona L. KYDD*[1], Michael J.DANSON[1], David W. HOUGH[1] &Christopher D. REEVE[2].
[1] Centre for Extremophile Research, Department ofBiology and Biochemistry, University of Bath, Bath, BA2 7AY, U.K.
[2] Zeneca LifeScience Molecules, Belasis Avenue, Billingham, Cleveland, TS231YN, U.K.

*Sulfolobus solfataricus* is a member of thehyperthermophilic Archaea with a growth optimum at 80°C and pH 3 [1]. This organism metabolises glucose by a modified,non-phosphorylated Entner-Doudoroff pathway [2]. Glucose undergoes an$NAD(P)^+$ dependent dehydrogenation to gluconatethat is dehydrated to form 2-keto-3-deoxygluconate [3]. This thenundergoes an aldol cleavage to yield pyruvate and glyceraldehyde. At nopoint is there a requirement for ATP [2].

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

$$\text{2-keto-3-deoxygluconate} \longleftrightarrow \text{pyruvate} + \text{glyceraldehyde}$$

The enzymehas been purified from *Sulfolobus solfataricus* and acomplete gene sequence obtained (AC AJ224174). Amino acid sequence alignments suggest that this enzymeis a member of the N-acetylneuraminate lyase (NAL) superfamily comprisingSchiff Base-dependent aldolases, dehydratases and decarboxylases [4, 5].The active enzyme has been recombinantly expressed in E. coli, and purified to homogeneity. Kineticcharacterisation shows the recombinant protein to have similar propertiesto the native enzyme. The enzyme is remarkably thermostable, with ahalf-life of 2.3 hours at 100°C. The substrate specifity of this enzyme iscurrently being investigated.

We believe that KDG aldolase has considerable potential as athermostable enzyme for the stereoselective synthesis of carbon-carbonbonds in biotransformations.

*(1) Zillig, W., Stetter, K., Wunderl, S., Schulz, W., Priess, H. andScholz, L. (1980) Arch. Microbiol. 125, 259-269.*
*(2) De Rosa, M., Gambacorta, A., Nicolaus, B., Giardina, P., Poerio E.and Buonocore, V. (1984) Biochem. J. 224, 407-414.*
*(3) Giardina, P., De Biasi, M-G., De Rosa, M., Gambacorta, A andBuonocore, V. (1986) Biochem. J. 239, 517-522.*
*(4) Lawrence, M., Barbosa, J., Smith, B., Hall. N., Pilling, P., Ooi,H., Marcuccio, S. (1997) J. Mol. Bio. 266, 381-399.*
*(5) Babbitt, P.and Gerlt, J. (1997) J. Biol. Chem. 272, 30591-30594.*

**15.55 SUGAR METABOLISM IN ARCHAEA**

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A comparative analysis of the sugar degradation pathways of >hyperthermophilic Archaea and Bacteria and of halophilic Archaea will be given. The Archaea Pyrococcus, Thermococcus, Desulfurococcus and the Bacterium Thermotoga ferment sugars to acetate as main product; the Archaea Thermoproteus , Sulfolobus and Halococcus oxidize glucose to CO2 with either sulfur and oxygen as electron acceptors, respectively. In particular the following partial reactions of glucose catabolism in these organisms will be discussed.

(1) Glucose degradation to pyruvate: All Archaea analyzed do not contain the classical versions of the Embden-Meyerhof (EM) and Entner-Doudoroff (ED) pathway. Instead, glucose is degraded either by modified versions of EM pathway (Pyrococcus, Thermococcus, Desulfurococcus), the non-phosphorylated ED pathway (Sulfolobus) or a combination of both pathways (Thermoproteus). Halococcus employs a partially phosphorylated ED pathway. Modifications of the EM pathways implicate novel types of hexose kinases and glyceraldehyde-3-phosphate oxidizing enzymes. In contrast, both the classical EM and (to a minor extent) the classical phosphorylated ED pathway are operative in the hyperthermophilic, phylogenetic ancestral, Bacterium Thermotoga (and in other Bacteria).

(2) Acetyl-CoA conversion to acetate: In all Archaea, both hyperthermophiles and halophiles, acetate formation is catalyzed by only one enzyme, an acetyl-CoA synthetase (ADP forming) (Acetyl-CoA + ADP + P Acetate + ATP + CoA), which is a novel prokaryotic enzyme of acetate formation and energy conservation. In contrast, in the Bacterium Thermotoga (and all other Bacteria) the formation of acetate and ATP involves two enzymes, phosphate acetyltransferase and acetate kinase.

Schönheit, P. and Schäfer, T. (1995) Metabolism of hyperthermophiles. World J. Microbiol. Biotechnol. 11: 26-57
*Selig, M., Xavier, K., Santos, H. and Schönheit, P. (1997) Comparative analysis of Embden-Meyerhof and Entner-Doudoroff glycolytic pathways in hyperthermophilic archaea and the bacterium Thermotoga. Arch. Microbiol.167: 217-232*

**09.00 MICROBIAL PHYSIOLOGY OF THE HYPERTHERMOPHILIC ARCHAEON, *PYROCOCCUS FURIOSUS*.**

Richard J. Sharp, Jean C. Carr and Neil D.H.Raven
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Progress in DNA sequencing has lead to the availability of complete genome sequences for a number of microorganisms, including *Pyrococcus furiosus* (Weiss *et al*., 1999). Fifty percent of the ORFs identified for this organism are of unknown function. Additionally, many other biomolecules of potential biotechnological interest are produced by *Pyrococcus furiosus* (e.g. organic solutes and ether-linked lipids) but their production and function may not be recognisable from genomics alone. *Pyrococcus furiosus* is a robust fast-growing heterotrophic archaeon and the most extensively studied hyperthermophile. It is, therefore, ideal for addressing the gap between genomics and phenomics in microorganisms able to grow above 100°C.

The growth of *Pyrococcus furiosus* has been investigated in continuous culture in a gaslift bioreactor. This has permitted steady-state conditions to be maintained indefinitely for *Pyrococcus furiosus*, eliminating the variability obtained with batch culture. For example, defined and minimal media have been developed using this approach. Additionally the production of *Pyrococcus furiosus* biomass in multigram quantities under specified conditions has been made routine, including under stress (e.g. 7% salt or temperatures above 100°C). The key steps in the development of this fermentation system will be described, along with examples of biomolecules identified and studied using the biomass produced.

**09.45 GROWTH OF THE STRICTLY AEROBIC, HYPERTHERMOPHILIC ARCHAEON, *AEROPYRUM PERNIX***

Clive M. Buswell and Neil D.H. Raven
CAMR, Porton Down, Salisbury, Wilts. SP4 0JG

The archaeon *Aeropyrum pernix* is the first strictly aerobic organism to be isolated which grows up to 100$^{o}$C. *A.pernix* is a new member of the Crenarchaeota and belongs to the *Sulfolobales*. Furthermore, this organism produces one of the most thermostable extracellular proteinases reported. Large-scale cultivation would therefore be invaluable for the study of this organism. In order to undertake large-scale culture, however, a medium is required which produces cell yields and culture supernatant suitable for subsequent down-stream processing. In this study the medium for *A.pernix* was optimised using small batch cultures (50ml). Initially, the components of the basal marine medium (Bacto marine broth, Difco) were reduced to the minimum level possible without adversely affecting biomass yields or growth rate. The effects of the addition of other nutrients were then determined on the growth of cultures, and any found to be limiting, added to the basal medium. Cultures of *A.pernix* using the newly modified medium were then scaled up, and with further adjustments being made to growth conditions, such as, air flow and agitation, cultures of up to 120 litres have been produced.

**10.05 MECHANISMS OF ENZYME AND GENOME STABILITY IN HYPERTHERMOPHILIC ARCHAEA**

Frank T. Robb[1], Dennis L. Maeder[1], Jocelyne DiRuggiero[1], David Rice[2], Douglas S. Clark[3] and Robert Weiss[4].
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2Krebs Institute, University of Sheffield, UK,
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[4] Dept of Genetics, University of Utah, Salt Lake City, USA

Comparative studies of stable enzymes from this group of Archaea reveals insights into protein structure and function at very high temperatures. The critical importance of ion pair networks and the contributions of extrinsic stabilizing factors, such as high pressure, will be discussed. Complete genomic sequences of two members of the hyperthermophiles, *P. furiosus* and *P. horikoshii*, have recently been completed and released. Genome integrity is maintained by a combination of protective mechanisms, such as histones and high salt, in concert with high-capacity DNA repair activities. Recent evidence for genomic rearrangement and intergeneric lateral gene transfer will be discusssed. The tryptophan, histidine and maltose-trehalose operons of *P. furiosus* have been lost in *P. horikoshii* , which has a reduced genome size (1.83 mbp), compared with *P. furiosus* (1.91 mbp). The maltose-trehalose operon of *P. furiosus* is contained in a 17.8 kbp region, closely associated with the tryptophan and histidine operons of *P. furiosus*. The maltose-trehalose element, flanked by putative transposons, is virtually identical in sequence in *P. furiosus* and in *Thermococcus litoralis,* a related strain growing optimally at 88°C. We propose that the region was exchanged recently between these hyperthermophiles.

### 13.45 STRATEGIES OF IMMUNE RECOGNITION AND DEFENCE AGAINST INFECTIOUS ORGANISMS

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The association between microbial infection and an inflammatory response, involving the action of multiple components of the "innate" immune system (granulocytes, macrophages, complement etc.) is one of the classical models of microbial immunity. Similarly, the role of the adaptive immune response (both T and B cell) in orchestrating and amplifying this innate response so as to result in protective immunity has been extensively documented.

The past five years, however, have witnessed a significant paradigm shift in immunology. The realisation that innate immunity (via germline encoded genetic elements selected to recognise molecular components of all the major classes of vertebrate pathogens) plays a fundamental role in the inductive, as well as the effector phase, of most immunological responses introduces a new check-point in the events leading to a protective immune response. This interface between innate and adaptive immunity operates at many levels; but a major role is played by the dendritic cell. These cells are a minor numerical component of the cellular infiltrate at the site of most infections, but play a crucial role as sentinels, alerting the adaptive system and regulating the subsequent B and T lymphocyte response qualitatively and quantitatively.

This paper will outline the dendritic cell's role in sensing and responding to microbial infection, ultimately leading to an adaptive immune response. Some of the available information on the molecular mechanisms which regulate dendritic cell activity will then be reviewed. Finally, some of the major implications of this new immunological paradigm for immuno-prophylaxis (vaccination) and immunu-modulation of chronic infection will be discussed.

### 14.25 BACTERIAL EVASION OF THE EFFECTS OF COMPLEMENT

M A Kerr
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The complement system is one of the most powerful components of innate immunity and is the major effector system of the humoral immune response. In both cases, opsonisation of microorganisms for uptake by phagocytic cells is a major function. Direct complement mediated lysis is also important but *in vivo* it is restricted to a few families of bacteria. There are now three recognised pathways of complement activation, the classical pathway is activated primarily by IgG and IgM antibodies bound to antigen, the alternative pathway is also activated by bound antibody but is also activated directly by (carbohydrate) bacterial cell wall components. The newly described lectin pathway uses C4 and C2 of the classical pathway but is activated by mannan binding lectin (MBL) and possibly other plasma derived lectins without the need of antibody. Clearly the composition of the cell wall is a major factor in the efficiency of activation of complement by innate pathways and therefore microorganisms can evade the complement system by changing their cell surface carbohydrates. The specificity and class of the antibody response to any infection will affect the degree of activation of complement. IgA antibodies have been reported to inhibit complement activation although this is now disputed. Some species have developed specialised mechanisms to avoid complement activation or its effects by inhibiting the biological functions of different complement components or the functions of phagocytic cells which are triggered by antibody and complement. There are numerous mechanisms such as the secretion of proteases which cleave and inactivate key components eg C3b or C5a, production of surface proteins such as the M proteins of group A streptococci which block C3 binding and the production of ammonia which inactivates C3. These mechanisms will be discussed.
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### 15.35 VIRAL EVASION OF COMPLEMENT

Peter Lachmann
University of Cambridge

Conventional immunological wisdom has it that immunity to viruses lies with neutralising antibodies that can prevent infection and cytotoxic T cells that promote recovery by killing virus infected cells. That these appear to be the most effective defence strategies reflects the successful evolution by viruses of mechanisms to subvert other modalities of the immune response and their capacity to evade complement mediated damage provides a good example of this.

Many viruses have ìlearnedî to avoid complement attack by evolving a distribution of epitopes on their surface glycoproteins that does not allow antibody reacting with them to activate complement. This has been known since the early days of clinical virology: complement fixation measures antibodies to nucleocapsids and haemagglutination inhibition those to the surface glycoproteins of myxoviruses.

Other viruses, notably dengue, have acquired the capacity to enter cells through complement receptors using fixed complement on their surfaces as the ligand. This is the phenomenon of complement mediated enhancement which underlies dengue haemorrhagic fever; and to a much smaller extent is seen also with HIV. This phenomenon is distinct from viruses using complement receptors as their own ligand ( as EBV does with CR2) which shows no need for the virus to interact with complement.

There are also a number of examples where viruses ñ usually those with substantial genomes such as pox and herpes viruses ñsynthesise proteins that protect against complement activation in an analogous way to the complement control proteins of mammalian cells. In the case of the complement control protein (CCP) of vaccinia and orf 4 of the murine gamma herpes virus these proteins are based on the ìshort consensus repeatî that makes up the all mammalian CCPs that act at the C3 stage of complement activation. Herpes simplex virus has in its Gc glycoprotein a complement inhibitor that is functionally analogous to these CCPs but is not structurally related. This is therefore clearly a case of convergent evolution and is in strong contrast to the situation in Herpes Virus Saimiri which has both an SCR-based CCP and a close homologue of mammalian CD59, a complement inhibitor acting on the membrane attack complex. HVS CD59 has closer sequence similarity to the CD59 of its natural host, the squirrel monkey, than this latter molecule has to human CD59. It is difficult to escape the conclusion that this is therefore an example of gene capture.

In some case it has already been shown that these viral complement inhibitors are required for pathogenicity and they are potential targets for antiviral chemotherapy

### 16.15 IDENTIFICATION OF A CYTOKINE-INDUCING LIPID A ASSOCIATED PROTEIN FROM PORPHYROMONAS GINGIVALIS: A DUAL ROLE FOR BACTERIAL EXTRACELLULAR PROTEASES?

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Lipid A-associated proteins (LAPs) of enteric bacteria stimulate pro-inflammatory cytokine synthesis and may be important virulence factors. Are similar proteins found in oral bacteria implicated in human pathology? Porphyromonas gingivalis is a major causative organism of the periodontal diseases whose surface proteins have been shown to be rich in proteolytic and agglutinating activities. Isolation and assay of the LAP fraction of this bacterium revealed that it had potent pro-inflammatory cytokine inducing activity. Fractionation studies showed a number of proteins in the LAP fraction and, using a modified SDS-PAGE system, it proved possible to identify the active protein. This was a 17kDa protein, and N-terminal sequencing revealed that this LAP was homologous to an internal region of a conserved adhesin domain found in the family of P. gingivalis extracellular proteins including the major R1 protease. In contrast, the purified R1 protease was able to completely degrade the key pro-inflammatory cytokine interleukin-1b and abrogate its biological activity. Thus different domains of this single protein have opposing effects on the levels of pro-inflammatory cytokines. The balance of the activities of these two domains of this major cell surface protein could play a significant role in controlling cytokine networks in the periodontal diseases. Moreover, inhibition of the activity of this protease, which would appear at first sight to be beneficial, could possibly enhance inflammation.

### 16.30 CYTOKINE DEGRADATION BY *PORPHYROMONAS GINGIVALIS*

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Department of Microbiology1, Cellular Microbiology Research Group2, Eastman Dental Institute, University College London, London WC1X 8LD, UK and Division of Endocrinology3 National Institute for Biological Standards andControl, South Mims, Potters Bar EN6 3QG, UK.

This study compared the ability of Porphyromonas gingivalis, an oral commensal organism which is a causative agent of the periodontal diseases, grown planktonically or as a biofilm to degrade the cytokines, interleukin (IL)-1b, IL-6 and interleukin-1 receptor antagonist (IL-1ra). Cytokines were incubated with either cell-free supernatants from liquid-grown P. gingivalis or with biofilm cultures of this organism and breakdown was assessed by subjecting cytokines to SDS-PAGE followed by western blotting with specific anti-cytokine antibodies. The ability of purified P. gingivalis R1 proteinase to degrade cytokines was also examined.

Both biofilms and planktonic culture supernatants were able to hydrolyse all three cytokines, with IL-6 being particularly susceptible to degradation. Cytokine degradation occurred even in the presence of an excess of other proteins, in this case serum, suggesting the bacterial proteinases have a specificity for degrading cytokines. Purified R1 proteinase was active in degrading these cytokines.

This study reveals that exported proteinases of P. gingivalis can degrade pro-inflammatory (IL-1b), anti-inflammatory (IL-1ra) and cytokines with both attributes (IL-6) even in the presence of serum proteins. If this occurs in vivo it may lead to a disruption of the highly complex cytokine network responsible for maintaining periodontal health.

### 16.45 PARAMYXOVIRUSES AND CELLULAR ANTI-VIRAL DEFENSE MECHANISMS: IMPORTANCE FOR VIRUS

## PATHOGENESIS

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Viruses have evolved their replication strategies *in vivo* in the face of a number of powerful anti-viral responses, including those induced by the interferons (IFN). Whilst many DNA viruses have the ability to inhibit IFN responses, they usually achieve this by blocking enzymes such as PKR. Much less is known about how RNA viruses interact with the IFN system. Evidence will be presented that the paramyxoviruses Simian Virus 5 and Sendai virus produce products which interfere with interferon (IFN) signalling, thereby preventing the induction of a classical anti-viral state in a cell. Their ability to block IFN signalling is dependent on the species from which the cells were derived, thus defining one major constraint which prevents paramyxoviruses from crossing species barriers.

## 09.00 VIRAL EVASION OF IFN REGULATED PATHWAYS

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Three known pathways mediate the antiviral activities of interferon (IFN). The key functional components of these pathways are the interferon-induced double-stranded RNA (dsRNA)-activated protein kinase (PKR), the 2-5A activated RNaseL and the Mx proteins. The activity the Mx proteins is restricted to some negative RNA stranded viruses but PKR and RNase L can inhibit the replication of different viruses. However, viruses have evolved several ways of inhibiting the functioning of these antiviral pathways. Virus-activated protease activity can degrade the antiviral enzymes, dsRNA can inhibit the activation of PKR and virus-encoded proteins can act as dsRNA decoys or substrate mimics. Despite these strategies IFNs remain the first line of defense against viral infections and provide an important link between innate and specific immunity. To determine whether other IFN regulated pathways exist which could substitute for PKR or RNaseL mediated antiviral activity, RNase L-/- mice and PKR-/- mice were crossed onto an Mx1-/- background to generate a strain of triply deficient (TD) mice. After infections with encephalomyocarditis virus (EMCV), the TD mice died three to four days earlier than infected, wild type mice. However, there was an IFN dose-dependent increase in survival times after EMCV infections for both the TD and wild type mice. Surprisingly, cultured embryonic fibroblasts lacking both RNase L, or PKR, or both proteins were still able to mount a substantial residual antiviral response providing unequivocal evidence for the existence of novel, innate immune pathways against viruses. To begin to address the nature of these pathways we have probed oligonucleotide arrays with cRNA prepared from IFN-a, -b or *g treated cells. Many novel IFN-stimulated genes were identified that are implicated in diverse functions and provide new insights into the antiviral activities of IFNs.

## 09.40 BACTERIAL CONTROL OF CYTOKINE NETWORKS

Brian Henderson, Cellular Microbiology Research Group
Eastman Dental Institute, University College London, UK

Inflammation, which is controlled by pro- and anti-inflammatory cytokine networks, is both a protective and a pathological mechanism. Bacterial infections invariably cause inflammation, and in conditions such as septic/toxic shock, meningitis, pneumonia and tuberculosis inflammation can be fatal. It is vital, in order to treat bacterial diseases, that we understand how bacteria control the production (and activity) of cytokines which manipule host inflammatory responses. All bacteria, at least when cultured, produce a range of cytokine-inducing virulence factors which we have termed modulins and which are responsible for the inflammatory response to bacteria. In spite of this, the enormous numbers of commensal bacteria which populate the mucosal surfaces of mammals generally do not induce inflammation, unless there is a defect in cytokine signalling. We have proposed the hypothesis that the failure of the commensal microflora to trigger mucosal inflammation is due to their production of proteins (microkines) which can regulate the induction and perpetuation of host pro-inflammatory cytokine networks. We further hypothesize that with opportunistic pathogens it is the balance between modulin and microkine production, which may in turn be under the control of host factors, that defines whether these organisms are harmless or pathogenic. The bacterial molecules which can induce cytokine synthesis and the host receptor systems that they utilise to do so will be discussed in the context of the stimulation and evasion of immune and inflammatory host responses to bacteria.**BACTERIAL CONTROL OF CYTOKINE NETWORKS**

**10.50 ADVERSARIAL RELATIONSHIP BETWEEN THE LEISHMANIA LIPOPHOSPHOGLYCAN AND PROTEIN KINASE C OF HOST MACROPHAGES**

Salvatore J. Turco
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The remarkable ability of Leishmania parasites to survive in macrophages is attributed to specialized molecules on their cell surface. One of these specialized molecules is a unusual lipid-anchored polysaccharide named lipophosphoglycan (LPG), the dominant glycoconjugate on the cell surface of all Leishmania promastigotes. Its relative abundance, unique structure, and cellular location have resulted in its determination as an essential virulence determinant. Numerous descriptive roles have been proposed for the Leishmania LPG. One pivotal role that we have been concentrating on is the function of LPG in survival of Leishmania upon entry of the parasite into a host macrophage. The underlying basis for surviving with impunity is accomplished, at least in part, by preventing macrophages from becoming activated, a process mediated by protein kinase C (PKC). In vivo, LPG and PKC are found on opposite sides of the membrane. During phagocytosis, LPG is intercalated on the macrophage surface while PKC binds to the cytoplasmic side of the plasma membrane. Despite the positions of LPG and PKC on opposing monolayers, inhibition of PKC activity is still observed. Importantly, full-length LPG is necessary for optimal inhibition of PKC, consistent with observations that LPG remains intact during phagocytosis of Leishmania. The mechanism by which LPG inhibits PKC activity appears to be related to the observation that integration of LPG into membranes stabilizes the bilayer against the formation of an inverted hexagonal phase structure. This bilayer stabilizing property of LPG is the converse of the bilayer destabilizing property of DAG, the physiological activator of PKC. This demonstrates that inhibition of PKC by LPG may be of biological importance in the initial stages of infection by Leishmania and that this inhibition must be a consequence of effects of LPG on membrane physical properties that can be transmitted across the bilayer.

**11.30 AN AFRICAN SWINE FEVER VIRUS PROTEIN WHICH INHIBITS THE CELLULAR PHOSPHATASE, CALCINEURIN: MAPPING FUNCTIONAL DOMAIN(S) OF THE PROTEIN.**

James E. Miskin, Charles C. Abrams, Lynnette C. Goatley & Linda K. Dixon
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African swine fever virus (ASFV) is a large dsDNA virus which replicates in the cytoplasm of macrophages. Nucleotide sequence analysis of the ASFV genome revealed a number proteins potentially able to interfere with host defence systems (1). The transcription factors NF B and NFAT control the expression of many immunomodulatory proteins. We have previously shown that ASFV inhibits proinflammatory cytokine expression in infected macrophages, and NF B activation is inhibited by a viral protein A238L (2). The protein also displays the activity of the immunosuppressive drug Cyclosporin A, by inhibiting NFAT-regulated gene transcription in vivo. This it does by binding the catalytic subunit of calcineurin and inhibiting calcineurin phosphatase activity (3). We have identified a 14 a.a. region of A238L which binds to calcineurin in the Yeast 2-Hybrid system. Full length clones of A238L were created with mutations spanning this 14 a.a. region. These were subsequently used to identify the calcineurin-binding motif in A238L.

References: 1 Yanez, R. J. et al. (1995) Virology 208, 249; Dixon L. K. et al. J. Gen. Virol. 75, 1655. / 2 Powell, P. P., Dixon, L. K. & Parkhouse, R. M. E. (1996) J. Virol. 70, 8527. / 3 Miskin et al. (1998) Science 281, 562.

**11.45 EXPRESSION OF CYTOKINE RECEPTORS BY ECTROMELIA VIRUS**

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Ectromelia virus is an orthopoxvirus which causes a severe and often fatal disease of laboratory mice called mousepox. This virus provides an excellent model for the study of poxvirus pathogenesis and immune evasion for a number of reasons. Firstly, the mouse is the natural host of ectromelia virus. Secondly, several loci in the mouse genome have been found to confer resistance to mousepox in common laboratory strains. Finally, the recent identification of a number of immunomodulatory gene products in poxviruses, including soluble cytokine receptors, should enable us to understand at the molecular level how ectromelia virus evades immune responses.

To facilitate our studies of immune evasion we have screened a number of ectromelia virus isolates for their expression of several cytokine receptors and characterised the binding specificity of those receptors which are expressed by the virus. We will report our findings on receptors for IL-1, TNF, interferons and chemokines.

*Presenting the paper. (28 years old, maybe eligible for the Promega Prize)*

**12.00 THE VACCINIA VIRUS A41L GENE ENCODES A NOVEL SECRETED IMMUNOMODULATORY FACTOR**

Aylwin Ng, David Tscharke, Patrick Reading and Geoffrey L. Smith
Sir William Dunn School of Pathology, University of Oxford, South Parks Road, Oxford, OX1 3RE

The A41L open reading frame (ORF) shows features typical of a secreted glycoprotein, namely a signal peptide, hydrophobic extracellular domain with sites for attachment of carbohydrate, but no anchor sequence. An antiserum raised against the A41L protein expressed from baculovirus detected an early, secreted glycoprotein in the supernatants of cells infected with all orthopoxviruses tested. Despite this conservation, the gene was non-essential for virus replication in cell culture and a vaccinia virus Western Reserve deletion mutant showed *in vitro* growth properties indistinguishable from wild type and revertant viruses. In a murine intranasal model of infection the mutant virus showed an unaltered virulence, but in a mouse intradermal model, the deletion mutant caused enhanced lesion size that was accompanied by enhanced infiltration of leukocytes and lower virus titres. The A41 protein shows 25% amino acid identity with vCKBP, a protein expressed by several poxviruses and which binds CC chemokines.

**14.00 VACCINIA VIRUS INHIBITION OF CYTOKINES AND CHEMOKINES**

Geoffrey L. Smith
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Vaccinia virus is the live vaccine that was used to vaccinate against smallpox. Like other poxviruses, vaccinia virus is a large, double stranded DNA virus that replicates in the cell cytoplasm and produces two forms of infectious virus particle. The genome contains about 200 genes with those near the genomic termini being more divergent between poxviruses. The genes in these regions are mostly non-essential for virus growth *in vitro* and encode factors that affect virus virulence and host range. A subset of these virulence factors includes proteins that are secreted from the infected cell and which bind host cytokines, chemokines, interferons (IFNs) or complement factors. In vaccinia virus, soluble receptors, or binding proteins, for complement factor 3b and 4b, interleukin (IL)-1β, IFN-α/β, IFN-γ and CC chemokines have been described. The properties of these proteins and their roles in virus pathogenesis will be reviewed. In addition the expression of tumour necrosis factor (TNF) receptors in a few strains of vaccinia virus will be described. Interestingly, these TNFRs are present in the extracellular medium and at the cell surface, unlike the TNF receptors described for other poxviruses which are not found at the cell surface.

### 14.40 CHEMOKINES AND CHEMOKINE RECEPTORS: INTERACTIONS WITH MICRO-ORGANISMS.

Philip M. Murphy
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The chemokine signaling system includes more than 50 secreted pro-inflammatory peptides and 15 G protein-coupled receptors that together orchestrate specific leukocyte trafficking in the mammalian immune system, ideally for antimicrobial defense and tissue repair processes. Paradoxically and perversely, some chemokines and chemokine receptors are also promicrobial factors and facilitate infectious disease, the result of either exploitation or subversion by specific microbes. Several modes of exploitation are known, including usage of *cellular* chemokine receptors for cell entry by intracellular pathogens, (e. g. CCR5 by HIV in AIDS and Duffy by Plasmodium vivax in malaria), and usage of *virally-encoded* chemokine receptors for host cell proliferation (e. g. ORF 74 of HHV8 in Kaposiís sarcoma). Two modes of subversion are known: virally-encoded chemokine *antagonists* (e. g. HHV8 vMIP-II and Molluscum contagiosum virus MC148), and virally-encoded chemokine *scavengers* (e. g. poxvirus 35 kDa proteins). Understanding how microbes turn the tables on the chemokine system may point to new methods to prevent or treat infection, or, more generally, to treat inappropriate chemokine-mediated inflammation.

### 15.50 BACTERIAL EVASION OF KILLING BY PHAGOLYSOSOMES

Kathleen A. McDonough
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Microbes employ a variety of mechanisms to avoid killing b phagolysosomes within macrophages. The means by which Mycobacterium tuberculosis survives within macrophages is central to our understanding of tuberculosis disease, which remains one of the world's leading killers. We have compared the intracellular trafficking patterns of single versus clumped mycobacteria by transmission and high voltage electron microscopy.We have also addressed the question of whether prior passage within macrophages affects the trafficking of tubercle bacilli upon reinfection of new macrophages. Two patterns of interaction with macrophages were identified. Both virulent and avirulent bacilli that entered macrophages singly rather than in small clumps nearly always underwent less fusion with lysosomal components than bacteria that entered as small clumps. In contrast, virulent, but not avirulent, mycobacteria that reentered macrophages as small clumps following intracellular passage within other macrophages were capable of restricting their interaction with phagolysosmal components. These virulent bacteria were sometimes also found free within the host cytoplasm when examined by stereo imaging using high voltage electron microscopy. These virulence-associated fusion avoidance and vacuolar escape phenotypes required de novo bacterial protein synthesis within macrophages and were inhibited by the presence of serum components at the time of reinfection. Two-dimensional polyacrylamide gel electrophoresis and mass spectrometry are currently being used to identify proteins that are specifically expressed by the virulent bacteria within macrophages and which may be of particular importance to the success of virulent Mtb bacteria within macrophages.

## 16.30 TYPE III PROTEIN SECRETION AND RESISTANCE TO PHAGOCYTOSIS

Åke Forsberg
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Type III secretion systems are used by several Gram-negative pathogens to deliver anti-host factors in the host during infection. Most of the components of the secretion apparatus show homology between different pathogens whereas the proteins secreted by the system show greater variability. This is not unexpected as the effect on host cells mediated by the secreted proteins of different bacteria show great variability. For instance, whereas one the type III system of *Salmonella* (SPI1) induces uptake of the bacteria into eukaryotic cells the system of *Yersinia* mediates resistance to phagocytosis.
Even though most of the components of the secretion apparatus show homology between different pathogens and in many cases also have been shown to be functionally conserved it is possible to "subgroup" some of these pathogens. For instance, the secretion systems as well as the secreted proteins involved in delivery of effector proteins across the eukaryotic cell membrane show great similarity between *Salmonella* (SPI1) and *Shigella* and between *Yersinia* and *Pseudomonas aeruginosa*. Common to *Salmonella* and *Shigella* is that the type III systems are involved in a cell contact dependent induction of uptake by host cells. The type III secretion systems of *Yersinia* and *P. aeruginosa* on the other hand have share the ability make these pathogens resistant to phagocytosis.
The delivery of effector proteins into the target cell (translocation) by *Yersinia* involves at least five of the secreted proteins YopB, YopD, LcrV, TyeA and YopK. Of these, YopB and YopD are absolutely required for delivery of all the translocated proteins. In all, six proteins (five in *Y. pseudotuberculosis*) are targeted into host cells by pathogenic *Yersinia* species and of these YpkA, YopH, YopM and YopE are essential virulence determinants in the systemic mouse infection model. YopE and YopH have been shown to act in concert to mediate resistance to phagocytosis. YopE mediates a dramatic effect on the actin microfilament of host cell and YopH has a protein tyrosine phosphatase activity (PTPase) and effectively dephosphorylates host proteins. Even though YopE has a drastic effect on the host cell morphology the effect on inhibition of phagocytosis is less powerful than that of YopH. The most likely explanation for this phenomenon is that YopH is able to dephosphorylate host proteins already within 30 seconds after infection of a macrophage with *Y. pseudotuberculosis.* In addition, YopH contains a domain which efficiently targets the protein to focal adhesions and the these structures are also disrupted in macrophages infected with *Yersinia* expressing YopH which also suggests that this is the main mechanism by which YopH blocks phagocytosis.

## 09.00 VIRUS EVASION OF NATURAL KILLER CELLS

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Natural killer cells are an important component of the innate cellular immune response. They are particularly important during the early immune responses following virus infection, prior to the induction of cytotoxic T cells (CTL). NK cells are sensitive to aberrant expression of cell surface molecules, in particular MHC class I. Thus, cells expressing low levels of surface MHC class I are generally susceptible to recognition by NK cells, with concomitant triggering of cytolytic and cytokine-mediate responses. Many viruses, including the cytomegaloviruses (CMVs), downregulate cell surface MHC class I: this is likely to provide protection against CTL-mediated clearance of infected cells, bur may also render infected cells sensitive to NK cell attack.
This presentation focuses upon CMV-encoded proteins that are believed to promote evasion of NK cell-mediated immunity. The MHC class I homologues, encoded by all CMVs characterised to date, have been implicated as molecular "decoys', which may mimic the ability of cellular MHC class I to inhibit NK cell functions. Results from studies *in vitro* are not uniform, but in general support the proposal that the CMV class I homologues engage inhibitory receptors from NK cells and other cell types which normally interact with cellular class I. Consistent with this, *in vivo* studies of murine CMV indicate that the class I homologue is required for efficient evasion of NK cell-mediated clearance. Recently a second murine CMV protein, a C-C chemokine homologue, has been implicated as promoting evasion of NK and T cell-mediated clearance *in vivo*.

**09.40 ESCAPE FROM CYTOTOXIC T LYMPHOCYTES (CTLS) BY DNA VIRUSES**

G Eric Blair
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In common with many viruses, the DNA viruses have evolved mechanisms to evade the cellular immune system and establish latent and persistent infections in their hosts. An overview will be given of the mechanisms adopted by small DNA viruses ( principally the adenoviruses) and herpesviruses to evade CTLs by down-regulating components of the antigen processing and presentation pathway in virally-infected and in virally-transformed cells.

Human adenoviruses (Ads) can establish latent infections in the host and certain Ads (the oncogenic Ads, such as Ad12) can induce tumours following inoculation into baby rodents. Latent infections may be due to a post-translational down-regulation of surface major histocompatibility complex (MHC) class I molecules by their retention in the endoplasmic reticulum through binding to an early Ad gene product, the E3-19kDa glycoprotein. In contrast, in highly oncogenic Ad12-transformed cells, down-regulation of surface MHC class I molecules is mediated by expression of the Ad12 E1A gene and may permit these cells to evade the T cell-mediated immune system, thus eliciting tumours. Much evidence points to a major role for Ad12 E1A-mediated transcriptional repression of all class I heavy chain genes ( H-2K, D and L in the mouse) in this process. In contrast, neither Ad2 nor Ad5 E1A participates in transcriptional repression, providing a possible explanation for the normal or elevated levels of class I molecules found on these cells and the non-oncogenic phenotype of these viruses. The mechanism whereby Ad12 E1A down-regulates class I transcription is of considerable interest. Several models have been proposed, including E1A-mediated targeting of upstream elements which negatively regulate transcription driven from the class I or heterologous promoters and also a well-characterised highly conserved enhancer element (the class I regulatory element, or CRE, located between -159 to -201). Two domains of the CRE , the region II domain (which binds cellular transcription factors such as RXR-$\beta$ and COUP-TF) and the region I domain (which binds factors of the NF-$\kappa$B/c-*rel* family) have been reported to be targets for Ad12 E1A.

Other genes located in the MHC play major roles in the biosynthesis of surface class I molecules. In particular, the products of two subunits of the proteasome (LMP2 and LMP7) and the TAP1 and TAP2 (Transporter associated with Antigen Processing) products are involved in generation and transport of antigenic peptides that bind to class I heterodimers in the endoplasmic reticulum. We have shown that the levels of TAP1 and LMP2 transcripts are also down-regulated in Ad12-transformed cells and that transcription of rat TAP1 and LMP2 transcripts is directed from a 564 bp intergenic region which is significantly less active in Ad12-transformed cells compared to those transformed by Ad5. Therefore in highly oncogenic Ad12-transformed cells there appears to be a global repression of several MHC genes whose products are involved in antigen presentation.

**10.50 HIV CONTROL BY AND ESCAPE FROM CTL**

Dr Sarah Rowland-Jones
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There is an increasing consensus that one of the principle factors involved in the control of HIV replication is the presence of circulating HIV specific cytotoxic T-lymphocytes (CTL). Support for this view has come from the recent availability of a technique to quantify antigen specific T cells directly from peripheral blood mononuclear cells (PBMCs), using peptide HLA tetrameric complexes. Using a complex of HLA A2 and the immunodominant A2 restricted peptide from HIV P17, we have observed a striking inverse correlation between plasma viral load and CTL numbers in untreated HIV infected patients at all stages of disease. The study using this technique to look at the dynamics of the primary immune response to acute HIV infection show a very close relationship between the appearance of HIV specific CTL and control of viral load in early infection. We have also observed that some highly HIV exposed but persistently seronegative subjects have both circulating and mucosal HIV specific CTL, suggesting that this response may occasionally be associated with protective immunity.

Further evidence for the role of HIV specific CTL in controlling viral replication has come from studies of how the virus is adapting to evade the CTL response. HIV is able to interfere with class 1 HLA expression and also with FAS/FAS ligand expression, reducing the susceptibility of infected cells to CTL lysis. Both in early and in late disease, viral variants which contain mutations in the epitopes recognised by CTL can arise and reach fixation. The dynamics of this relationship between HIV control and viral variation will be discussed further.

**11.30 PROTEIN GLYCOSYLATION BY MYCOBACTERIA: A MECHANISM FOR MASKING T CELL EPITOPES?**

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The glycosylation of proteins by mycobacteria has been well established although the biological function of this post-translational modification is unknown. The complex mycobacterial antigen PPD, which is used for tuberculin skin testing in cattle, is prepared from culture filtrates of *Mycobacterium bovis* and contains several glycosylated proteins. In preliminary experiments to test whether glycosylation of proteins by mycobacteria affects antigen processing and presentation, stronger responses against chemically deglycosylated PPD were observed with T cells isolated from *Mycobacterium bovis* infected guinea pigs and cattle. To investigate this observation further we studied the effect of protein glycosylation on T cell recognition of the glycoprotein MPB83, which is a major component of bovine PPD. O and N-linked glycosylation sites within MPB83 were characterized by the use of site-directed mutagenesis and ConA blotting. Immunization of BALB/c mice with recombinant, non-glycosylated MPB83 revealed the presence of four T-cell epitopes within the molecule. In contrast immunization with *Mycobacterium smegmatis* expressing glycosylated MPB83 failed to induce a specific T cell response whilst immunization with *Mycobacterium smegmatis* expressing non-glygosylated MPB83 induced T cells reacting with at least one of these peptide epitopes. Results from these initial experiments are consistent with the hypothesis that protein glycosylation may effect antigen processing and presentation.

**11.45 TRANSCRIPTIONAL REGULATION OF ANTIGEN PRESENTATION GENES BY THE E1A PROTEIN FROM ONCOGENIC ADENOVIRUSES.**

P. Cotterrell, J.A. Proffitt* and G.E. Blair
School of Biochemistry and Molecular Biology and * Institute of Pathology, University of Leeds, Leeds, LS2 9JT
The E1A gene products of adenovirus (Ad) 12 pleiotropically regulate cellular gene expression in virally-transformed cells. Ad12-transformed cells escape immune surveillance by E1A-mediated repression of antigen processing genes, such as class I heavy chain, Transporter associated with Antigen Presentation (TAP1 and TAP2 genes) and the Low Molecular Proteins (LMP2 and LMP7 genes). TAP1 and LMP2 genes are divergently transcribed from a 564 bp bi-directional promoter comprising 3 regulatory elements, NFκB, SP1, and an ISRE that enables interferon-inducibility of both genes.

The mechanism of Ad12 E1A-mediated transcriptional repression has been studied by transient transfection, site directed mutagenesis and DNA-binding analysis. The activity of the TAP1/LMP2 bi-directional promoter was reduced around 10-fold in highly oncogenic Ad12- compared to non-oncogenic Ad5- transformed cells. Mutation of either the SP1 or NFκB sites partially relieved repression, suggesting Ad12 E1A acted at these sites. This could be directly attributed to E1A expression since co-transfection of promoter-reporter constructs and E1A expression plasmids gave similar results. The effect of interferon treatment and highly oncogenic simian adenovirus 7 (SA7) E1A expression, on the class I heavy chain and TAP1/LMP2 promoters will also be presented.

**12.00 IMMUNOSUPPRESSION BY MORBILLIVIRUSES**

J. Heaney[1,2], T. Barrett[2], S. L. Cosby[1]
School of Biology and Biochemistry, The Queen's University of Belfast[1].
Institute for Animal Health, Pirbright Laboratories, Surrey[2].
A marked suppression of immune function is well documented as the major cause of high mortality and morbidity associated with morbillivirus infections in vivo. A hallmark of virus induced immunosuppression is the reduced capability of isolated peripheral lymphocytes to proliferate in response to mitogens and recall antigens. An in vitro system, developed by Schlender et al. (1996 Proc. Natl. Acad. Sci. USA 93 13194-13199), showed the impaired proliferation response of naive peripheral blood lymphocytes (PBLs), as well as a lympoblastoid B cell line (BJAB), to a variety of stimuli, after co-cultivation with MV-infected, UV-irraditated presenter cells. These experiments suggest that surface contact with one or more viral proteins is required. Initial experiments, based on this system, show that all members of the morbillivirus genus can impair proliferation of BJAB cells in vitro. In vivo proliferative responses of bovine PBLs to vaccine and wild-type strains of rinderpest (RPV) and peste-des-petits ruminants virus (PPRV) were also investigated.

**13.45 MALARIA**

C I Newbold
University of Oxford
Abstract not received

**14.25 VIRUS ANTIGENIC VARIATION.**

Rod Daniels
National Institute for Medical Research, Mill Hill, London
For viruses which have not evolved mechanisms to circumvent the immune surveillance of their hosts, antigenic variation represents a mechanism whereby the virus can "stay one step ahead" of the immune response in order to reproduce and maintain a presence in the host population by either transmission to new individuals and/or persistence in an infected individual. The best examples of this are seen in RNA viruses, which have an inherently high rate of mutation due to the lack of "proof-reading" during RNA replication, such as influenza and HIV. The major antigenic components of these two viruses are their surface glycoproteins which serve important functions during the infection process in terms of receptor binding and membrane fusion. To maintain these functions certain domains on the proteins have to be conserved and those that can vary, usually the antigenic domains, do so within limits consistent with preservation of function. The variation we observe in viable viruses is a reflection of the interplay between this need for preservation of function and the focus of the immune response mounted by the host. In the course of this presentation I will attempt to review antigenicity in terms of structural information available on these glycoproteins and how this is influencing vaccine strategies.

### 15.35 BACTERIAL PHASE-VARIATION

Nigel J Saunders
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One mechanism used by pathogenic bacteria to alter their phenotypes is phase-variation. This is a process of reversible switching between phenotypes, mediated by a genetic re-organisation or mutation, which is not associated with a loss of coding potential, and occurs at a comparatively high frequency. Phase-variation results in the continuous generation of alternative phenotypes which allow a bacterial population to adapt to changing environmental conditions. In this context, genes that are phase variable have been called 'contingency genes'. Phase-variation has complex effects upon the composition of bacterial populations and many bacterial species have a number of phase-variable genes that are varied independently – enabling a single colonising strain to express many diverse phenotypes and sub-populations. Switching adapts the bacteria for interactions with a variety of epithelial surface types in and between hosts, different microenvironments within an individual host, and also confers the ability to evade immune responses.

The contribution of phase variation of surface structures to the normal life cycle of bacterial species and their role as virulence determinants will be discussed. *Neisseria meningitidis* will be used as an example - in this bacterium almost all of the cell surface components that are available for interaction with the host are antigenic- and phase-variable structures. Similarities between the repertoire of phase-variable genes in different bacterial species will be highlighted. These reveal common strategies for interactions with the host and establishing stable colonisation. These include switching of immunogenic surface proteins, host-mimicry, immune evasion through generation of capsules and substitution of cell surface components, mediation of adhesion and uptake into epithelial cells and macrophages, alterations in the induction of NO production, and susceptibility to phagocytic killing.

### 16.15 MUTAGENESIS AND CHARACTERISATION OF ALKYL HYDROPEROXIDASE GENE *ahpC* IN *HELICOBACTER PYLORI*

Chrystala Constantinidou[1], Sally Plummer[1], Elaine Allen[2], Brendan W. Wren[2] and Charles W. Penn[1]

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A gene sequence encoding an antigen specific to *H. pylori* was first cloned and sequenced by O'Toole *et al.* but not identified. It was subsequently clear from homology searches that the gene was a homologue of alkyl hydroperoxidase AhpC. This enzyme mediates repair of peroxidised cellular molecules following oxidative damage, by their reduction to alcohols. In screening an expression library of genomic DNA from *H. pylori* NCTC 11637 in lambda Zap (Stratagene) for antigen-expressing clones using hyperimmune rabbit antiserum, we identified a clone expressing AhpC (revealed from its sequence) at a high level in recombinant *E. coli*. In this clone we have insertionally disrupted the gene with an antibiotic resistance marker, and used transformation and allelic replacement followed by selection for antibiotic resistance to isolate mutant clones of strains NCTC 11637 and the Sydney strain SS1. The mutant phenotype was explored and the organisms shown to be moderately but significantly more sensitive to growth inhibition by exposure to the alkyl hydroperoxide cumene hydroperoxide, suggesting a role for the enzyme in mediating resistance to oxidative damage. In contrast to our findings in *Campylobacter jejuni*, there is no evidence for iron regulation (stimulation of expression) or for the existence of a fur box or adjacent ferritin gene in the upstream promoter region of the DNA encoding AhpC.

### 16.30 TWO *SALMONELLA* [CU-ZN] SODS AND THEIR ROLE IN PATHOGENESIS

A. Sansone[1], P. Watson[2], T.S. Wallis[2], F. Fang[3], P.R. Langford[1] and J.S. Kroll[1]
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[2] Institute for Animal Health, Compton, UK
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Superoxide dismutases (SODs) are virtually ubiquitous among living organisms, playing an essential protective role against the action of cytotoxic free radicals. In bacteria, cytosolic Mn/Fe-cofactored SODs (SodA/SodB) fulfil a "house-keeping" function, removing superoxide produced endogenously in the context of aerobic metabolism, but the source of the substrate for periplasmic [CuZn]-cofactored SOD (SodC) in many cases remains obscure. Since $O_2^-$ does not cross the cytoplasmic membrane, periplasmic SOD must protect bacteria from reactions involving $O_2^-$ produced locally or exogenously (e.g. in the course of phagocytic cell host-defence). Exploring this, a role in pathogenesis has been established for SodC in some Gram-negative pathogens including *Salmonella typhimurium*. *Salmonella* spp. mutant in *sodC*, a gene apparently acquired by horizontal gene transfer, are more susceptible to the toxicity of superoxide-derived free radicals in vitro, and less virulent in a mouse model of infection, than wild-type[1]. Uniquely among bacteria so far characterised, *Salmonella* spp. have a second [CuZn]-SOD gene, *sodC2*, this time highly homologous to the *E.coli sodC*. This too encodes an enzymatically-active periplasmic [Cu-Zn]SOD. *sodC*2 is regulated by RpoS[2], a global regulator of gene expression responsive to starvation and stress conditions, while *sodC* appears not to be under RpoS control. *S. choleraesuis* A50 strains have been constructed carrying a knockout mutation either in *sodC*, *sodC2* or both genes. The *sodC2* mutant appears less sensitive to superoxide in vitro than the *sodC* mutant. Comparative studies using these strains are in progress to assess the role of SodC2 in the interaction of Salmonella with superoxide, and in the pathogenesis of systemic salmonellosis.

*1 Farrant JL, Sansone A, Canvin JR, Pallen MJ, Langford PR, Wallis TS, Dougan G, Kroll JS. 1997. Bacterial Copper- and Zinc*
*cofactored superoxide dismutase contributes to the pathogenesis of systemic Salmonellosis. Mol. Microbiol. 25: 785-796*
*2JW Foster, 97th annual meeting of the American Society for Microbiology, 1997. Abstract K-132 and personal comm.*

**16.45 PROTECTIVE EFFECTS OF *SALMONELLA TYPHIMURIUM* FLAGELLA AGAINST PHAGOCYTOSIS BY AVIAN POLYMORPHONUCLEAR CELLS**

P Wigley, S D Hulme and P A Barrow
Institute for Animal Health, Compton, UK

Avian polymorphonuclear cells are knowN as heterophils and are the functional equivalents of mammalian neutrophils. Phagocytosis of several bacterial species including *Salmonella* has been demonstrated by heterophils *in vitro* by microscopy. The level of phagocytic activity may be expressed as the phagocytic index (PI), based upon the precentage of cells phagocytosing multiplied by the average number of bacteria phacocytosed by each phagocytosing heterophil. The PI of non-*flagellated Salmonella typhimurium* mutants with heterophils isolated from peripheral blood from adult birds of a range of chicken breeds and *Salmonella* resistant and susceptible in-bred lines was increased by 36% to 50% over that of the wild-type. This suggests that flagella protect against phagocytosis by heterophils. An increase in PI of only 17% above the wild-type with non-motile flagellated *Salmonella typhimurium* mutants compared to a 36% increased for non-flagellated mutants with heterophils isolated from adult Light Sussex chickens indicate that both motility and presence of flagella contribute to this protective effect.

**09.00 BACTERIAL LATENCY AS A STRATEGY FOR IMMUNE EVASION**

Yanmin Hu, Philip D Butcher, Joseph A Mangan, Jasvir Dhillon, Kath Sole, Denis A Mitchison, Marie-Adele Rajandream[1], Anthony R M Coates
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Bacteria cause a substantial amount of human disease. Some bacteria can evade the immune response and can persist in the human body for long periods of time. Persistence is usually associated with entry of the organisms into a stationary growth phase in which the bacteria are more resistant to the killing mechanisms of the immune system than log-phase growth organisms. One of the most extreme examples of this form of symbiosis is the persistence of *Mycobacterium tuberculosis*. This bacterium can exist for decades in infected people. Little is known about this dormant type of *M. tuberculosis*, because it is generally regarded as invisible to microscopy and non-cultivable. We have studied the characteristics of dormant *M. tuberculosis in vitro* and *in vivo*. The transition period between log-phase and the dormant phase is associated with global down-regulation of protein synthesis, and by upregulation of the transcription of certain specific genes. The stationary phase is accompanied by continuing metabolic activity, a decline in protein synthesis, acquisition of the ability to survive in complete anaerobiosis, viability and immune evasion. These dormant bacteria consist of at least two subpopulations. The first and much the largest subpopulation is relatively sensitive to antibiotics and is cultivable on agar plates. The second population is profoundly tolerant to antibiotics, although it is genotypically sensitive and is not cultivable on agar plates. However, it can be cultured in low numbers in liquid broth. In addition, it is metabolically active, contains messenger RNA, is responsive to changes in its environment and can persist for longer periods *in vivo*. These data suggest that stationary phase bacteria persist in a metabolically active state which is resistant to immune attack.

**09.40 PERSISTENT INFECTION WITH EPSTEIN-BARR VIRUS - THE STEALTH VIRUS OF HUMAN B LYMPHOCYTES**

David A. Thorley-Lawson
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Epstein-Barr virus is a pathogenic herpesvirus associated with a number of human lymphomas and carcinomas. Yet the virus persists benignly in >90% of the adult population. Traditionally the virus has been best known for its ability to infect any resting normal B lymphocyte in culture and drive it to become a proliferating latently infected blast. This proliferative state is believed to be the predisposing factor in EBV associated neoplasia. Recently, however it has been shown by our group that EBV in vivo, in the peripheral blood behaves completely differently. It persists in a specific subset, memory B cells expressing switched immunoglobulin isotypes. The cells are resting not proliferating and the virus is probably transcriptionally silent. This accounts for the usually beginning state of EBV infection. We have developed a model to explain these apparently contradictory behaviours. In this model EBV uses the normal biology of B cells to establish and maintain latency. Specifically the lymphoblastoid form of latency is equivalent to the activated state that results from exposure to cognate antigen and T cell help. Just like antigen activated B cells, EBV activated B cells exit the cell cycle by differentiating into memory cells. Therefore EBV gains access to the memory compartment by driving latently infected B cells to become activated and then differentiating using the normal pathways of B cell activation and differentiation. Once in the memory compartment the virus shuts off gene expression and is invisible to the immune response. Maintenance of the infected memory cells is through a mechanism that mirrors a secondary antibody response. The production and release of infectious virus occurs when B cells terminally differentiate in mucosal lymphoid tissue. Thus the entire life cycle of the virus can be understood in terms of the normal biology of its host the B lymphocyte. Experimental support for this model and a discussion of its relevance to understanding the origin of the EBV associated lymphomas will be presented.

**10.50 POXVIRUS INHIBITION OF APOPTOSIS: VIRUS-ENCODED ANTAGONISTS OF TNF FAMILY LIGANDS.**

David J. Pickup
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The Tumor Necrosis Factor family of ligands, which includes TNF, lymphotoxin β (LTβ), LTα/LTβ, FasL, CD40L, TRAIL, and CD30L, play pivotal roles in host immune defenses against viral infections. Each of these ligands has different functions in the generation of immune responses, and each of these ligands is pleiotropic in its effects. On the one hand, many of these ligands have the capacity of inducing cell proliferation in cells of specific types, and on the other hand, each of these ligan ds is capable of inducing apoptosis. In response, many viruses, notably large DNA viruses such as the adenoviruses, herpes-viruses and poxviruses, have evolved mechanisms to control some of the processes normally governed by ligands of this family. Viruses may affect these processes either positively or negatively to gain some advantage for replication *in vivo*. For example, some viruses, such as Epstein-Barr virus, employ this strategy to induce the proliferation of latently-infected cells. In contrast, other viruses, such as cowpox virus, employ this strategy to inhibit potentially antiviral and apoptotic effects of these ligands.

Among the viruses that inhibit the effects of TNF family ligands, cowpox virus, an orthopoxvirus encoding ~150 proteins, encodes an unusually sophisticated and extensive system of cytokine-response modifiers. These include at least five proteins capable of modifying the effects of several different TNF family ligands: the CrmA protein, a potent inhibitor of caspase-1 (interleukin-1β converting enzyme), caspase-8 (FLICE), and certain other caspases; not one, but three, different, soluble, secreted, TNF receptors (CrmB, CrmC, and CrmD); and a soluble, secreted receptor for CD30 ligand. Thus the virus is capable of using each of these proteins in ways that may interfere with ligand-induced processes, including apoptosis. Although the exact biological roles of these viral proteins *in vivo* have yet to be determined, this virus system provides a unique model that should further the development of therapies designed to control cytokine-mediated processes in a variety of diseases. In addition, investigation of the functions of these viral proteins should advance our understanding of the molecular mechanisms of apoptosis.

**11.30 THE EFFECT OF TEMPERATURE ON EXPRESSION OF VIRULENCE DETERMINANTS IN *PORPHYROMONAS GINGIVALIS.***

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Bacteria persisting in periodontal pockets are exposed to elevated temperatures during periods of inflammation. Temperature is an environmental factor that can modulate gene expression. Consequently, in the present study we examined the effect of temperature on the expression of virulence determinants by the periodontopathogen, Porphyromonas gingivalis. P. gingivalis W50 was grown in a complex medium under haemin excess at pH 7.0, at a constant temperature of either 37, 39 or 41 C; cultures were monitored for protease and haemagglutinin activity. P gingivalis grew well at all three temperatures. An increase in growth temperature from 37 to 39 C resulted in a 65% reduction in both total arginine- and lysine-specific activities ($p<0.01$). A further rise in growth temperature to 41 C led to an even greater reduction in arginine-specific (82%; $p<0.001$) and lysine-specific (73%; $p<0.01$) activities. This reduction was also associated with an altered distribution of individual arginine-specific enzyme isoforms. At 41 C, there was a disproportionate reduction in the level of the heterodimeric R1 protease, which also contains adhesin domains. The reduction also correlated with a markedly diminished haemagglutination activity of cells especially in those grown at 41oC, and a reduced immunoreactivity with a monoclonal antibody which recognises gene products involved in haemagglutination. Thus, as the environmental temperature increased, P. gingivalis adopted a less aggressive phenotype, while retaining cell population levels. The co-ordinate down-regulation of virulence gene expression in response to an environmental cue linked to the intensity of the host inflammatory response is consistent with the clinically-observed cyclical nature of disease progression in periodontal diseases.

**11.45 AFRICAN SWINE FEVER INFECTION OF PORCINE AORTIC ENDOTHELIAL CELLS LEADS TO APOPTOSIS**

Isabelle Vallée and Penelope Powell
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African swine fever (ASF) is a haemorrhagic disease of domestic pigs caused by a large double-stranded DNA virus, ASFV, and characterised by a disseminated intravascular coagulation. ASFV replicates in primary porcine macrophages, where it down regulates proinflammatory cytokine responses *in vitro*. Using primary porcine aortic endothelial cells (PAECs) we demonstrate that ASFV replicates in PAECs with the same kinetics of early and late protein expression, and production of viral factories as seen in macrophages. In response to ASFV-infection, PAECs do not up-regulate expression of MHC class-I molecules nor expression of E-selectin, consistant with the lack of inflammatory responses reported in *in vivo* studies of the disease. Moreover, we have found that ASFV-infected PAECs die by apoptosis. Taken together, our results demonstrate that ASFV infects and replicates in porcine endothelial cells, inactivates the endothelial cell response to infection and induces cell death by apoptosis.

**12.00 TUMOUR NECROSIS FACTOR-a-DEFICIENT MICE RESIST PERIPHERAL CHALLENGE WITH SCRAPIE**

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In most peripheral infections of rodents and sheep with scrapie, infectivity is first found in the spleen and later in the central nervous system (CNS). Germinal centres (GCs) in the spleen are an important site of extraneural replication from which infection is likely to spread to the CNS along peripheral nerves. Despite possessing functional T and B lymphocytes, tumour necrosis factor-α-deficient ($TNF^{-/-}$) mice lack GCs and mature follicular dendritic cells (FDC) in lymphoid tissues. When infected with ME7 scrapie directly into the CNS, $TNF^{-/-}$ and wild-type mice were equally susceptible. However, when infected peripherally (intraperitoneally), most $TNF^{-/-}$ mice failed to develop scrapie up to 500 days post-infection. Wild-type mice succumbed to infection 300 days post-challenge. While strong PrP accumulations in association with FDCs were detected in the spleens of infected wild-type mice, none were detected in tissues from $TNF^{-/-}$ mice. These experiments provide further evidence that for the ME7 scrapie strain, disease after peripheral challenge, occurs only in the presence of mature FDCs.

**13.45 HOW DO GAMMA HERPESVIRUSES MAKE CELLS PROLIFERATE BUT AVOID APOPTOSIS?**

Paul J. Farrell
Ludwig Institute for Cancer Research, Imperial College School of Medicine, St Maryís Campus, Norfolk Place, London W2 1PG

The human gamma herpesviruses Epstein-Barr virus (EBV) and HHV8 (KSHV) both have viral genes that are capable of causing cell proliferation and suppressing apoptotic signals. The growth of B lymphocytes that occurs in cell culture in response to EBV infection is mirrored in vivo during primary EBV infection but long term persistence of EBV is thought to involve a more restricted pattern of viral gene expression in which most of the immortalisation genes of the virus are not expressed. Early events in EBV infection involve activation of the B cells both through signal transduction induced by the binding of virus to its receptor and through the subsequent expression of viral genes. Considerable progress has been made in understanding the mechanism of human B cell immortalisation by EBV, which involves the viral genes EBNA-1, 2, 3A, 3C, LP and LMP-1. Expression of anti-apoptotic genes such as BCL-2 and A20 correlates with the relative resistance to apoptosis induced by low serum levels in EBV immortalised B cell lines. Although EBV encodes a homologue of the BCL-2 gene, this is mainly expressed during the virus lytic cycle rather than in the immortalised B cell lines.

Less is understood about the control of cell proliferation by HHV8 but several viral genes are able to cause transformation in culture and the virus has been reported to maintain the growth of human endothelial cells, possibly by a paracrine mechanism. HHV8 also encodes genes with sequence similarity to regulators of apoptosis, such as BCL-2 and FLIP. Regulation of cell growth and apoptosis by these viruses and herpesvirus saimiri, a monkey gamma herpesvirus, will be explored in the context of an overall herpesvirus strategy.

**14.25 BACTERIAL SUPERANTIGENS**

Malak Kotb
Veterans Administration Medical Center, Memphis, USA

A group of microbial proteins known as superantigens, have generated tremendous interest among scientists of different disciplines because of their potential involvement in a wide variety of human infectious and autoimmune diseases as well as their possible utility as anti-cancer drugs. Superantigens are bifunctional molecules that utilize at least two types of receptors expressed on different mononuclear cells of the immune system. The receptor for superantigens on T cells is the aß heterodimeric T cell receptor (TCR) for antigen, while the MHC class II molecules expressed primarily on B cells, monocytes, and dendritic cells serve as receptors for superantigen on these cells. The binding of superantigens to the TCR and/or to class II molecules triggers intracellular biochemical signals that program a number of events leading to cell activation, differentiation, proliferation, and the release of large amounts of inflammatory cytokines.

Several bacterial, viral, and mycoplasma species have been found to produce superantigens. Some are secreted toxins; whereas, others are membrane associated proteins. Amongst the best characterized superantigens are the family of pyrogenic exotoxins produced by *Staphylococcus aureus* and *Streptococcus pyogenes*. Despite differences in their primary structure, these superantigens share conformational features that allow them to interact with both the TCR and class II molecules and utilize a common mechanism of immune stimulation to elicit very potent immune responses.

Despite the many remarkable common features of superantigens, differences exist in the mode by which they interact with their receptors on the surface of T cells and antigen presenting cells. These differences may influence the magnitude of immune stimulation by superantigen and may account for the observed variations in their biological activity and disease inducing ability. In addition, recent studies suggest that host immunogenetic factors, namely the class II haplotype and TCR Vß repertoire, can differentially modulate superantigen responses. The preferential presentation of superantigens by distinct HLA class II allotypes may have important biological implications particularly in modulating host susceptibility to certain diseases in which these toxins play a role. Isotypic and allelic variations in class II molecules that influence the interaction of antigen presenting cells and/or T cells with superantigens are likely to affect the magnitude and profile of the cytokine response and potentiate the severity of systemic manifestation in certain infections. In addition to the effect of class II polymorphism on superantigen responses, recent studies revealed that the presentation of these toxins by a particular class II allele can be greatly influenced by the nature of MHC-associated peptide and antigen presentation co-factors. These findings suggest that superantigens are likely to elicit different levels of responses in different hosts as well as in different tissues of the same host. Elucidating the mode by which superantigens interact with immune cells can help us decipher their role in disease, identify host factors that potentiate their effects, and design drugs that specifically block their activity.

**15.05 MOUSE MAMMARY TUMOR VIRUS SUPERANTIGENS**

Hans Acha-Orbea
Ludwig Institute for Cancer Research , Lausanne Branch and Institute of Biochemistry, University of Lausanne, 1066 Epalinges, Switzerland

Mouse mammary tumor virus (MMTV) is a retrovirus that exploits the immune response. Initially it infects B lymphocytes which after infection express a superantigen (Sag) on the cell surface. SAgs are molecules which bind to MHC class II molecules and stimulate a large proportion of T cells by interacting with the T cell receptor Vb sequence of the T cell receptor. Expression of the Sag leads to interaction with $CD4^+$ T cells which help the infected B cells to divide and to differentiate. Each division leads to duplication of the infected B cell pool and differentiation leads to increased survival of the infected B cells. In the absence of this vigorous immune response the virus cannot establish an efficient infection. Furthermore the Sag response induces an efficient neutralizing immune response which, however, is not capable of controlling infection. In the absence of this neutralizing immune response the final target of infection, the mammary gland, is infected at much higher levels. These higher infection levels do not alter virus transmission via milk to the next generation but might strongly increase the risk for mammary tumor generation. We propose that the virus also exploits this immune response to establish a long lasting infection and due to longer survival allows successful transmission to the next generation.

## POSTERS

### M1 INVESTIGATION OF A *FLHB* HOMOLOGUE GENE AND ITS MUTANT PHENOTYPE IN *CAMPYLOBACTER JEJUNI*

C. Matz[1], A.H.M. van Vliet[2], M.-L. Baillon[1], C. Constantinidou[1], J.M. Ketley[2], and C.W. Penn[1]

[1]School of Biological Sciences, University of Birmingham, Birmingham B15 2TT, UK

[2]Department of Genetics, University of Leicester, Leicester LE1 7RH, UK

The FlhB protein is believed to be a structural component of the flagellar hook as well as being involved in the regulation of flagellar assembly in *Salmonella typhimurium*. A clone from a *Campylobacter jejuni* (NCTC 11828) genomic library was shown to contain a *flhB* gene homologue showing high similarities to the *flhB* genes of *Salmonella typhimurium* and *Helicobacter pylori*. Downstream of *flhB*, a divergent *motB* homologue was identified, which is a component of the flagellar motor. The cloned *flhB* was interrupted by the insertion of a chloramphenicol cassette and this construct used for allelic replacement in *C. jejuni*. The resulting deletion mutants showed a drastically reduced motility, and Western blot analysis showed reduced although not totally abolished expression of flagellins. Electron microscopic analysis revealed that they were also altered in cell morphology. The mutant cells were straight in distinction to the spirally curved wild type, and lacked flagella. However, a small percentage of mutants showed a truncated flagellar filament. The transcriptional start site of *flhB* was mapped using primer extension analysis, but no apparent consensus promoter sequence could be identified. The *flhB* gene appeared to be monocistronic, and confirmation that it is not co-transcribed with the upstream *ahpC* gene is being sought. Northern blot analysis did not reveal a distinct signal for *flhB* in wild type cells (transcript levels were probably to low to detect), but was positive for the major flagellin *flaA* transcript. Expression of the *flhB* under various growth conditions is currently being studied using transcriptional *lacZ* fusions.

### M2 DELETION OF ALTERNATIVE SIGMA FACTORS IN *HELICOBACTER PYLORI*

Chrystala Constantinidou[1], Nick Dorrell[2], Brendan W. Wren[2] and Charles W. Penn[1]

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*Helicobacter pylori* exhibits very limited apparent capacity for regulation of gene expression, with minimal evidence from the genome sequence for regulatory DNA-binding proteins or two-component sensor-regulator gene pairs, and only three apparent RNA polymerase sigma factors for recognition of alternative promoter sequences: $\sigma^{70}$, $\sigma^{54}$ and $\sigma^{28}$. In other organisms, $\sigma^{70}$ is the predominant 'housekeeping' factor while $\sigma^{54}$ is often utilised in specialised responses to environmental change or stress and $\sigma^{28}$ for motility and chemotaxis gene expression. In view of the evident paucity of mechanisms for regulation of transcription in this organism, it might be predicted that the alternative sigma factors would be critical for a wide range of cellular responses or functions. We have made deletion mutants in $\sigma^{28}$ and $\sigma^{54}$ by insertional disruption of the cloned genes with antibiotic resistance markers, and transformation and allelic replacement followed by selection for antibiotic resistance to isolate mutant clones. Against our expectation, the phenotypes are obviously affected only in expression of flagellar genes, shown by studies of their morphology and expression of flagellins. The mutants appeared otherwise generally normal, in their growth and survival under standard laboratory conditions.

**M3 PROMOTER RECOGNITION IN *HELICOBACTER PYLORI***

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[1]National Institute of Biological Standards and Control, South Mimms, Herts EN6 3QG, UK

*Helicobacter pylori* is now well established as a significant human pathogen, and many genes have been characterised and expressed as recombinant products. Little is known however, of how *H. pylori* gene transcription is initiated and subsequently regulated. In this study transcription start sites of selected *H. pylori* genes, namely those encoding urease, glutamine synthetase, a nickel transport protein (NixA), and alkyl hydroperoxide reductase (AhpC) were mapped by primer extension analysis using *H. pylori* total cellular RNA. Deduction of upstream promoter recognition sites indicated conservation of -10 Pribnow regions and extended -10 TG motifs but no -35 regions comparable with the *E. coli* s70 consensus sequence. The transcriptional start identified for *ureAB* was not compatible with a s54 promoter sequence previously postulated for this operon. Northern blotting was used to confirm the size of the urease transcript predicted from our data. In addition, primer extension analysis was also used to locate the transcription start site of cloned *ahpC* and urease genes in *E. coli*, which proved to be similar or adjacent to those recognised in *H. pylori.* The promoter sequences published for other transcriptionally mapped *H. pylori* genes were analysed and aligned against the data obtained in this study. Results consistent with those reported here were observed, indicating that there is some consensus in -10 sequence for the s80-containing RNA polymerase.

**M4 DIFFERENTIATION WITHIN CLINICAL ISOLATES OF EPEC AND EHEC USING RAPD AND IDENTIFICATION OF MOLECULAR MARKERS SPECIFIC TO SEROGROUP O:157.**

K.L. Hopkins, A.C. Hilton[1] & C.W. Penn
School of Biological Sciences, University of Birmingham, Birmingham. B15 2TT
[1]Institute of Public & Environmental Health, University of Birmingham, Birmingham. B15 2TT

The ability to differentiate between strains of *Escherichia coli* causing enterohaemorrhagic disease has become increasingly complicated by the emergence of clonal strains responsible for a majority of the cases of infection, serotype O:157,H:7 in particular. As a result attention has turned to the development of molecular methods to target the often minimal amount of variation available for strain differentiation. In this study the polymerase chain reaction was used to amplify randomly primed genomic DNA from *E. coli* using a primer previously reported to be discriminatory in an optimised RAPD protocol. Clinical isolates of *E. coli* representing blood and urine isolates, EPEC, and EHEC of serotype O:157 were analysed. Following electrophoresis the RAPD profiles were captured in a digital form for analysis with GelCompar software. The method was able to reproducibly discriminate between all the EPEC, and within the O:157 isolates investigated, thus proving to be a useful technique for the epidemiological analysis of *E.coli*. Computer based analysis of the RAPD-generated fragments facilitated the identification of a 500bp amplicon from O:157 isolates which was absent from the other isolates investigated. Such a molecular marker may prove useful as a probe or in a PCR specifically targeted to the identification of O:157.

**M5 ISOLATION AND PARTIAL CHARACTERISATION OF A 190 kDa PRO-INFLAMMATORY SURFACE PROTEIN FROM STREPTOCOCCUS SANGUIS**

J. Banks[1], S. Poole[3], S. Nair[1], M. Wilson[2], & B. Henderson[1]
[1]Cellular Microbiology Research Group & [2]Dept of Microbiology, Eastman Dental Institute, University College London.
[3]Division of Endocrinology, NIBSC, Potters Bar, Middlesex.

The oral commensal *Streptococcus sanguis* expresses few obvious virulence factors, yet is a common causal agent of infective endocarditis, a chronic inflammatory disease. The aim of this work was to determine whether exported proteins from *S. sanguis* were capable of inducing pro-inflammatory cytokine release from human cells, and to isolate and purify the active proteins.

Surface material containing exported proteins was extracted from plate-grown bacteria by gentle stirring with 0.15M NaCl at 4°C. The extract was dialysed against distilled water and freeze-dried. Pro-inflammatory cytokine-stimulating activity was assayed by incubation of material with either peripheral blood mononuclear cells (PBMC) or the HUVEC-like cell line, ECV304. The levels of cytokines in the supernatants were measured by two-site ELISA. Individual proteins were isolated by anion exchange and gel filtration HPLC, and visualised by silver-stained SDS PAGE.

Surface material from *S. sanguis* stimulated PBMC, but not ECV304 cells, to produce IL-1b, IL-6, IL-8 and TNF-a in a dose-dependant manner. Anion exchange and gel filtration HPLC yielded a fraction containing a 190kDa doublet which stimulated PBMC to produce IL-6. This activity was blocked by heating.

In conclusion, *S. sanguis* possesses secreted surface-associated proteins, including a 190kDa protein, which are potent stimulators of pro-inflammatory cytokines and as such may play a role in the pathogenesis of infective endocarditis.

**M6 CHAPERONIN 60-DERIVED PEPTIDES: A POSSIBLE ROLE IN INFLAMMATORY DISEASE**

S. Khan[1], S P. Nair[1], M. Preuss[2], S. Poole1, P. Tabona[1], J. Lewthwaite[1], A.R.M. Coates[3], P. Mascagni[4], A. Miller[2] and B. Henderson[1]

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[2] Dept. of Chemistry, Imperial College, London,

[3] Dept. of Medical Microbiology, St. George's Hospital Medical School, London

[4] Department of Chemistry, Italfarmaco Research Centre, Milan, Italy

Chaperonins are proteins that catalyse the folding and refolding of intra-cellular proteins. However, in recent years, chaperonins have begun to reveal additional non-folding biological activities. We have recently shown that trypsinised chaperonin 60 of *Escherichia coli*, stimulates peripheral blood mononuclear cells (PBMC) to produce cytokines. This suggests, that the biological activity of this chaperonin resides within a constituent peptide or peptides. Several synthetic chaperonin 60-derived peptides were tested for biological activity. Peptide 195-217 from Mycobacterium tuberculosis chaperonin 60.1 was particularly active, stimulating PBMC's to produce the pro-inflammatory cytokines IL-6, TNF-* and IL-8. The same sequence from *M. tuberculosis* chaperonin 60.2 (hsp 65), which differs by only one non-identical amino acid did not stimulate cytokine production. This suggests that bacterial chaperonins, despite having a highly conserved sequence, can still differ sufficiently in structure to account for the different biological actions found. In addition to testing synthetic peptides we are also separating and identifying active peptides by reverse chromatography.Thus chaperonin 60 proteins behave as inter-cellular signalling molecules mediating cytokine-like cellular responses. The range of their biological actions remains to be determined.

**M7 CHARACTERISATION OF THE DT104 CLONE OF *SALMONELLA TYPHIMURIUM***

Nicola Martin, [1]Roy P. Betts and Charles W. Penn

School of Biological Sciences, University of Birmingham and [1]Campden and Chorleywood Food Research Association, Chipping Campden, Glos GL55 6LD

During the past few years, isolation of *Salmonella typhimurium* from foodborne enteritis cases in the UK and elsewhere has increased relative to that of *S. enteritidis*. The strains of *S. typhimurium* involved have become increasingly dominated by a single phage type, DT104, believed to represent a highly successful clonal population which is presumably exceptionally fitted in some way to fill this niche. Other than in its carriage of chromosomally determined multiple antibiotic resistance (to ampicillin, chloramphenicol, streptomycin, sulphonamides and tetracycline), there has to date been no clearly demonstrable phenotypic property of the clone which might account for its extraordinary success. We have therefore investigated, in comparison with an older, virulent strain TML, the growth rate in laboratory media, survival under stress (high temperature, acid, high salt and exposure to hydrogen peroxide), and 2D electrophoretic profile of cellular proteins. We are currently also assessing the sequence of the gene *rpoS* encoding global regulator of stress responses RpoS. Contrary to our expectations, no firm evidence has emerged to date of any significantly enhanced fitness characteristics in the DT104 organisms, which appear very comparable to TML in growth and survival under stress. There are however indications of proteome differences between the strains which will be the subject of future investigation.

**M8 THE BIOTREATMENT OF SPENT CAUSTIC WASTE**

A. Ferguson / Supervisor: Dr. M. Larkin.

Questor Centre, Queens' University, Belfast

Spent caustic waste is a common organic by-product produced from the petroleum industry. Such caustic liquid wastes are produced from the treatment of the naptha fraction with caustic lime. This fraction is produced from the catalytic and thermal cracking process and contains a mixture of contaminants such as phenols, cresols and propanoic acids as well as thiocyanate and metal salts.

Chemical analysis of spent caustic waste from three different sites was carried out and a suitable synthetic waste was developed. Concentrations of each constituent were formulated from both the chemical characterization data and also the likely limits for microbial growth. A number of enrichments have shown that growth of a mixed microbial population can occur in an environment containing phenol, thiocyanate, and nickel at pH values up to pH 10.5. Chemostat enrichments using the synthetic waste produced a mixed microbial population, which degraded 98.6% of 5 mM phenol in 146 hours. Subsequently, an unidentified fungus was isolated which appears to be the sole phenol utilizer (as C - source) present in the mixed population. Fungal growth is optimal at pH 10, which is the pH at which the spent caustic waste, buffers.

We present here further characterization of the fungus with respect to its substrate range, phenol, thiocyanate and nickel tolerance.

**M9 CYTOKINE PRODUCTION BY PERIPHERAL BLOOD MONONUCLEAR CELLS IN RESPONSE TO CHAPERONIN 60 FROM *E. COLI* (GroEL)**

JC Lewthwaite+, P Tabona+, S Khan+, S Poole[1] M Preuss[2], A Miller[2] and B Henderson
Eastman Dental Institute, University College London, London, WC1X 8LD,
[1] NIBSC, Potters Bar, Hertfordshire, EN6 3QG,
[2] Imperial College, London, SW7 2AY, UK.
+These authors contributed equally to this work.

Chaperonin 60 (cpn60) is a member of a family of intracellular protein-folding proteins. A number of these proteins, including cpn60, have recently been shown to have activities that are unrelated to their protein-folding function. These include the ability to stimulate the synthesis of pro-inflammatory cytokines from a variety of cells. Earlier work from our laboratory showed that human peripheral blood mononuclear cells (PBMC) produce IL-1b and IL-6 in response to cpn60. In this study we have investigated the production of other cytokines. PBMC were separated from whole blood by histopaque fractionation and the adherent cells stimulated with cpn60 or LPS as a control. Culture supernatants were harvested and assayed for cytokines by ELISA. In addition to IL-1b and IL-6, PBMC were found to produce IL-8, IL-10, IL-12, TNFa and GM-CSF but not IL-4 or IFNg. Therefore cpn60 induces the production of pro-inflammatory cytokines (IL1b, TNFa, IL-6 and IL-8), a number of which stimulate the development of a T helper type 1 immune response (TNFa, IL-12). The production of the anti-inflammatory cytokine IL-10 may serve to down regulate the production of these pro-inflammatory cytokines.

**M10 A LETHAL ROLE FOR LIPID A IN SALMONELLA INFECTIONS**

Shahid Khan, Romina Emilianus, Simone Titus & Duncan Maskell,
Centre for Veterinary Science, Department of Clinical Veterinary Medicine, University of Cambridge, Madingley Road, Cambridge, CB3 0ES, UK. Tel: 44 1223 339868. Email: djm47@cam.ac.uk

*Salmonella typhimurium* infects mice to cause an invasive typhoid-like disease. In susceptible mice, the infection grows rapidly in the liver and spleen, reaching the "lethal load" of approximately log 8 bacteria per organ. Much evidence suggests that death is not a consequence of systemic septic shock. However, no toxins have been identified in *S. typhimurium* = that are responsible for causing death. We have thus attempted to investigate the role of lipid A in causing death in mouse typhoid by cloning and mutagenising the msbB (waaN) gene from the virulent *S. typhimurium* C5. The resulting mutant bacteria appear normal and synthesise a full-length O-antigen. By compositional analysis, the lipid A molecule lacks the expected secondary fatty acid in its lipid A structure. Heat-killed = mutants are much less able to induce a range of cytokine and chemokine responses, = as well as inducible nitric oxide synthase. On i.v. infection of susceptible BALB/c mice the mutant bacteria grow identically to their wild-type = parents.

The wild-type bacteria kill all infected mice when lethal loads of around log 8 are attained, whereas the mutant bacteria continue to grow, with no apparent effect on the animals. Less than 10% of the mice die, with bacterial loads approaching log 9.5 in their organs. *In vivo* cytokine and iNOS responses are greatly reduced. This provides strong evidence that lipid A is responsible for lethality in mouse typhoid infections.

## PHYSIOLOGY, BIOCHEMISTRY & MOLECULAR GENETICS GROUP
## REGULATION OF COMPLEX PROCESSES IN BACTERIA

*Friday 16 April 1999*

**09.00 THE MOLECULAR MACHINERY OF BACTERIAL CYTOKINESIS**
J Lutkenhaus
University of Kansas Medical Center, USA
Abstract not received

**09.35 ASYMMETRIC CELL DIVISION AND DIFFERENTIATION IN *BACILLUS SUBTILISJ***
J Errington
University of Oxford
Abstract not received

**10.30 ONE BRICK AT A TIME: MAKING A PROTEIN SECRETORY APPARATUS**
D Nunn
University of Illinois at Urbana-Champaign ,USA
Abstract not received

**11.05 MAKING A FLAGELLUM WITH STYLE AND EFFICIENCY**
K Hughes
University of Washington, USA
Abstract not received

**11.40 MAKING A SEX PILUS**
L Frost
University of Alberta, Canada
Abstract not received

**14.00 BACTERIA IN SYMBIOSIS: FACTORS AFFECTING NODULATION BY RHIZOBIUM**
A Downie
John Innes Centre, Norwich
Abstract not received

**14.35 BACTERIA ATTACKING ANIMALS: CURRENT APPROACHES TO THE STUDY OF YERSINIA VIRULENCE REGULATION**
H Wolf-Watz
University of Umea, Sweden
Abstract not received

**15.10 BACTERIA ATTACKING PLANTS: UNUSUAL REGULATORY MECHANISMS**
M Daniels
The Sainsbury Laboratory, Norwich
Abstract not received

**16.05 BACTERIAL DIFFERENTIATION AND POPULATION MIGRATION: PROTEUS SWARMING**
G Fraser
University of Cambridge
Abstract not received

**16.40 GENETIC BASIS OF NICHE SPECIALISATION IN EXPERIMENTAL POPULATIONS OF *PSEUDOMONAS FLUORESCENS***
P Rainer
University of Oxford
Abstract not received

### 09.10 PROBING ECOLOGICAL QUESTIONS WITH A MICROBIAL MODEL

James M. Tiedje, Center for Microbial Ecology,
Michigan State University, East Lansing, MI 48824, USA

Major ecological questions address the relationships between stability and diversity, perturbation and resilience and persistence, and selection and persistence. These questions have not traditionally been addressed for microbial communities, but, with new methods, they can be and perhaps with better chances of success than with macrobiological communities. Bioreactors are advantageous ecosystems for some of these studies because they are controllable both with regard to microbial inocula and environmental conditions. We have used anaerobic reactors fed glucose to compare long-term functional stability with the population stability. Over a 2 year period the reactor function was stable but the populations changed dramatically, especially among the *Bacteria*. Succession was observed at family levels as well as a cycling within an organism group. Similar reactors were also used to address the functional and population responses of two different communities to a shock-loading of substrate. The community dominated by Spirochetes had greater functional resilience and resistance than the more conventional anaerobic digestor community and had more persistence of its original population. ARDRA, T-RFLP, isolation, image analysis, rRNA hybridization and activity measures were used to analyze these communities. Stirred, liquid ecosystems, such as reactors, can change community composition much more rapidly than ecosystems such as soil which is dominated by attached organisms with an infinite array of protective niches. We have examined by ARDRA, T-RFLP, RSGP, isolation and functional and biomass measures how long-term (10 yr.) selection for 2,4-D has altered a soil community. Furthermore, we have addressed how rapidly the community returns to a new state once the 2,4-D selection is removed. The 2,4-D addition selected a soil community in which the dominant culturable members are 2,4-D degraders. At 2,4-D additions 100 x agricultural practice, the microbial biomass and some ecosystem functions are reduced and the dominant members are nearly a monoculture. After 2,4-D use is stopped the dominant strains continuously decline over a 2 year period. The signature of the altered population is still detectable, however, after 2 years of non-selective conditions.

### 09.55 SOIL MICROBIAL DIVERSITY - WHICH APPROACH?

Allison E. McCaig, L. Anne Glover and James I. Prosser
Dept Molecular and Cell Biology, Institute of Medical Sciences, University of Aberdeen, Foresterhill, Aberdeen, AB25 2ZD, UK.

Many approaches are now available for studying soil microbial diversity. These range from broad-scale techniques, such as DNA reassociation kinetics and community cross-hybridisations, to fine-scale approaches, such as 16S ribosomal DNA sequencing. These approaches are often targeted at very different aspects of the microbial community and, when a range of methods is used in parallel, a more complete understanding of population structure and function may be gained. In MICRONET, a 9 year initiative funded by the Scottish Office Agriculture, Environment and Fisheries Department (SOAEFD), a number of techniques have been employed to investigate genetic and functional differences between microbial communities associated with grasslands under different land management regimes. In addition, DGGE of specific bacterial groups has been used to study the importance of sample size and spatial variation.

### 11.10 ANALYSING ACTIVITY *IN SITU* – SINGLE CELLS TO POPULATIONS

Andrew S. Whiteley
NERC Institute of Virology and Environmental Microbiology. Mansfield Road, Oxford. OX1 3SR. U.K.

Fundamental to the analyses of bacterial populations is the assessment of the distribution of activities within the assemblages. Bacterial populations within the environment undergo transitions between activity and non-activity modulated by environmental stimuli. To gain a thorough understanding of bacterial functionality and its variability requires an activity assessment of the individual species/group components or the population as a whole. The presentation will focus on methods to assess bacterial activity at the single cell level and extensions of these methods to the population by examples derived from single cell and population analyses in cultures and natural environments. Specific emphasis will be placed upon the use of redox methods to assess bacterial respiration, in situ hybridisation for dual identification and activity assessment and the applications of luminescence techniques. Future perspectives will also be discussed involving molecular and marker gene developments and methods aimed at targeting specifically the active component of bacterial assemblages.

## 11.55 DIVERSITY AND ACTIVITY OF MICROBIAL POPULATIONS INVOLVED IN ANAEROBIC DEGRADATION OF LANDFILL WASTE

Michele I. Van Dyke and Alan J. McCarthy
School of Biological Sciences, University of Liverpool, Liverpool, UK, L69 7ZB

To effectively monitor and optimise stabilisation of waste in landfill sites, the biological systems involved must be better understood. Anaerobic degradation of landfill waste is performed by key functional communities of bacteria, involving cellulolysis, acetogenesis, sulphate reduction and methanogenesis. In the study of anaerobic microorganisms, problems encountered with traditional culture based techniques can be overcome by using molecular biological approaches. Phylogenetic analysis using 16S rRNA genes can be particularly useful for assessing the composition, dynamics and activity of microbial communities in landfills.

Using direct nucleic acid extraction from landfill leachate and PCR amplification of 16S rDNA with specific primers, we could detect and identify cellulolytic clostridia, sulphate-reducing bacteria and methanogens at each site analysed. TTGE analysis was used to profile community diversity and RT-PCR could detect and profile active methanogenic strains. Subgroup and species identification revealed dominant members in each functional group of bacteria. This rapid assessment of diversity will be useful in monitoring the response of microorganisms to changes in operating conditions, and demonstrates the potential for molecular biological techniques in landfill environments.

## 12.10 MOLECULAR IDENTITY AND SURVEY OF ANAEROBIC AMMONIUM-OXIDIZING BACTERIA

Mike Jetten[1], Marc Strous[1], Katinka van de Pas-Schoonen[1], Gerard Muyzer[2], Michael Wagner[3], and J. Gijs Kuenen[1]
[1] Dept. of Biotechnology TU Delft, Julianalaan 67, NL 2628 BC Delft, TheNetherlands;
[2] Netherlands Institute for Sea Research, Den Burg,Netherlands;
[3] Dept. of Microbiology3, TU Munich, Germany.

The anaerobic ammonium oxidation process (ANAMMOX) is one of the most innovating advances in the removal of ammonium from wastewater. In this novel biological process nitrite and ammonium are combined to dinitrogen gas under anoxic conditions, with hydrazine and hydroxylamine as important intermediates. The feasibility of the ANAMMOX process has been demonstrated in several biofilm reactor systems. Enrichments on ammonium, nitrite, and bicarbonate resulted in the dominance of one morphotypical bacterium, which resisted molecular identification due to a strong PCR bias against amplification of its 16S rRNA gene. Only after single cell separation via density gradient centrifugation and RT-PCR of the extracted RNA could a dominant 16 S rRNA clone be obtained. Based on this sequence, 18 fluorescent probes were designed, of which 11 gave bright and specific hybridisation signals with the dominant bacterium. Phylogenetic analysis identified the Anammox bacterium as a deeply branching planctomycete. *In situ* analysis showed that these typical planctomycete cells were present in different wastewater systems for which high anoxic ammonium losses have been reported.

## 12.25 COMPETITIVE PCR TO MONITOR AMMONIA OXIDISERS IN A 150 YEAR OLD FIELD EXPERIMENT

T.A. Mendum, R.E. Sockett and P.R. Hirsch
IACR-Rothamsted, Harpenden, Herts, AL5 2JQ

The oxidation of ammonia to nitrate is central to the nitrogen cycles of terrestrial environments. Ammonia, which is bound by soil particles, is converted to nitrate which is mobile within the soil, thus nitrogen becomes available to crops and susceptible to loss from the soil via leaching and denitrification. The initial and rate-limiting step in this process, the oxidation of ammonia to nitrite is carried out mainly by a monophyletic group of $\beta$-group ammonia oxidising bacteria (AOB). These organisms contain the unique enzyme ammonia monoxygenase (amo) which catalyses the primary step in ammonia oxidation. We have examined the effects of fertiliser addition on the population sizes and activities of the AOB in plots on the Broadbalk experiment. These plots have been under unchanging fertiliser regimes since 1852. Using $^{15}N$ dilutions methods, the rate at which soils from the plots nitrify, that is produce nitrate, was determined. AOB population sizes were determined by competitive PCR based upon both the 16S and *amo* genes. Phylogenetic trees based upon sequence information from these PCR were constructed to examine the AOB community structure.

**14.00 APPLICATION OF DGGE TO DUTY MICROBIAL COMMUNITIES IN SOIL**

Kornelia Smalla, Angela Zock, Jens Schönfeld, and Holger Heuer
Federal Biological Research Centre for Agriculture and Forestry, Messeweg 11/12, D-38104 Braunschweig

Denaturing gradient gel electrophoresis (DGGE) and the related technique, temperature gradient gel electrophoresis (TGGE) are now frequently applied in microbial ecology to compare the structure and dynamics of complex microbial communities. This technique has the potential needed for monitoring techniques since it offers the chance to analyze large sample numbers by generating fingerprints of the dominant constituents. We have applied DGGE to study microbial communities of the rhizosphere of different crop plants and to follow succession during the vegetation period. Furthermore, the effect of T4-lysozyme expressed by transgenic potato plants on the structural composition of the bacterial rhizosphere community was analyzed. The steps in the procedure are the recovery of the bacterial fraction followed by DNA extraction and purification, amplification of a 16S rDNA fragment by PCR, and electrophoretic separation by DGGE or TGGE. The diversity can be explored by a "top to bottom" analysis with primers of varying phylogenetic specificity. Besides primers conserved for bacterial 16S rDNA, primers to specifically enrich sequences of a- and b-proteobacteria or Actinomycetales have been developed and applied in a nested PCR to follow community shifts in the rhizosphere by DGGE. The application of the group-specific primers reduces the complexicity of the banding pattern and allows to analyze minority populations. One striking observation was the high stability of the DGGE fingerprints between parallel samples. A few bands differed between different crop plants studied indicating that plants enrich different bacterial rhizosphere communities. Individual bands in the DGGE fingerprints were excised and sequenced. However, the ecological role of an organism cannot be concluded from its 16S rDNA sequence. Therefore, we have recently developed a tool to link bands from community profiles to strains isolated from the same environment. Digoxigenin-labeled polynucleotide probes targeting a hypervariable region of the 16S rRNA genes (V6) were generated by PCR, using bands excised from T/DGGE community fingerprints as a template. The specificity of the probes could be increased by enzymatic removal of the flanking conserved bases. Probes generated from differentiating bands have been utilized to detect bacterial isolates corresponding to bands of the T/DGGE-community fingerprint.

**14.45 IMAGE ANALYSIS AS A TOOL TO STUDY MICROBIAL ECOLOGY**

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There are many methods to study microbial processes, including batch and continuous cultivation or probing of natural environments for key genera or key genes. However, the study of biofilms does not lend itself to many of these methods. By their very nature biofilms are dynamic areas of microbial activity and the sites of many microbial processes in natural environments. They have complex spatial arrangements which can be destroyed by many sampling or visualisation techniques. It is therefore crucial to study living biofilms in real time to be able to understand these processes.

We use image analysis and computer enhanced microscopy to study biofilms as we find that these meet the above criteria. The work presented will cover two different systems that have been developed by our research group, firstly, the detachment of bacteria from biofilms under different flow conditions and secondly the study of biofilm formation by thermophilic anaerobic consortia, including sulphate reducing bacteria. For example, we will report on the detachment of cell clusters from mature biofilms, including the dimensions of the clusters and their detachment frequency under steady growth conditions.

**16.00 BACTERIAL VIABILITY ASSESSMENT**

Jonathan Porter and Roger Pickup
Schl of Biological Sciences, University of Liverpool, Liverpool and Institute of Freshwater Ecology, Ferry House, Far Sawrey, Ambleside, Cumbria

Microscopic examination of samples from most natural environments reveals bacterial cells that are not routinely cultured. Evidence exists to suggest that many of these cells are alive. Direct methods for bacterial viability assessment require validation before being accepted as true indicators of viability. It is also necessary to evaluate which cellular functions should be used for effective viability determination, and which functions may provide ambiguous data. Correlated data from multiple probes that assess different cellular functions may increase the acceptability of the methods. Data are presented on the use of a range of viability probes applied to a variety of situations. Probing was performed to assess membrane potential, integrity and permeability and included use of novel nucleic acid dyes with differing charges. Laboratory strains and wild-type strains of several bacterial species in laboratory microcosm studies, continuous flow microcosms and from a range of freshwater lakes of differing trophic levels. Cells were also fractionated by size before probing. nalysis of labelled cells was achieved by flow cytometry and fluorescence microscopy.

The validity of the probes as satisfactory indicators of viability was supported in some ases but not others. As may be expected, bulk cell reparations from natural environments showed that the direct probes labelled as viable a proportion of uncultured cells. However, bacterial cells were always present that failed to respond to either approach with some instances of high proportions of the total bacteria being unaccounted for. It must follow that either these cells are dead, or that methods for assessing them are inadequate.

**16.45 MOLECULAR ANALYSIS REVEALS DIVERSITY IN FORM AND FUNCTION OF UNCULTURED BACTERIA.**

Neil D. Gray[1,2], Richard Howarth[1,2], Roger W. Pickup[3,4], J. Gwyn Jones[4] and Ian M. Head[1,2].

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For many years the unusual, morphologically conspicuous bacterium *Achromatium oxaliferum* has been considered as a single species. However, recovery of 16S rRNA gene sequences from purified *A. oxaliferum* cells obtained from different environments consistently produced multiple related 16S rRNA sequences. The use of whole cell *in situ* hybridization techniques demonstrated that these sequences were present in different populations of cells, morphologically identifiable as *A. oxaliferum*. Detailed analysis of the composition of the *A. oxaliferum* populations revealed that different sub-populations fell into distinct size classes that could not be discerned without the use of whole cell hybridization procedures. The divergence of 16S rRNA sequences derived from *A. oxaliferum*-like cells was consistent with different *Achromatium* species being present at a single site. Species-level diversity was also apparent between geographically distinct sampling locations. Microautoradiographic studies also revealed physiological differences between different populations. This was supported by the ability to amplify functional genes involved in inorganic carbon fixation (*rbcL*) and sulfur oxidation (*aprBA*) from cells present in populations that contained a high proportion of cells capable of fixing inorganic carbon and a failure to detect these genes in populations where inorganic carbon fixation was not evident. The presence of these genes correlated with the ability of the resident *Achromatium* species to fix inorganic carbon.

**09.05 DETECTION OF MICROBES IN THE NATURAL ENVIRONMENT BY *IN SITU* HYBRIDISATION.**

Rudolf Amann

Molecular Ecology Group, Max-Planck-Institut für marine Mikrobiologie, Celsiusstr. 1, D-28359 Bremen, Germany.

The ribosomal RNA (rRNA) approach to microbial evolution and ecology has become an integral part of environmental microbiology. The comparative analysis of rRNA gene sequences is today the standard method for the initial classification of newly isolated bacteria and has created a stable framework in which microorganisms are no longer lumped into artificial taxonomic entities but placed according to their true evolutionary relationships. Large publicly available database exist that encompass besides the 16S rRNA sequences of the majority of the validly described bacteria also numerous so-called "environmental" 16S rRNA sequences. rRNA-targeted oligonucleotide probes with defined specificities can now be designed in a directed way. When such probes are labelled with fluorescent dyes or enzymes they can be used to identify single microbial cells by fluorescent *in situ* hybridisation (FISH) directly in complex environmental samples. An up[date on available methods will be given including possibilities to identify individual cells of strongly autofluorescent cyanobacteria. With optimized methods and proper controls the FISH technique can yield exact cell numbers and spatial distributions for defined bacterial populations. Examples will be discussed that show the potential but also current limitations of the method. Furthermore, it will be outlined how FISH together with other methods can be used to study the diversity, structure and function of complex microbial communities, e.g. how microsensor measurements can be combined with FISH to study the in situ structure and activity of nitrifying biofilms at high spatial resolution.

**09.50 MOLECULAR METHODS FOR THE DETECTION OF METHANOTROPHS IN THE ENVIRONMENT**

J. Colin Murrell

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Methanotrophs are a fascinating group of bacteria that have the unique ability to grow on methane as their sole carbon and energy source. They appear to be widespread in nature and have been isolated from a number of different environments including soils, sediments, freshwater, marine sediments, seawater, acid peat bogs, hot springs and cold environments such as the Antarctic. There are now eight recognised genera of methanotrophs. Methanotrophs have attracted a great deal of interest over the past thirty years since they have considerable potential for the production of bulk chemicals, fine chemicals and in bioremediation processes such as the degradation of the groundwater pollutant trichloroethylene. More recently they are being extensively studied in a wide variety of environments since methanotrophs play a critical role in the global methane cycle. Polymerase chain reaction-based methods have been used to study the ecology and diversity of methanotrophs. We review here molecular ecology methods available or being developed for methanotrophs. These are based on 16S ribosomal RNA technology and specific amplification of 'functional genes', such as those encoding unique enzymes in the metabolism of these organisms including methane monooxygenase and methanol dehydrogenase. The use of $^{13}C$ substrates coupled to DNA-based detection methods for methanotrophs is also explored.

**11.00 ANALYSIS OF NATURAL COMMUNITIES OF AMMONIA OXIDISING BACTERIA**

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[2] Department of Zoology, Natural History Museum, Cromwell Road, London, SW7 5BD.

The analysis of microbial species diversity in environmental samples traditionally involves laboratory cultivation of natural isolates. In addition to the generic limitations of methods requiring laboratory cultivation, this approach is of little value for ecological studies of autotrophic ammonia oxidising bacteria, due to difficulties in obtaining and identifying pure cultures of these organisms. Molecular approaches, in particular sequence analysis of 16S rDNA and of functional genes, following direct nucleic acid extraction from samples and PCR amplification, provide alternatives which are independent of culturability. Application of 16S rDNA sequence analysis has proved particularly valuable for community analysis of autotrophic ammonia oxidising bacteria which, with the exception of apparently rare marine strains, belong to a monophyletic group within the â-subgroup of the proteobacteria. Ammonia oxidiser specific PCR primers have been used to amplify 16S rDNA from a range of environmental samples and subsequent denaturing gradient gel electrophoresis and phylogenetic analyses have been used to answer a number of ecological questions:

What is the extent of diversity of autotrophic ammonia oxidisers in natural environments?
Does laboratory cultivation select for specific groups of ammonia oxidisers?
Do marine aggregates and polluted sediments select for particular groups of ammonia oxidisers?
Do changes in soil pH influence the community structure of ammonia oxidising bacteria?

**11.45 QUANTIFICATION OF ACTINOMYCETES**

T P Curtis, W Sloan, R Davenport, C Coskuner, J Upton and M Goodfellow
University of Newcastle, UK

Abstract not received

**14.00 INCORPORATION OF 13C LABELLED SUBSTRATES INTO BACTERIAL FATTY ACID BIOMARKERS : A NEW APPROACH TO STUDYING NATURAL MICROBIAL POPULATIONS**

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Application of molecular genetic approaches have underlined the huge underestimate of microbial diversity based on culturable organisms in a range of natural environments. These approaches usually, however, provide little direct information about the physiology and biogeochemical impact of these organisms as phylogenetic position and physiological characteristics may not be closely related, especially in terms of detailed metabolic activity. Utilisation of radiolabelled substrates, however, provides accurate and quantitative information about bacterial metabolism in situ although no information about the organisms involved. The development of GC-IRMS techniques provides the potential of measuring the incorporation of 13C-labelled substrates into biomarkers for specific bacterial groups or types, thus coupling in situ bacterial activity with characterisation of thebacteria responsible. This approach, therefore, provides the opportunity to obtain unique biogeochemical information about microbial activity in natural environments andwill be demonstrated in estuarine sediments dominated by anaerobic sulphate reduction occuring via acetate metabolism. Although 13C-labelled acetate was incorporated into a range of relatively common bacterial fatty acids (16:1w7, 16:1w5, 16:0 and 18:1w7) interestingly these did not include the C17 fatty acids or mid chain branched fatty acids of the sulphate reducing bacteria thought to be dominant in sediments (Desulfovibrio & Desulfobulbus and Desulfobacter spp, respectively). Use of a specific inhibitor confirmed that the bulk of labelled fatty acids were derived fromsulphate-reducing bacteria and the fatty acid profile was most closely related to Desulfotomaculum acetoxidans, a spore former which could be readily isolated from the sediment. In addition, there is considerable 12C enrichment in the fatty acids of sulphate reducing bacteria over substrate, which might enable their contribution to bacterial biomass to be recognised without the need for isotopic labelling.

**14.45 MERCURY RESISTANCE IN BACTERIAL COMMUNITIES**

K Bruce
University of Liverpool

Abstract not received

**16.00 WHAT ARE THEY DOING THERE? *IN SITU* ANALYSIS OF FUNCTIONAL PROPERTIES OF BACTERIA WITHIN COMPLEX ENVIRONMENTS**

Michael Wagner[1], Natuscka Lee[1], Per H. Nielsen[2], Michael Schmid[1], Holger Daims[1], Stefan Juretschko[1], Jeppe L. Nielsen[2], and Karl-Heinz Schleifer[1].
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Two novel in situ analytical approaches yielding information about identity and function of a microbial cell in its environmental niche will be presented. Firstly, a new microscopic method for simultaneous in situ analysis of the identity, activity and specific substrate uptake profiles of individual bacterial cells within complex microbial communities has been developed by combining fluorescent in situ hybridization using rRNA-targeted oligonucleotide probes and microautoradiography. This method was applied to study nitrifying and denitrifying bacterial populations in activated sludge and biofilm systems by visualizing the uptake of organic and inorganic radiolabeled substrates in probe-defined populations under aerobic, anoxic and anaerobic incubation conditions. Most interestingly, in situ physiological data for abundant but yet not successfully cultured novel *Nitrospira*-like nitrite-oxidizing bacteria as well as novel *Azoarcus*-like denitrifying bacteria were obtained.

Secondly, a protocol for the *in situ* identification of mRNA of a virulence factor of *Listeria monocytogenes* has been developed. m-RNA-targeted transcript probes carrying multiple digoxigenin molecules were combined with anti-digoxigenin Fab antibody fragments labeled with horse-radish peroxidase to detect, via the catalytic deposition of fluorescein-tyramide, the *iap*-mRNA in single *Listeria monocytogenes* cells.

These techniques should help to identify key functional microbial groups active in the environment. Possible applications range from fundamental studies in medicine and microbial ecology to identifying bacteria involved in biotechnological processes such as in sewage treatment and bioremediation. In addition, knowledge on in situ physiology of novel bacterial taxa identified by molecular techniques should facilitate the design of appropriate isolation techniques for many of the hitherto uncultured microorganisms.

**16.15 MARINE BACTERIOPLANKTON IDENTIFICATION AND ACTIVITY ASSAYS WITH 16S RRNA PROBES AND MICROAUTORADIOGRAPHY**

Jed Fuhrman and Cleber Ouverney,
Department of Biological Sciences, University of Southern California, Los Angeles, CA 90089-0371, USA.

Among the main questions we have about native microbial communities are the identity and activity of individual microbes, and how these activities combine to form the community activity. For several years we have been using cloning and sequencing of 16S rRNA genes to learn what kinds of organisms are present in marine plankton communities, and to our surprise we have learned that archaea are common in the plankton, especially from deep waters. Several novel bacterial groups are present as well. Cloning gives an idea of what is there, but to learn the relative abundances of different organisms it is necessary to use methods with less chance of bias. We have adopted the use of FISH (fluorescent in situ hybridization) with 16S rRNA-based probes to count individual organisms from particular groups. With image-intensified epifluorescence microscopy and chloramphenicol signal amplification we have been able to detect the large majority (typically 90%) of total DAPI-countable cells, and we find that our local communities are made up largely of members of the alpha-proteobacteria (particularly a marine subgroup) and CFB groups, with some dynamics detectable. Deeper waters can be >50% archaea. The final question is how to assay activity of the groups. We have developed the means to combine microautoradiography and probe analysis to learn the activity of individual cells that can be identified by probe binding. Uptake of tritiated amino acids added at tracer levels is a reasonable general indicator of heterotrophic activity, and we find that most of the countable cells from most groups (including archaea) are indeed active. This suggests marine communities are highly active and dynamic.

# POSTERS

**S1 MICROBIAL DIVERSITY- AN INDICATION OF SOIL QUALITY?**

Sile O'Flaherty, Steve McGrath and Penny Hirsch
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Soil is not only important as a means of supporting crop production. It is an essential natural resource which should be maintained for future generations. A measure of soil quality is important for determining whether soil is being degraded. Microorganisms may constitute less than 0.5% of the total soil mass, but they are essential for nutrient cycling and exert a major influence on soil fertility. It is not clear whether the genetic diversity of soil microbes is an indicator of soil quality, or if the diversity of microbial functions is the most important factor. These are not necessarily the same. The toxic effects of heavy metals on soil microorganisms are well reported. However, a few studies have examined the more subtle effects of heavy metal pollution on the genetic diversity of particular groups of microorganisms. Studies that have been conducted tend to concentrate on the loss of microbial function in soil, with little consideration of the effect of bacterial biodiversity. The experimental work reported in this paper will assess the population shifts of microorganisms in heavy metal contaminated soils from the Market Garden Experiment at Woburn Experimental Farm, Woburn, U.K. These plots received contaminated sewage sludge between 1942 and 1961 and their history is well documented. A number of conventional methods were used to compare populations in these soils with different levels of contamination, while functional diversity was tested using BIOLOG™. We have applied a molecular technique, ERIC DNA fingerprinting, which allows us to study biodiversity at a molecular level, with the resolution required to see subtle changes in community structure.

**S2 THE SHORT AND LONG TERM EFFECTS OF SEWAGE SLUDGE APPLICATION TO SOIL ON b-SUBGROUP AMMONIA-OXIDISER COMMUNITY STRUCTURE.**

Graeme R. Campbell, Ken Killham and Jim Prosser
[1]University of Aberdeen, Dept Molecular and Cell Biology, Institute of Medical Sciences, Foresterhill, Aberdeen. AB25 2ZD. UK

Current European Union legislation banning the dumping of sewage sludge in marine environments has forced responsible authorities to come up with alternative strategies for disposal. One cost effective means of disposal has been to utilise sewage sludge as a fertiliser and soil conditioner. This practice is well documented in agricultural systems and owing to the changes in European legislation, disposal of sewage sludge on forest land has also been proposed as a attractive option. Despite the wide attention sewage addition to land has received, there has been relatively few investigations looking at its effects on ecologically important groups of microorganisms, including the b-subgroup ammonia oxidising bacteria. To investigate the impact of sewage sludge on the community structure of these bacteria, a short term mesocosm experiment was set up involving control (unamended) and sewage amended forest and agricultural soils. Long term effects on community structure was also assessed by comparing control and amended agricultural soil that received a four year field supply of sludge. PCR was used to selectively amplify b-subgroup ammonia oxidiser 16S rDNA sequences from total soil DNA. Potential differences between retrieved 16S rDNA populations were assessed by DGGE analysis and oligonucleotide probing. Results indicated that in the long term field experiment, no differences between control and amended soils could be detected. However, differences were observed between treatments in the short term incubations. These studies highlight the usefulness of DGGE and oligonucleotide probing for the detection of subtle population changes within ammonia oxidiser populations.

**S3 STABLE ISOTOPIC LABELLING OF SOIL MICROBIAL POPULATIONS TO DIRECTLY LINK TAXONOMIC IDENTITY WITH FUNCTION**

N R Parekh[1], P I Ineson[1], I Bull[2] and D Sleep[1]
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[2] University of Bristol

Abstract not received

**S4 DEVELOPMENT OF A PCR-BASED DETECTION SYSTEM FOR THE WATERCRESS PATHOGEN *SPONGOSPORA SUBTERRANEA* F. SP. *NASTURTII***

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*Spongospora subterranea* f. sp. *nasturtii* is the causal agent of crook root disease of watercress, and is the vector for two damaging viruses. It is an obligate biotroph, classified in the Order Plasmodiophorales, which also includes the pathogens *Plasmodiophora brassicae* (clubroot of crucifers), *S. subterranea* f. sp. *subterranea* (powdery scab of potato) and *Polymyxa* species.

Crook root is controlled by addition of zinc to watercress beds as a drip feed. Due to environmental concerns, it is desirable to rationalise the use of zinc. In this study a PCR-based approach has been adopted, using ITS rDNA to develop a diagnostic test for *Spongospora subterranea* f. sp. *nasturtii*.

Using a universal ITS primer, ITS5, with a primer based on known plasmodiophorid sequences, it was possible to isolate a product of 450-500 base pairs from crooked roots, which was identified as *S. subterranea* by BLAST sequence similarity searching. Following alignment with other published plasmodiophorid sequences, unique DNA regions within the ITS regions were used to design two specific primer combinations (SSN1, and SSN2 or 4). These are capable of amplifying conserved 300-350 base pair fragments from crooked root DNA from various locations around the U.K.

Initial data suggest that these primers will not amplify DNA from 16 other species tested, including closely related organisms. The sensitivity of detection of these primers is currently being evaluated.

**S5 MICROBIAL COMMUNITIES AS INDICATORS OF THE ECOLOGICAL IMPACT OF INDUSTRIAL POLLUTION**

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Industrial pollution is often quantified by expensive chemical analyses to determine the concentration of individual contaminants in the environment. Unfortunately, this gives no measure of the ecological impact posed, as factors such as bioavailability and the effect of combinations of pollutants are not taken into account. In this study, various measures of microbial community diversity and size were used to evaluate the ecological impact of heavy metal contamination. Thirty soil cores were taken from an industrial site covering approximately 25000 m2. Traditional plate counts of total `culturable' bacteria and those resistant to a variety of heavy metals were undertaken. Results from these simple analyses were supported by recently developed methods such as community level physiological profiling, total fatty acid profiling and DNA-based approaches. The latter involved comparison of sequence differences in amplified 16S rDNA fragments by denaturing gradient gel electrophoresis (DGGE). All microbiological data were compared to chemical determinations of heavy metal content. This revealed that microbial communities were generally smaller and less diverse in those samples with the highest levels of contamination. This was also associated with a significant shift in the both the culturable community composition and DGGE banding patterns. Two-dimensional representations of the data allowed the patchy nature of the impact to be visualized.

**S6 UV-C INDUCED KILLING IN *SALMONELLA TYPHIMURIUM* CULTURES DURING PROLONGED STARVATION**

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[1] University of Liverpool
[2] Insitute of Freshwater Ecology, Windermere

Abstract not received

**S7 DEVELOPMENT OF MOLECULAR BIOLOGICAL TOOLS FOR MONITORING SULFATE - REDUCING BACTERIA IN LANDFILL SITES**

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Landfill sites are essentially bioreactors in which the activity of anaerobic bacterial communities mediates the mineralization and stabilization of organic matter. They are extremely heterogeneous environments, rich in organic carbon sources and comprising large populations of a diversity of microbial species. Sulfate-reducing bacteria (SRB) are important anaerobes in a variety of environments, however, their activity in landfills is undesirable as they compete with methanogenic bacteria for available electron donors. This leads to inhibition of methane production and this interferes with the stabilization of landfill sites. SRB have been extensively studied in environments such as marine sediments and anaerobic biofilms, however our knowledge of SRB occurrence and distribution in landfill is extremely limited. Here we describe the development and application of molecular biological techniques to provide information on SRB populations that inhabit landfill sites. Nucleic acid PCR primers and oligonucleotide probes were designed to detect 16S rDNA of SRB genera and subgroups in DNA extracted directly from samples of landfill leachate. The results demonstrate the presence of populations of SRB in landfill sites and enable conclusions to be drawn on the relative distribution of SRB subgroups in different landfill sites. Temporal Thermal Gel Electrophoresis (TTGE) fingerprinting supported by sequence analysis of cloned DNA fragments permitted a more detailed phylogenetic analysis of these SRB communities. This study therefore provides the first insight into the occurrence and population structure of sulfate-reducing bacteria in landfill.

**S8 DETECTION OF METHANOTROPHS IN LANDFILL SITES BY PCR AMPLIFICATION OF 16S rRNA AND FUNCTIONAL GENE TARGETS**

Andrea E. Kelly and Alan J. McCarthy
School of Biological Sciences, University of Liverpool, Life Sciences Building, Crown Street, Liverpool, L69 7ZB.

Methanotrophs are a subset of the diverse physiological group known as methylotrophs that have been shown to be important in the global regulation of methane. They are obligate methane utilisers and contain methane monooxygenase as a defining property. Two types of methanotrophs are known, those that possess only the high affinity particulate form of the methane monooxygenase enzyme, pMMO (Type I) and those that also contain a low affinity soluble form, sMMO (Type II).
Methane oxidation has been observed in top soils of landfill sites but methanotrophic populations have not yet been isolated from landfill soils or leachate.

To date methanotrophs have been detected in a variety of environments using molecular methods for direct analysis of environmental samples.

In this study, the detection of methanotrophs in landfill samples by PCR amplification of DNA extracts is described. Amplification primers specific for the alpha subunit of the particulate methane monooxygenase (*pmo*A) and for the 16S rRNA genes were designed and applied to assess the diversity of methanotrophic populations.

**S9 QUANTIFICATION OF AMMONIA-OXIDISING BACTERIA BY COMPETITIVE PCR**

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The ammonia oxidising bacteria play a key role in the cycling of nitrogen in natural environments. Although culture-based methods, in particular most probable number techniques, have been used to quantify ammonia oxidisers from environmental samples their low growth rate means that results can only be obtained after several months of incubation. In addition, there is concern that the laboratory media used may not support growth of all or even representative bacteria. We have developed a competitive PCR (CPCR) technique to detect and quantify the ammonia oxidising bacterial populations in a range of environmental samples. Competitors have been constructed to allow nested amplification of ammonia oxidiser DNA thereby allowing their quantification in environmental samples where they are present in very low numbers. A range of calibration methods has been evaluated in order to obtain the most accurate means of interpreting the data obtained.

**S10 SPECIES AND FUNCTIONAL GENE DIVERSITY OF AMMONIA-OXIDISING BACTERIA IN GRASSLAND SOIL**

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[2] Department of Zoology, Natural History Museum, London, SW7 5BD, UK.

Ammonia-oxidising bacteria are responsible for the first stage of nitrification, the oxidation of ammonia to nitrite. Molecular analysis, based on 16S rDNA sequences of natural populations of ammonia-oxidising bacteria from a range of environments, has shown large variation in diversity, which was previously uncharacterised. Phylogenetic analysis of environmental 16S rDNA sequences demonstrated the existence of a number of clusters whose presence and abundance are related to environmental factors. The physiological basis for such differences in relative abundance of different clusters is unknown. The recent development of primers specific for the functional gene, *amoA* (ammonia monooxygenase), provides an opportunity to investigate the relationship between species and functional gene diversity within ammonia-oxidiser populations. Specific primer sets have been used to amplify, by PCR, both 16S rDNA and *amoA* gene sequences from both soil and laboratory enrichment cultures obtained from unimproved and improved grassland sites. PCR products have been analysed by denaturing gradient gel electrophoresis (DGGE) and oligonucleotide probing, coupled with sequencing and phylogenetic analysis.

**S11 MONITORING THE SURVIVAL OF A LUX-MARKED PAH-DEGRADING BACTERIUM IN POLLUTED AND NON-POLLUTED SOIL MICROCOSMS**

Mashreghi M., and J. I. Prosser

DeptMolecular and Cell Biology, University of Aberdeen, Institute of Medical Sciences, Foresterhill, Aberdeen AB25 2ZD, UK

The introduction of either genetically engineered (GEMs) or native microorganisms to contaminated environments has been used to accelerate bioremediation in situ. The success of such introductions depends on the survival and subsequent activity of the newly introduced species. To investigate survival of biodegrading GEMs in soil, a luminescence pseudomonad, *Pseudomonas stutzeri* P16 *lux* AB4, was constructed by insertional mutagenesis using mini-Tn5 *lux* transposon with high levels of luminescence, high stability and degradation activity. Survival and activity of this strain were studied in non-polluted soil microcosms inoculated with $10^7$- $10^9$ cells $g^{-1}$ at three matric potentials ( -30kPa, -750 kPa, and - 1500 kPa), in the presence and absence of indigenous populations, and also in sterile and non-sterile soil amended with 1 mg or 1 g phenanthrene $kg^{-1}$ soil, using drop plate techniques and luminometry. In non-polluted, non-sterile microcosms increases in viable cell number and luminescence activity were observed at -30 kPa.Viable cell number and luminescence activity in microcosms contaminated with 1 mg phenanthrene $kg^{-1}$ soil showed similar decrease in non-polluted microcosms but addition of 1 mg phenanthrene $g^{-1}$ of soil increased viable cell number and luminescence activity in sterile and nonsterile conditions after 5 days incubation. Results obtained showed the advantage of bioluminescence marker systems for detection and monitoring of the activity of GEMs in the environment and the effect of nutrient availability on degradation of pollutants by introduced bacterium.

**S12 DETERMINATION OF AMMONIA-OXIDISER POPULATION STRUCTURE IN A HYPEREUTROPHIC LAKE.**

Meade;[1] C. Whitby;[1] G. Basnett;[1] J.R. Saunders;[1] G. Hall;[2] R. Pickup[2] and A.J. McCarthy[1].

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Autotrophic ammonia-oxidising bacteria oxidise ammonia to nitrite and play a key role in the nitrification of freshwater lakes. Both 16S rRNA and ammonia monooxygenase (AMO) genes can be used as targets for the detection and differentiation of these organisms. Priest Pot, situated in the English Lake District, is a small hypereutrophic lake (3-4m deep, 1 hectare in area) which undergoes well-defined seasonal stratification associated with high ammonia-oxidising activity.

Lakewater and sediment obtained throughout the seasonal cycle were studied by a combination of most probable number (MPN) determination, PCR amplification and oligonucleotide probing of the 16S rDNA and temperature gradient gel electrophoresis (TGGE). *Nitrosospira* species were found to be ubiquitous, and the genotypic profiles of this community in the water column and sediment were distinct. Enrichment cultures at different ammonium concentrations were monitored using fluorescent *in situ* hybridisation (FISH). Ammonia-oxidisers from the sediment grew in both high (12.5mM) and low (0.65mM) ammonia concentrations but those enriched from the water column exhibited a preference for the low ammonia concentration. FISH was used to demonstrate the relative abundance of *Nitrosospira* and *Nitrosomonas* spp in the enrichments. We have also designed and optimised a range of PCR primers that target the AMO genes and these primers are also being applied to study the ammonia-oxidiser community structure in Priest Pot.

**S13 SOIL MICROBIAL DIVERSITY WITH RESPECT TO POLLUTANT PRESSSURE**

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Athough the microbial biomass in the soil is the driving force of most terrestrial ecosystems, (Killham, 1994), it has remained largely unstudied, due to the fact that most soil bacteria are unculturables (Alexander, 1977). With the advent of 16S rDNA PCR techniques, it has become possible to look at their genetic structure directly and so partially circumvent the need for culturing.

The aim of this project is to characterise the eubacterial population of a chosen soil, perturb it with a toxin (benzene) and monitor the changes in community structure through time. This was performed with specific regard to its ability for recovery (i.e. return to the original community structure). The main hypothesis of the experiment was that the eubacterial community would be sufficiently robust that it would recover its diversity without the aid of immigration, even though certain species numbers may fall below detectable limits. Secondary to this was the question: are culturable bacteria dominant in a community or merely suited to *in vitro* culture ?

To perform this, a destructively sampled microcosm was set up, the unculturable population was monitored by the technique of DGGE (denaturing gradient gel electrophoresis). The culturable population was monitored by traditional isolation, identification was achieved using BIOLOG.

**S14 INVESTIGATING THE SPATIAL DISTRIBUTION OF MICROBIAL DIVERSITY IN SOIL USING 16S rDNA ANALYSIS**

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Molecular techniques have revealed microbial communities in soil to be vast in both diversity and complexity. It is important to understand how this diversity is distributed when considering a sampling regime for a soil based study. Denaturing gradient gel electrophoresis (DGGE) of 16S rDNA PCR fragments is now used commonly to examine the community structure of uncultured organisms in soil and other environments. Spatial homogeneity of total bacterial communities in soil has been demonstrated previously using this approach. In this study, the microbial diversity in bulked and undisturbed rhizosphere soil of three characteristic grassland types has been examined. Various primer sets targeting archaea, pseudomonad and actinomycete communities have been used. This increase in resolution may indicate spatial differences between samples that were not observed when examining the total bacterial community.

**S15 DIVERSITY OF REPLICATIVE DNA HELICASES IN CULTURABLE AND NON-CULTURABLE BACTERIA**

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A range of methods are available for analysing genetic diversity in situ within both culturable and non-culturable bacteria, and there is now significant evidence that only approximately 1% of all bacteria have been isolated and characterised. Analysis of signature gene sequence diversity of 16S rRNA has indicated that there are several bacterial divisions which are not represented in cultivated bacteria but are widely distributed in the environment. Some may play crucial roles in geochemical cycles while others represent uncharacterised pathogens. For example, the causative agent of Whipples disease has been characterised taxonomically by analysis of 16S rRNA from clinical specimens, yet the causative agent has not yet been cultured. Our aim is to use culture-independent techniques to determine the diversity within bacterial replicative DNA helicase genes (dnaB) in culturable and non-culturable bacterial populations. Degenerate oligonucleotides have been designed to allow PCR-detection of dnaB genes from clinical specimens and environmental samples. A range of techniques will be used to assess the extent of diversity within PCR-amplified dnaB gene fragments and to determine the distribution of dnaB within different populations. Some existing bacterial dnaB genes contain putative intein-coding regions, hence we are also interested in the distribution of these regions within dnaB. This work complements DnaB protein structure/function studies being carried out by our collaborating partners. By this combined approach we aim to generate a map of potential inhibitable surface targets for DnaB, with the view to the empirical and rational design of novel helicase inhibitors. We also hope to further our knowledge of the distribution of unculturable bacteria in the environment.

**S16 SPECIES DIVERSITY OF AGGREGATE ASSOCIATED MARINE NITRIFIER POPULATIONS**

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Autotrophic nitrification is an integral part of marine nitrogen cycling. This process is performed by physiologically specialised ammonia oxidising bacteria (AOB). As marine systems are increasingly subjected to nitrogenous anthropogenic input from terrestrial sources, the study of the population ecology of this group of micro-organisms is of extreme ecological relevance.

Within the marine water column, AOB populations may exist as free living, planktonic populations or as aggregate associated communities, colonising particulate material, often generically termed marine snow. Significant differences exist between aggregate associated and free living populations in terms of activity and preliminary data also indicate that the autotrophic AOB community structure differs between the two environments.

The remit of this project was to assess the phylogenetic diversity of natural populations of AOBs within a transect of the NW Mediterranean Sea. Molecular analysis of aggregate associated and planktonic natural populations of AOBs was performed using rDNA based methodologies, including direct sequencing, denaturing gradient gel electrophoresis and genus specific oligonucleotide probing. Such experimental approaches were applied to investigate the succession of attached AOB community structure according to depth, seasonality and proximity to fresh water input. Molecular analysis of AOB community structure present in the underlying sediment was also performed to complement the community profiles obtained from the overlying water.

**S17 EVALUATION OF THE USE OF STABLE ISOTOPES IN DNA-BASED DETECTION METHODS FOR METHYLOTROPHS IN SOIL**

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Methylotrophs are a specialised group of microorganisms which utilise reduced carbon substrates with no C-C bonds as their sole source of carbon and energy. We have exploited the physiology of these microorganisms by exposing forest soil to 99% atom enriched $^{13}CH_3OH$. As a consequence of obligate growth on a labelled substrate, the DNA from active methylotrophs will be labelled with $^{13}C$ thereby increasing its density relative to that of $^{12}C$-DNA. Following equilibrium centrifugation in CsCl-ethidium bromide gradients $^{13}C$-DNA was collected and used for PCR amplification with phylogenetic (16S rDNA) and functional gene (*mxaF*, *pmoA*, *mmoX*) primers. In $^{13}CH_3OH$ exposed soil, ammonia monooxygenase-like sequences and novel methanol dehydrogenase sequences were detected in conjunction with several 16S rDNA sequences. This ability to link physiological activity with taxonomic identity should make the use of stable isotopes a powerful technique for studying methylotroph ecology.

**S18 MOLECULAR ECOLOGY OF METHANESULFONATE-UTILISING BACTERIA**

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Methanesulfonic acid (MSA) is a photooxidation product of dimethyl sulfide. It is degraded in the environment by methylotrophic bacteria that possess a methanesulfonate monooxygenase (MSAMO). The genes encoding the novel enzyme MSAMO from the terrestrial bacterium *Methylosulfonomonas methylovora* have been cloned and sequenced. MSAMO genes are clustered on the chromosome and encode a two-component hydroxylase, a ferredoxin and a reductase. The genes encoding MSAMO from the marine MSA-utiliser *Marinosulfonomonas methylotropha* have also been analysed. In this organism, the hydroxylase genes are present in two copies. Comparison of the MSAMO hydroxylase genes has shown that these genes are highly conserved in these MSA-utilisers and this has enabled the design and use of MSAMO gene-specific PCR primers to analyse new MSA-utilising bacteria from a ariety of different environments. 16S rRNA analysis has revealed that these new isolates are of the genera *Methylobacterium* and *Hyphomicrobium* and that their MSAMO genes have a high degree of identity to those found in *Methylosulfonomonas* and *Marinosulfonomonas*. The properties of these new isolates which grow on MSA as their sole carbon and energy source, phylogenetic analysis and comparison of their MSAMO hydroxylase genes will be presented.

**S19 BACTERIONEUSTON: FACT OR FICTION?**

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The bacterioneuston is the community of bacteria housed within the sea-surface microlayer, between 10 and 100µm thick. The microlayer contains considerably greater numbers of bacteria than the subsurface waters. Surface tension forces provide a physically stable environment, but one which is subjected to greater environmental and climatic variation than the water column. Most biological and chemical processes of importance occur at surfaces or interfaces between differing environments. The sea covers 71% of the surface of the Earth and this interface controls the exchange of natural and man-made substances between the atmosphere and hydrosphere, such as methane. The bacterioneuston is thought to have an affect upon gaseous exchange and transport mechanisms from the water column to the atmosphere and vice-versa.

By looking at microbial diversity within the bacterioneuston and comparison with subsurface water, we are able to identify significant differences within the two community structures. The top 30µm of the sea was sampled and examined using molecular ecology techniques combining 16S rRNA sequence data with specific gene probes. The affect of the bacterioneuston on gas exchange was investigated using gas flux measurements in both a laboratory tank and sea-going floating tank. The transfer velocity (k) for three tracer gases ($CH_4$, $N_2O$ and $SF_6$) was measured simultaneously. Preliminary results from the molecular analysis of the bacterioneuston and the pelagic water samples showed that only one type of methanotroph was present in both locations. Data from both the laboratory and field gas flux experiments will also be presented.

**S20 LINEAR ALKANESULFONATES AS A SOLE SOURCE OF CARBON AND ENERGY FOR GRAM POSITIVE AND GRAM NEGATIVE BACTERIA**

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Several bacteria were enriched and isolated from soil, activated sludge and rainwater samples using ethanesulfonate, propanesulfonate or butanesulfonate as the sole carbon and energy source. Most of the strains isolated utilized non-substituted alkanesulfonates with a chain length of C3 to C6 and the substituted sulfonates taurine and isethionate as carbon and energy source. A Gram positive isolate, P40, and two Gram negative isolates, P53 and AE4 , were characterized in more detail. Phylogenetic analysis grouped strain P40 in the genus *Rhodococcus*. Strain P53 was identified as *Comamonas acidovorans* and strain AE4 as an *Alcaligenes* sp. These bacteria also utilized a wide range of sulfonates as a sulfur source. Strain P40 and strain AE4 but not strain P53, released sulfite into the medium during dissimilation of sulfonate compounds. Cell-free extracts of strain P53 exhibited high sulfite oxidase activity when assayed with ferricyanide but not with cytochrome c. The dissimilation of 1-propanesulfonate by whole cells of P40 and P53 strains was induced during growth on this substrate and was not present in cells grown on propanol, isethionate or taurine. Whole cell suspensions accumulated acetone when oxidizing the non-growth substrate 2-propanesulfonate. Strain P40 also accumulated sulfite under these conditions. Stoichiometric measurements with 2-propanesulfonate as a substrate in oxygen electrode experiments indicate that the non-substituted alkanesulfonates were degraded by a monooxygenase. When strain P53 grew with non-substituted alkanesulfonates as carbon and energy source, cells expressed high amounts of yellow pigments, supporting the proposition that an oxygenase containing iron sulfur centres or flavins was involved in their degradation. The most probable mechanism for initiating alkanesulfonate degradation is by conversion to the homologous aldehyde by monooxygenation.

**S21 MICROBIAL DIVERSITY AND METHANE OXIDATION OF DRAINED AND RE-FLOODED FENLAND PEAT.**

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In the UK approximately 50% of peatlands have been altered for agricultural and forestry purposes. Reconversion of agricultural peatland to wetlands provides conditions favouring a short-term pulse in methane production until the community of methane oxidising bacteria has stabilised. We aim to quantify the changes in methane fluxes and methanotroph populations at a drained fenland in Suffolk which is being managed by the Royal Society for the Protection of Birds to develop reedbeds and wetland habitats. Methane oxidation measurements from depth profiles of arable (drained), reedbed (flooded) and woodland soils identified maximal rates in the top 10 cm of the woodland and reedbed soils, and at 15-25 cm depth within the arable soil. No methane production was recorded with any of the soils. DNA extracted from these soils was used for PCR amplification with phylogenetic (16S rDNA) and functional gene (*pmoA*, *mxaF*, *mmoX*) primers. Analysis of *pmoA* libraries identified a greater diversity of *amoA/pmoA* sequences in the arable soil as compared to reedbed soil. These preliminary results indicate that re-flooding of fenland peat may cause a shift in the population of methanotrophs.

**S22 DETECTION OF METHANOTROPHS IN DANISH SOILS USING MOLECULAR ECOLOGY TECHNIQUES**

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Aerobic soils, such as forest soils, play an important role in the global methane ($CH_4$) cycle by acting as a major sink for atmospheric $CH_4$ in the atmosphere. $CH_4$ consumption is a biological process carried out by aerobic $CH_4$-oxidising bacteria. At present it is not known what organisms consume atmospheric concentrations of $CH_4$ *in situ*. In order to determine which organisms may be responsible for the oxidation of atmospheric concentrations of $CH_4$, three soils from Danish forest (oak and sitka) and heathland, each demonstrating rates of atmospheric methane oxidation, were sampled. DNA extracted from the soils was used to prepare Eubacterial 16S rRNA and particulate methane monooxygenase (*pmoA*) gene libraries. The libraries were initially screened by RFLP analysis and clones representative of each of the Operational Taxonomic Units (OTUs) identified were sequenced. Phylogenetic analysis of these sequences revealed the diversity of the bacterial communities, the diversity of the methanotrophs, and also the type of *pmoA* sequence which may be associated with atmospheric methane oxidation. DGGE analysis was also used to study the community structures of the soils.

**S23 CHLOROMETHANE METABOLISM IN *HYPHOMICROBIUM* STRAIN CM2**

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Chloromethane ($CH_3Cl$) is a small, volatile organic compound, produced by predominantly natural sources in the environment, which is thought to account for 20% of the depletion of the ozone layer. Bacteria of the genus *Hyphomicrobium* are restrictive facultative methylotrophs that utilise a variety of C1 compounds, including methylsulfates and halomethanes. *Hyphomicrobium* CM2, isolated from contaminated soil, has the ability to utilise $CH_3Cl$ as a sole carbon and energy source. Three approaches were used to investigate the mechanism of $CH_3Cl$ degradation in this strain : i) protein profiling ii) molecular analysis iii) biochemical analysis. The results suggest that CM2 uses a methyltransferase system to degrade this compound which is similar to that found in *Methylobacterium* CM4. Novel strains capable of utilising $CH_3Cl$ have been isolated from pristine terrestrial, estuarine and marine sites, suggesting that these bacteria are widely distributed in the environment.

**S24 16S RDNA DIVERSITY WITHIN AN ANOXYGENIC PHOTOTROPHIC BACTERIAL COMMUNITY FROM AN EUTROPHIC LAGOON (LAKE VILAR, NE SPAIN).**

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We studied the 16S rDNA composition of the anoxygenic bacterial community in Lake Vilar during a mass development of purple sulphur bacteria (June 20th, 1996). Lake Vilar (Banyoles, NE Spain), is a meromictic karstic lake, with a surface area of 10,770 $m^2$. A stable chemocline is established between 4.0 and 4.5 m depth.

*acteria* specific oligonucleotides (27f, 1492r) were used to amplify by PCR the SSU rRNA genes from the genomic DNA extracted from this community. After cloning, 22 sequences were obtained and their phylogenetic affiliation was determined after comparison with different databases. The sequences identified as purple sulphur bacteria were identical and accounted for 55% of all clones. These were supposed to belong to *Chromatium minus*, which was recognised as the dominant species accounting for the 21% of total cells counts and 92% of the prokaryotic biovolume. ß–*Proteobacteria* and *Cytophaga-Flavobacteria-Bacteroides* groups both contained 9% of the recovered sequences.

ame groups were also identified in the anaerobic bacterial community from Wintergreen Lake (MI, USA), which was studied previously. As a remarkable fact, just one of the retrieved clones from Lake Vilar shared more than 90% similarity with sequences currently deposited, pointing to a high degree of unknown diversity within the anaerobic assemblage.

## S25 MOLECULAR DETECTION OF TYPE II POLYKETIDE SYNTHASE GENES IN CUBAN SOILS.

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Polyketides are a group of complex and diverse natural products, many of which are produced by actinomycetes and are synthesised by polyketide synthases, which are multifunctional enzyme assemblies. Two types of PKSs have been identified. Modular type I PKSs such as those responsible for erythromycin production and type II PKSs responsible for the synthesis of aromatic polyketides such as actinorhodin. Consensus primers have been designed to amplify a region spanning two genes found in all type II PKS so far characterised. These primers were used to amplify PKS genes from DNA extracted from freshly sampled Cuban soil. The distribution of PKS genes was found to vary depending on the nature and location of the sampling site. A clone library was constructed consisting of PKS PCR products amplified from soil DNA. Sequence data from these soil clones showed considerable diversity compared to published sequences. Bacteria containing PKS genes have been isolated from Cuban soils and shown to be actinomycetes on the basis of 16S rRNA and recA sequence data. PKS sequences from actinomycete isolates showed less diversity compared to sequences obtained from soil clones.

## S26 MOLECULAR DETECTION OF CHITINOLYTIC ACTINOMYCETE COMMUNITIES IN SOIL.

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Actinomycetes are found in a wide range of different soil types from diverse geographical locations and play an important role in the degradation of polysaccharides, cellulose and chitin. Chitinases are produced in abundance by bacteria and majority of the streptomycetes have chitinolytic activity.

*Streptomyces chitinase* genes appear to cluster into two groups, one consisting of *Streptomyces lividans chiA,chiB* and the *Streptomyces olivaceoviridis exo-chi01* exochitinase. The second group consists of *S. lividans chiC*, *Streptomyces plicatus chi63* and *Streptomyces thermoviolaceus chi40* (Miyashita *et al.,* 1997). Two sets of PCR primers have been designed from alignments of the nucleotide sequences of these groups and amplified the respective genes from *S. lividans* and numerous other streptomycetes. Nearly all the streptomycetes screened were shown to contain at least one of the *S. lividans* chitinase genes. Sequencing of the PCR products has shown that these genes have high sequence identity within the region amplified to the *S. lividans*-like chitinase genes. These results indicate that the two clusters of streptomycete chitinases are highly conserved and distributed within the streptomycetes. A soil was chosen that was known to have high chitin content and a bacterial community that was almost entirely dominated by actinomycetes. Total community DNA and isolates were screened with these primers.

Primer sets have been designed to amplify sequence from the group A and group B bacterial chitinases. These primer sets amplified a product of the expected size from bacteria known to contain group A or B chitinases. Using these primer sets new sequence data has been retrieved from several chitinolytic bacterial strains.

**S27 DISTRIBUTION OF AMMONIA OXIDIZER MICROCOLONIES IN ACTIVATED SLUDGE FLOCS BY *IN SITU* CHARACTERIZATION**

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Samples were collected from a plug-flow sewage treatment plant and fluorescence in situ hybridization (FISH) technique was used to identify ammonia oxidizing bacteria (AOB) with confocal scanning laser microscopy (CSLM). Nitrosospira genus was found dominant in this plant. Distribution of AOB microcolonies was studied along the depth of activated sludge flocs (ASF) with vertical optical sectioning. Most of the AOB microcolonies were observed at about 55-60% and 75-80% of ASF depth with Nso1225 and Nsv443 and their distribution was normal. Furthermore, there was no or very few AOB microcolonies in the 10% bottom and top of the ASF in both cases. Therefore, it was demonstrated that AOB microcolonies were not distributed randomly in the ASF. In addition, AOB microcolonies number per unit volume of different sections of ASF was found heterogeneous. It was also demonstrated that size of AOB microcolonies are related to their location in the ASF.

**S28 LOSS OF BACTERIAL DIVERSITY IN ARABLE SOIL DUE TO THE APPLICATION OF ZINC-CONTAMINATED SEWAGE SLUDGE**

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We have used restriction enzyme analysis of 16S rDNA molecules (**C**loned **A**mplified **R**ibosomal **D**NA **R**estriction **A**nalysis) to measure the diversity of the extractable bacterial fraction of polluted and control soils. The contaminated soil received two doses of zinc-enriched sewage sludge raising zinc levels to 400 mg $kg^{-1}$: this has reduced crop yields of pea and barley in this loamy sand by at least 50%. CARDRA results from 250 clones from each soil have been analysed. Initially, all fragment patterns obtained with *Hpa* II were sub-divided on the basis of the number of restriction fragments >45bp. Then principal components analysis was used to compare the relative diversity of patterns in each soil. Application of zinc changed the two main measures of diversity, reducing both the number of different patterns present and their equatability (the way in which individuals are distributed among those species). This loss of biodiversity has important implications for the continued disposal of sewage sludge on arable land as the functioning of the soil may also have been disturbed.

**S29 IDENTIFICATION OF MEMBERS OF THE NOVEL CANDIDATE DIVISION "OP5" AS ACTIVE MEMBERS OF A FRESHWATER SEDIMENT BACTERIAL COMMUNITY**

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Recently 16S ribosomal RNA gene sequences that may represent novel division-level diversity within the bacterial domain (e.g. Hugenholtz et al., *J. Bacteriol.* **180**, 366-376.) have been detected in natural microbial populations. These sequences were recovered from DNA extracted from a number of environmental samples. Because the sequences were derived from DNA it is difficult to infer whether the bacteria from which they were derived, were active members of the resident microbiota, and there is evidence that PCR-amplifiable DNA may survive for long periods of time in natural samples even though the source organisms are unlikely to be active in the environment from which they were recovered (e.g. Coolen and Overmann, 1998. *Appl. Environ. Microbiol.* **64**, 4513-4521). In a study of the bacterial diversity of an anoxic freshwater lake sediment we recovered rRNA sequences from an RNA template that were most closely related to members of the recently described OP5 candidate division providing evidence that bacteria from OP5 were active *in situ*. This is possibly the first instance of these bacteria being identified as metabolically active in the environment.

## S30 SPECIFIC DETECTION OF THE THREE MAIN PATHOGENS OF POTATO BLEMISH DISEASES

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Tuber skin blemish diseases are of increasing importance due to the demand for washed potatoes with a high quality appearance for the fresh 'pre-pack' market. The bacterial disease common scab (*Streptomyces scabies*) and the two fungal diseases, silver scurf (*Helminthosporium solani*) and black dot (*Colletotrichum coccodes*) are the most frequently encountered blemish diseases in the UK. Wastage from these diseases can cost the British potato industry up to £14 million a year. Providing practical solutions to wastage problems is therefore regarded as a high priority throughout the potato industry. An improved understanding of the epidemiology of these diseases will assist in their control thus improving the quality and efficiency of ware production and ensuring supplies of healthy seed. It is therefore essential to develop methods to detect and quantify these pathogens in potatoes and soil to screen seed stocks and predict the probability of infection from field soils.

PCR primers were designed to unique sequences within the internal transcribed spacer (ITS) regions for specific detection of *H. solani* and *C. coccodes*. In the case of *S. scabies*, primers were designed to detect the proposed pathogenicity gene *(nec1*). Nested PCR was used to increase specificity and sensitivity of single-round PCR and the assay could detect purified DNA of *H. solani*, *C. coccodes*, and *S. scabies* down to levels of 10 fg per reaction. It was also possible to detect all the pathogens in one reaction by the incorporation of the three primer sets into a multiplex PCR assay. Comparisons of each primer to DNA and protein databases of other fungi and bacteria revealed no, or low levels, of similarity. The specificity of primers was confirmed as no PCR products were amplified using each primer set and DNA from a range of different fungal and bacterial plant pathogens. A simple and rapid
procedure for direct extraction of DNA from soils was modified to yield DNA of a high purity suitable for PCR within 3 h. The sensitivity of PCR for the specific detection of *H. solani*, *C. coccodes*, and *S. scabies* in seeded soils was tested in parallel for both the single primer sets and in multiplex PCR. The level of sensitivity for both PCR systems was set at 3 spores per gram of soil, the lowest level of inoculum added. However, the multiplex PCR assay had the advantage of reducing the time and cost of theprocedure. The potential of these techniques for the detection and monitoring of these pathogen populations in soil and tubers for epidemiological studies will be discussed.

## S31 CHARACTERISATION OF SOIL MICROBIAL COMMUNITIES IN UPLAND GRASSLANDS: COMPARISON OF PHENOTYPIC TECHNIQUES

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Successful ecosystem management depends on an understanding of the mechanisms controlling the functioning and stability of these ecosystems. It is recognised that soil microorganisms mediate important ecosystem processes such as nutrient cycling. However, the identity and functional significance of these organisms is poor due to the lack, until recently, of methodologies to study microbial diversity. The SOAEFD MICRONET project is quantifying the spatial and temporal diversity of soil microbial communities across a gradient of characteristic grassland types Mg6-U4b-U4a at ten sites throughout the UK. As part of this co-ordinated project, microbial communities were characterised using Biolog, PLFA, biomass and culturing. Canonical variate analysis of the Biolog and PLFA data showed significant discrimination of soil microbial communities between sites and pasture types. The intensive (Mg6) and extensive (U4a) grasslands were bacterial and fungal dominated communities, respectively. Samples were taken over a one-year period to determine the degree to which temporal variation and management influence microbial community variability. It is apparent that soil microbial communities are strongly influenced by vegetation type and site. It is also clear that broad scale measures of microbial biomass show little temporal variation as compared to measures of community structure, which vary significantly with time.

## S32 THE RELATIONSHIP BETWEEN THE CONVOLUTED COLONY MORPHOLOGY AND SURFACE SURVIVAL IN SALMONELLAS

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The convoluted colony morphology has been associated with enhanced stress tolerance and virulence in *Salmonella enteritidis* and *Salmonella typhimurium*. The aim of this study was to determine if a similar correlation could be seen in isolates of *Salmonella binza*, a salmonella which is frequently isolated from food animals but which rarely features in human infection.

To establish the prevalence of the convoluted colony type in *Salmonella* serovars, isolates were screened for their ability to produce such colonies by growing them on modified brilliant green agar and blood agar at 25°C. Selected isolates were screened for their ability to survive in dried blood droplets on Formica squares at 20°C for 24 hours.

One hundred and nine *S. enteritidis*, 102 *S. typhimurium* and 25 *S. binza* isolates were screened for colony morphology. Colony morphology could be divided into three groups, those with a high degree of convolution on both media, those which remained smooth and those which exhibited only slight convolution. Thirty six percent of isolates were screened for surface survival. Strains of *S. enteritidis* PT4 and *S. typhimurium* DT104 demonstrated that the convoluted morphology was related to enhanced levels of survival. Convoluted colonies were identified in strains of *S. binza* yet no correlation between colony morphology and surface survival was seen.

## S33 SURVIVAL OF *CAMPYLOBACTER JEJUNI* ON FOOD PREPARATION SURFACES SUBJECT TO DRYING

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Campylobacter has previously been observed to persist only on wet surfaces in the kitchen environment and to die rapidly in dry conditions. This study investigated the
persistance of Campylobacter on naturally and artificially contaminated catering surfaces using improved recovery techniques.

Fifteen households were each given a fresh chicken purchased from a local retail outlet to prepare for cooking on a formica worksurface or plastic board. After preparation, the surface was examined for the presence of Campylobacter at time intervals up to 60 minutes following preparation. A similar experiment using 12 chickens was performed under controlled laboratory conditions. Isolates from these experiments were biotyped and screened for their *in vitro* survival ability.

*C. jejuni* could be recovered from dry surfaces in kitchens and the laboratory after these surfaces had been observably dry for up to 50 minutes. Survival times varied between individual isolates and did not relate to their ability to survive air drying on surfaces in subsequent laboratory experiments.

It is important that dry surfaces are now considered to be possible sources of viable Campylobacter cells.

## S34 THE MOLECULAR EPIDEMIOLOGY OF CAMPYLOBACTERS ISOLATED FROM POULTRY BROILER FLOCKS USING *fla* TYPING

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Up to 70% of cases of human enteric campylobacteriosis are attributed to the consumption of poultry meat. The majority of cases are due to *Campylobacter jejuni*. Poultry flocks are frequently contaminated with this organism 2-3 weeks after hatching. The sources and routes of transmission of campylobacters within the broiler house are unknown. With this objective in mind, a molecular sub-typing method (PCR/RFLP of the *flaA* and *flaB* genes) has been applied to provide definitive identification of campylobacter strains isolated during regular sampling of birds through four successive crops and from the environment of one commercial broiler house.

No campylobacters were recovered from the internal environment of the broiler house after cleaning and disinfection.

Various genotypes were recognised amongst strains isolated from puddles, wild bird and cow faeces. The first crop of birds was shown to be infected with the same genotype of *Campylobacter jejuni* as had earlier been recovered from cow faeces. Campylobacters were also isolated from the soles of workers boots after dipping had taken place suggesting this may be an important route of transmission into the broiler house.

## S35 COMPARISON OF THE RECOVERY OF *SALMONELLA TYPHIMURIUM* DT104 IN LIQUID AND SOLID MEDIA FOLLOWING HEAT DAMAGE IN THE PRESENCE OF GLUCOSE AND FRUCTOSE.

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*Salmonella typhimurium* DT104 is of concern to the food industry due to the severe human disease caused, its multiple drug resistance and extensive animal reservoirs. Heat treatment and lowering the water activity ($a_w$) of products are established methods for reducing microbial contamination. In this study, recovery of *S. typhimurium* damaged by heating at reduced $a_w$ was optimised to allow a reliable and fail-safe assessment of survival.

Stationary phase cells of DT104 strain 30 grown at 37°C in tryptone soya broth (TSB), were inoculated (final density ~$10^8$cfu/ml) into aliquots of TSB with glucose-fructose ($a_w$s of 0.99, 0.9 and 0.65) at 54°C. Samples were taken at timed intervals and recovery on horse blood agar [BA], nutrient agar [NA], and by most probable number count in buffered peptone water (BPW) were compared. Recovery methods were performed in duplicate and incubated at 30 and 37°C for 144 hours, with plate reading at 24, 72,and 144 hours.

BA recovered more damaged salmonellae than NA or BPW. Incubation at 30°C gave no advantage over 37°C but severely damaged cells required prolonged incubation times at either temperature for maximal detection. Use of the optimised method described here will increase sensitivity of detection of DT104 damaged by heating at low $a_w$.

## S36 CULTURE OF *CAMPYLOBACTER* SPECIES IN MICROTITRE PLATES FOR THE ASSESSMENT OF MEDIA EFFECTS

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There is a need for rapid and improved methods for isolation of campylobacters from food and environmental samples. In particular, when only few injured cells are present, they must be protected from further damage and encouraged to repair and multiply as quickly as possible to facilitate detection by conventional or molecular methods. Many enrichment protocols have been described and testing even a few by traditional methods is expensive in media and time.

A Most Probable Numbers (MPN) method designed for enumeration of *Salmonella* species has been used to assess media effects on damaged campylobacter. Heat damaged cultures were serially diluted in broths under test and appropriate dilutions dispensed into wells of one or more microtitre plates. These were sealed with transparent film and incubated for up to five days to allow growth to be assessed visually. A multichannel pipette was used to transfer 5µl volumes from a representative number of wells to CCDA plates, to establish that turbidity was caused by growth of campylobacter. The number of wells in which growth had occurred was counted and MPN values estimated using a computer program (Oxoid).

Using this method, and strains of *C jejuni* and *C coli*, it was possible to screen a number of campylobacter base broths for those promoting best recovery of damaged cells. Subsequent microtitre MPN testing indicated best enrichment additives and the effects of selective supplements.

Microtitre MPN testing is an economical method for screening and comparative testing of broths for growth of campylobacter.

## S37 CHARACTERISATION OF STRESS TOLERANCE AND RPOS STATUS IN WILD TYPE *SALMONELLA ENTERICA* SUBSP. *ENTERICA* TYPHIMURIUM DEFINITIVE TYPE 104 AND ENTERITIDIS PHAGE TYPE 4 ISOLATES.

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The heat and acid tolerance and the ability to survive airdrying on commenly used kitchen surfaces were assessed in clinical and environmental isolates of the important *Salmonella enterica* subsp. *enterica* Typhimurium definitive type (DT) 104. Two (5 %) out of thirty eight DT 104 isolates were identified as being markedly more sensitive to all stresses examined. In comparison, data from a previous study showed that seven (18%) out of forty Enteritidis phage type (PT) 4 isolates were more sensitive to these stresses. Expression of the RpoS protein was examined in selected isolates of DT 104 and PT 4. In those with normal stress tolerance a 100-fold induction of the RpoS-dependent *spvR/A'*::*luxCDABE* fusion was observed upon entry into stationary phase. The four sensitive strains examined showed no induction or a reduced level of *spvR/A'*::*luxCDABE* expression. The *rpoS* gene was sequenced from these isolates and three were found to harbour mutations including one deletion, and two other mutations resulting in amber nonsense codons. Mutations in the *rpoS* allele in clinical and environmental isolates of DT 104 and PT 4 appear to arise relatively frequently despite providing a less virulent and less resistant phenotype as determined by standard methods.

## S38 EXTENDED CULTURE OF *SALMONELLA ENTERITIDIS* PHAGE TYPE (PT) 4 ISOLATES AT LOW TEMPERATURE: EFFECTS UPON CELL MORPHOLOGY AND TOLERANCE.

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*Salmonella enteritidis* PT4 was responsible for 47% of salmonella infections in England and Wales, in 1997. Intimately associated with this problem is the increasing tendency to consume chilled foods rather than frozen or chemically preserved foods. However, little work has been undertaken to determine the physiological changes induced in salmonella by chilling and to establish whether these changes could be relevant to food safety.

*Salmonella enteritidis* PT4 strains that were either RpoS positive or negative were examined. Stationary phase cultures were held at 6°C for up to 6 weeks. Morphological analysis was performed upon chilled cells and also cells warmed to 37°C. The effect of chilling upon salmonella physiology was determined by establishing relative tolerances to stressful conditions typical of food preparation, namely acidity, heat and airdrying on surfaces.

In response to sustained cold stress, isolates showed increased cell length due to perturbation of cell septation, but viability was not affected. This elongation or filamentation was most marked in RpoS-positive strains. Upon warming, single filaments produced many daughter cells. Significantly RpoS-positive status was associated with resistance to the above stresses.

RpoS-positive strains showed enhanced tolerances to some conditions encountered during food preparation. A functional RpoS protein may allow an increase in biomass without an increase in viable counts. The influence of cell density and the implications of these findings for public health are discussed.

**S39 THE ROLE OF RHEOLOGY IN THE STRUCTURE AND FUNCTION OF BACTERIAL BIOFILMS**

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A non invasive technique for measuring the rheological properties of biofilms growing in pipe flows has been developed. We used short term fluctuations in fluid shear to structurally deform both mixed (*Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, *Klebsiella pneumoniae*, and *Stenatrophomonas maltophilia*) and pure (*P. aeruginosa*) culture biofilm structures. Strain measurements were made by direct microscopic observation. Using this technique we conducted simple stress-strain and creep experiments. Our results revealed that both the pure and biofilms were highly compliant materials and readily deformed by changes in fluid shear. The biofilms behaved like elastic or viscoelastic solids below the shear at which they were grown but viscoelastic liquids at elevated shears. These characteristics are similar to those of Bingham fluids, a non Newtonian class of fluid which only exhibits viscous flow once strained beyond their elastic yield point. Implications for biofilm rheology on processes of mass transfer, detachment and energy losses in pipelines are discussed. The ability of the biofilm to flow may also help explain the formation of migratory biofilm ripples which we have recently documented in mixed culture biofilms. Biofilm ripples travelled downstream across the substratum at velocities up to approximately 1 mm/hr.

**S40 IMAGE ANALYSIS AS A TOOL FOR STUDYING SUBSURFACE MICROORGANISMS.**

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Some of the most economically important microorganisms originate from subsurface environments. Studying these organisms *in situ* is impossible for environments thousands of meters below the earth's surface. Image analysis provides a partial solution. Both flow cells and micromodels, which simulate rock pore systems, can be used to observe laboratory grown organisms received from fluids originating in the subsurface. It is possible to recreate the slow flow rates occurring in ground water with a pressure head generated flow and high temperatures with the use of a heated microscope stage. Organisms may therefore be studied microscopically in an environment that is as close to natural as is feasible. This allows investigation of biofilm formation and bacterial movement through rock matrices. Current research is directed to the problems associated with growth of sulphate reducing bacteria in oil field reservoirs. Quantitative studies have been carried out on the ecology of mesophilic and thermophilic consortia, denoted EX246 and EX258, which were originally isolated from sponsors wells.

**S41 ANTIMICROBIAL SUSCEPTIBILITIES OF METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* (MRSA) BIOFILMS**

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In Europe, Methicillin resistant *Staphylococcus aureus* (MRSA) accounts for 60% of nosocomial infections, and 30% of nosocomial infections in the U.S and South America. Prosthetic heart valve infections and urinary tract infections are frequently caused by single population biofilms of MRSA. These biofilms protect the bacterial cells from the effects of various antimicrobial agents. Therefore, MRSA biofilms are notoriously difficult to eradicate. The aim of this research was to administer vancomycin and rifampicin at clinically significant concentrations against a pre formed MRSA biofilm and assess the efficacy of these antimicrobial agents. This was achieved by using silastic rubber as the surface and artificial urine as the growth medium. The effect of the antimicrobial agents on the MRSA biofilm was assessed by using image analysis and scanning electron microscopy. In addition, the efficacy of vancomycin and rifampicin was also monitored against the free floating form (planktonic) of MRSA via a killing curve protocol. Comparisons were made between the susceptibilities of the planktonic and biofilm forms of MRSA.

**S42 IMAGE ANALYSIS TO MONITOR FUNGAL-BACTERIAL BIOLFILMS**

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Abtract not received

**S43 USE OF MORPHOLOGICAL AND MOLECULAR MARKERS TO PREDICT SECONDARY METABOLITES**

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Abtract not received

**S44 FORMATION OF FILAMENTS BY CHILLED *SALMONELLA ENTERIDITIS***

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The processing and storage of foods under refrigerated conditions is becoming more common due to the decreased use of preservatives. However, only recently has the effect of chilling been studied on food pathogens such as *Listeria monocytogenes* and *Salmonella* species. *Salmonella enteriditis* Phage Type (PT) 4 has been reported to form elongated cells at 4°C exceeding 150μm in length. It is suggested that the formation of these filaments is under the control of *rpoS.* This gene encodes a sigma factor that induces tolerance phenotypes under stress conditions, including for example, acid tolerance, at stationary phase. The aims of this study are to investigate the filament formation of wild type *S.enteriditis* PT4 isolates E and I using flow cytometry and image analysis techniques. These isolates are *rpoS* positive and *rpoS* negative, respectively and will help elucidate the functions of this gene in the elongation of *S.enteriditis* cells.

**S45 THE FORMATION, RECOVERY AND SURVIVAL OF CHILLED *SALMONELLA ENTERITIDIS* PHAGE TYPE 4 ISOLATE E IN FOODSTUFFS AND ON SURFACES**

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Work from our laboratories has reported that *Salmonella enteritidis* Phage Type 4 (PT4) isolate E elongates during chilling at 4°C. These filaments can reach lengths of up to 150μm and they complete cell division when warmed, increasing cell numbers substantially. The aim of this study was to investigate the formation of these filaments at 6°C and their subsequent recovery in Buffered Peptone Water at 22°C and 37°C and in milk and chicken broth at 37°C. In addition, experiments concerning the survival of these cells on glass slide surfaces at 22°C were also conducted. Elongated cells were formed in all media used and recovery was slower when compared to non-chilled *S.enteritidis* cells. Initially, there was no difference in survival between elongated cells and control cells on the glass surfaces. Increased exposure on the slides showed that non-chilled cells were able to survive for longer. The implications of these findings are immense when concerning possible food spoilage in food preparation, storage and consumption.

**S46 MONITORING RAPIDLY GROWING MYCOBACTERIA IN BIOFILMS**

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Abstract not received

**S47 BIODEGRADATION OF CHLOROPHENOXYALKANOIC ACID HERBICIDES.**

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Chlorinated phenoxyalkanoic compounds are widely applied as herbicides. However, due to their persistence in the environment, there has been extensive interest in their biodegradability. It has been well documented that the less recalcitrant herbicides such as 2,4-D and MCPA are readily decomposed by both mixed and single bacterial isolates, facilitated by the presence of the pJP4 plasmid.

However, fewer studies have focused on the biological activity of the more recalcitrant herbicides 2(2-methyl-4-chlorophenoxy)propionic acid (Mecoprop), 4-(2,4-dichlorophenoxy)-butyric acid (2,4-DB) and 4-(2-methyl-4-chloro)phenoxybutyric acid (MCPB). Through their capacity to utilise these more structurally complex herbicides, consortia of bacteria were isolated through enrichment. Crude cell extracts were monitored by spectrophotometric and phenolic assays, along with chloride ion determination to assess degradation. Such activity could either be plasmid or chromosomally mediated and so modified alkaline extraction methods were adopted for analysing the presence of degradative plasmids. We report that the presence of large plasmids in the isolated consortia may play a role in the degradation of these more complex compounds.

**S48 METAL UPTAKE AND GROWH OF HYPERACCUMULATING PLANTS IN FUNGUS-INOCULATED SOIl**

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Abstract not received

**S49 THE EFFECT OF INCREASED SALINITY ON TOLUENE-DEGRADING MICRO-ORGANISMS**

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Toluene is a component of some wastewaters from the chemical/petrochemical industries and is also a common contaminant of ground water and soil. Although there has been much research into its biodegradation, there has been very little study of the effects of salinity on this process. Process waters from the chemical and petroleum industries are often highly saline and are prone to rapid changes in salinity. Such changes in salinity without pre-treatment dilution are likely to adversely affect the biodegradation of compounds like toluene.

The aim of this work was to study how increasing salinity affects growth and physiology of toluene-degrading micro-organisms. Three mixed microbial cultures were developed at 0.2, 4 and 7% NaCl from mixed inocula from a diverse variety of environments including activated sludge, soils, river water and oil field produced water. From these enriched cultures, pure cultures were isolated. The toluene-degrading ability and salt tolerance of these pure cultures has been investigated. Toluene-degrading bacteria were isolated at all salinities but the number of isolates decreased with increasing salt concentration. Isolates varied considerably in the ability to tolerate changes in salinity and in the rate of adaptation to salt stress.

**S50 TRANSPORT FUNCTIONS AND PROTEIN INTERACTIONS IN MERCURIC IRON RESISTANCE**

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Abstract not received

**S51 LEAD RESISTANCE IN ALCALIGENES EUTROPHUS**

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Abstract not received

**S52 NITRATE REDUCTASE SEQUENCE DIVERSITY IN A SEDIMENT COMMUNITY**

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Nitrate respiring bacteria are a taxonomically diverse and ecologically significant group. They possess the membrane bound nitrate reductase (Nar) which reduces nitrate to nitrite and is expressed under anoxic conditions. Using the PCR and degenerate primers directed against the genes encoding Nar, sequence data have been obtained from DNA extracted directly from various depths of a freshwater sediment core, and from cultured isolates from the same sample. Sequence variation is observed within the two types of template, and between sequences recovered from samples at each depth. Isolates are currently being identified using 16S rDNA techniques. Comparison of genus information and nar sequence variation will provide an insight into the types and abundance of nitrate respiring bacteria at each depth and this will indicate whether these techniques can be used to characterise populations of nitrate respiring bacteria. Furthermore, a periplasmic nitrate reductase (Nap) which is active during micro-oxic or fully oxic conditions has been described in a number of laboratory strains. Using the PCR and degenerate primers, genes encoding this enzyme are observed to be widespread within the sediment isolates, and thus may further contribute to the removal of nitrate from the environment.

**S53 AN IMMUNOFLUORESCENCE ASSAY FOR ASSESSING THE NUTRIENT STATUS OF CYANOBACTERIAL PICOPLANKTON *IN SITU*.**

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Oxygenic photoautotrophic microorganisms of the genera Synechococcus and Prochlorococcus are cyanobacteria which contribute significantly to primary production and are now widely accepted as the most abundant members of the picoplankton in the world's oceans occupying a key position at the base of the marine food web. In the euphotic zone of oligotrophic oceans one of the main factors `limiting' the production (growth rate or growth yield) of these organisms is the availability of nutrients. However, we have a poor understanding of which nutrients limit productivity due largely to a lack of suitable methodologies. Molecular techniques for assessing the nutrient status of these organisms offer the possibility of analysing the status of individual cells, populations or communities in situ without perturbation or manipulation of the environment. The idea is to use the physiological response of the organism to identify, and subsequently raise antibodies against, protein markers whose synthesis is modified during depletion for a given nutrient. Cell surface proteins specific to a particular nutrient stress can be rapidly identified in vivo by labelling of cells using 35S-methionine and cell fractionation studies, or using biotinylation reagents which specifically label surface exposed proteins. The use of antibody probes has several advantages over classical methods including the avoidance of sample manipulation, the high sensitivity and taxon specificity of the procedure as well as the potential to detect and quantitate expression
in single cells. We present data describing the suitability of PstS, a component of the high affinity phosphate acquisition machinery, as a molecular marker of the P status of these photosynthetic picoplankton. Currently, markers for assessing the N status of these organisms are being sought so as to simultaneously interrogate natural picoplankton populations for their N and P status.

**S54 DETECTION OF METHANE MONOOXYGENASE GENES IN METHANOTROPHS FROM POLLUTED ENVIRONMENTS**

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Sixteen methanotrophic cultures isolated from different environments in Canada and Germany, were monitored for the possession and expression of the sMMO gene. The presence of the sMMO gene was monitored by the amplification of template DNA with sMMO-specific primers. Out of the 16 isolates, 10 expressed sMMO activity. Primers mmoX1-mmoX2 amplified the DNA extracted from these 10 cultures. In addition, the pmoA, particulate MMO, gene was amplified by PCR, cloned and sequenced, and phylogenetic analysis identified the isolates as being closely related to Methylosinus/Methylocystis type II methanotrophs, with the exception of FI-Diko which grouped closely with the type I methanotroph, Methylococcus capsulatus. Analysis of partial 16S rDNA genes revealed a similar phylogeny to that produced by analysis of pmoAgenes.

**S55 MOLECULAR CHARACTERIZATION OF A METHYL TERT-BUTYL ETHER (MTBE) DEGRADING CONSORTIUM.**

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Methyl *tert*-butyl ether (MTBE) is a widely used additive, it is blended with gasoline to improve combustion efficiencies and reduce carbon monoxide emissions.The use of this compound has associated human health risks due to its toxicity and potential carcinogenesis. Groundwater sources have been contaminated with MTBE mainly as a result of spills from underground storage tanks (UST's).

Transformation of MTBE and other ether oxygenates including ethyl *tert*-butyl ether (ETBE) and *tert*-amyl methyl ether (TAME) have been achieved by a mixed culture (CH1) utilizing cyclohexane as a sole source of carbon and energy. Seven members of the CH1 consortium were characterized by molecular techniques including Amplified Ribosomal DNA Restriction Analysis (ARDRA), Arbitrary Primer Polymerase Chain Reaction (AP-PCR) and partial 16s rDNA sequencing. Sequencing and phylogenetic analysis of the 16s rDNA gene identified isolates PC1, NA1 and NA6 as *Aureobacterium sp*., isolates PC2, NA2 and NA5 as *Alcaligenes sp*. and isolate NA4 as a *Micrococcus luteus*. Although isolates NA2, PC2 and NA5 appears to be *Alcaligenes sp*. by 16s rDNA sequencing, AP-PCR analysis reveals slightly differences in the banding profiles suggesting minor genetic differences. In addition, genes involved in petroleum hydrocarbon degradation pathways including *alkB*, *xylE* and *TodC1* were amplified by PCR suggesting the presence of degradation capabilities in some of these isolates.
The consortium is being developed as a biofilm as a potential *in-situ* treatment system. Electron micrographs of the biofilm and individual isolates of the CH1 consortium show morphological characteristics that support their characterization by the molecular techniques described.

**S56 MOLECULAR GENETICS OF IRON UPTAKE IN THE SYMBIOTIC BACTERIUM *RHIZOBIUM LEGUMINOSARUM*.**

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Rhizobia, the microsymbiont in the N2-fixing interaction with legumes have special requirements for Fe. The abundant enzyme nitrogenase contains Fe, as do $e^-$ donors in the bacteroids in root nodules. Further, bacteroids compete with the host for Fe; leghaemoglobin is the most abundant protein in the plant cytoplasm. As with most bacteria, rhizobia make siderophores, excreted molecules that bind $Fe^{3+}$ and are internalised by dedicated transporters. *R. leguminosarum* bv. *viciae*, the symbiont of peas, makes a cyclic peptide, trihydroxamate siderophore called vicibactin. We identified the *fhu* genes that are involved in vicibactin uptake and showed that their transcription is regulated by Fe availability. There are two copies of *fhuA*, the determinant of the vicibactin receptor. Unusually for bacteria, one of these has the hallmarks of a pseudogene. Adjacent to the functional *fhuA* is a gene, *rpoI*, which, when cloned causes overproduction of vicibactin. RpoI is similar to PvdS, a Pseudomonas protein which also regulates Fe uptake and which is a member of the ECF family of s factors of RNA polymerase. The defect in siderophore production in *fhu* mutants was corrected by cloning the *dpp* genes which, in other bacteria, are involved in dipeptide uptake. A model to explain this is presented along with a description of symbiotic phenotypes of *R. leguminosarum* mutants that are defective in Fe uptake.

## S57 AMMONIA OXIDATION IN *PARACOCCUS DENITRIFICANS*

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Chemolithoautotrophic nitrifying bacteria such as Nitrosomonas europaea derive energy by oxidising ammonia to nitrite via hydroxylamine. The initial reaction is catalysed by ammonia monooxygenase (Amo), which is homologous to the particulate methane monooxygenase (pMmo) of methanotrophs. Chemoheterotrophs such as Paracoccus denitrificans are also capable of nitrification although this phenomenon has been largely ignored. Heterotrophic nitrification does not conserve energy and may be involved in dissipating excess reductant.

The genes required for heterotrophic nitrification were cloned from Pa. denitrificans on a cosmid (pLCC5) which conferred on Pseudomonas putida the ability to accumulate nitrite from ammonia. In addition, Ps. putida (pLCC5) oxidises ethene to epoxyethane, reflecting a relaxed substrate specificity of Amo. pLCC5 was introduced into Methylobacterium extorquens which grows on methanol but not methane. M. extorquens (pLCC5) accumulated nitrite, oxidises ethene and grows methanotrophically. This indicates that the Pa. denitrificans Amo oxidises methane to methanol. An internal membrane system was expressed, reminiscent of that seen in lithotrophic nitrifiers and methanotrophs. M. extorquens (pLCC5) accumulates copper and excretes a copper binding peptide similar to those produced in association with the pMmo. Therefore, Pa. denitrificans Amo shares similarities with the pMmo, and Amo from lithotrophs. Sequencing of the nitrification genes is underway which will assist studies to ascertain the impact of heterotrophic nitrification in the environment.

## S58 BIOFILM FORMATION ASSOCIATED WITH ORAL ACRYLIC PROSTHETIC MATERIALS

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Biofilm accumulation on acrylic dentures is responsible for denture-associated stomatitis. However, the formation and composition of denture biofilms has been poorly studied and, because approximately half of the oral microflora is as yet unculturable, currently unknown bacteria may play a major role in the condition. The aim of this study was to perform cultural and molecular analysis on the developing biofilms in an in-vivo denture model. A mucosally-supported, heat-cured, upper prosthesis was constructed with six 5-mm diameter, removable acrylic discs on the fitting surface. The prosthesis was worn by a healthy dentate volunteer for 1h. The effect of different sonication times for the removal of bacteria from the discs was determined. Following sonication, the samples were divided, and one portion was cultured on non-selective media under aerobic and anaerobic conditions for 7 days. DNA was extracted from the remaining portion and the 16S rRNA genes amplified by PCR, with universal primers 27F and 1525R. Amplified genes were cloned and 60 randomly-selected clones were partially sequenced, using the 519R primer. The optimum sonication time for the removal of organisms from the discs, without killing them, was found to be 25s. The cultural analysis revealed total counts in the range $10^4$-$10^6$ cfu/disc. Predominant cultivable organisms were oralis-group streptococci (35%), other streptococcal species (10%) and neisseria (15%). Anterior sites on the prosthesis were more heavily colonised than posterior sites. A comparison of the partial sequences with those held on the 16S rRNA databases identified the predominant bacteria in the sample to be members of the *Streptococcus oralis* group (33%), *Peptostreptococcus prevotii* (17%), *Veillonella* spp (13%), *Neisseria flavescens* (7%), *Abiotrophia adjacens* (4%) and *Lactobacillus catenaformis* (4%). In conclusion, a combined molecular and cultural analysis allows a more comprehensive characterisation of the microflora of developing denture biofilms than culture alone.

## S59 MOLECULAR ANALYSIS OF MICROFLORA ASSOCIATED WITH ENDODONTIC INFECTIONS

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Endodontic infections arise primarily as a result of dental caries and may lead to the formation of dentoalveolar abscesses and / or tooth loss. Comprehensive cultural studies of endodontic infections have been performed and revealed a diverse microflora with the genera Streptococcus, Eubacterium, Prevotella, Porphyromonas, and Peptostreptococcus predominant. However, because 50% of the oral microflora is unculturable it is likely that as yet uncultured and therefore uncharacterised organisms play a role in the infection. The aim of this study was to use culture-independent molecular methods to characterise the microflora associated with endodontic infection. Three patients with infected root canals were included in the study. Access cavities were made into the pulp chamber of the affected teeth using sterile burs. Samples were collected by introducing 100ul of sterile saline into the root canal and gently aspirating the infected material. Samples were split and one portion cultured on blood agar incubated anaerobically and aerobically for 7 days. DNA was extracted from the second portion of the sample as well as from the cultured isolates and 16S rRNA genes amplified by PCR using the universal primers 27F and 1525R. Genes amplified directly from the samples were singularised by cloning. Amplified genes from 130 isolates and 310 clones were partially sequenced using primer 519R. Sequences were submitted to the Ribosomal Database Project and the Similarity_Rank program used to determine the most closely related sequences in the database. Phylogenetic trees were also constructed from the sequence data. A diverse microflora was found to be associated with the lesions with 46 different taxa detectable. The molecular analysis revealed a more diverse microflora than culture although some species were detected by culture alone. Three frequently detected groups of molecular sequences did not match with any known culturable organisms. These were related to the genera Dialister, Eubacterium and Prevotella. There was marked variation in the distribution of species in the 3 samples; no species was found in all of the samples. In conclusion, molecular analysis used in addition to culture provides a more complete description of the microflora associated with endodontic infections. 3 unculturable phylotypes were detected that were related to but distinct from known oral pathogens.

**S60 MOLECULAR ECOLOGY ANALYSIS OF METHANOTROPHIC POPULATIONS IN NEWPORT BAY ESTUARY, CALIFORNIA**

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An estuarine sediment from the Newport Bay estuary, California, was sampled for analysis of methane oxidising bacteria. Previously, a methanotrophic bacterium, Methylobacter sp. strain BB5.1, was isolated from this environment (Smith et al. 1997. Appl. Environ. Microbiol. 63,4617-4620). This study was carried out to determine the overall diversity of methane oxidising bacteria in the estuarine environment by measuring methane oxidation activity and analysing total sediment DNA and DNA extracted from methane enrichments using molecular ecological techniques. DNA samples were used to prepare methanotroph-specific 16S rRNA and particulate methane monooxygenase (pmoA) gene libraries. The libraries were initially screened by RFLP analysis and clones representative of each of the OTUs identified were sequenced. Phylogenetic analysis of these sequences revealed the diversity and relative abundance of methanotrophs using both functional and phylogenetic genes. The majority of cloned sequences detected were from Type I methanotrophs similar to Methylobacter sp. strain BB5.1. However other Type I methanotroph sequences from the genera Methylomicrobium and Methylomonas, and Type II methanotroph sequences from the genera Methylosinus, were also detected.

S61 FUNGAL COMMUNITY ANALYSIS: MOLECULAR CHARACTERISATION OF FUNGI INFECTING AMMOPHILA ARENARIA ROOTS IN NATURAL SAND DUNE ECOSYSTEMS

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A molecular strategy based upon specific PCR of 18S rRNA genes, denaturing gradient gel electrophoresis (DGGE), and sequence analysis was developed for the characterisation of fungi infecting plant roots. This strategy was applied in the analysis of the fungal community associated with the dune-stabilising plant, *Ammophila arenaria*, in several natural coastal sand dune systems in the Netherlands. It was demonstrated that these techniques could be applied to the detailed detection of fungi within individual roots. These methods were subsequently scaled up to allow for the concurrent analysis of multiple roots, thus providing a more rapid view of fungal community structure. Although several recovered sequences matched those of fungal strains isolated fromthe same sand dune systems, many of the recovered fungal sequences were not closely related to sequences from previously analysed culture strains. Sequence analysis of DNA fragments recovered after PCR and DGGE separation revealed an increased presence of the potentially pathogenic genera *Leptosphaeria, Phoma*, and *Fusarium* in the roots of A. arenaria at stands in a state of degeneration. Furthermore, the incidence of *arbuscular mycorrhizal* (AMF) infection decreased in degenerating stands with respect to healthy ones, suggesting that these ancient fungal symbionts may also play an important role in this nutrient-poor environment. The development of competitive PCR strategies for the quantification of fungal target DNAs and the use of improved PCR primers show promise for future progress.

**S62 MYCOPARASITIC GROWTH BY NON-FILAMENTOUS CHITINOLYTIC SOIL BACTERIA**

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The possession of chitinase genes is taxonomically widespread among non-filamentous soil bacteria. Yet, the ability of these bacteria to degrade chitin particles is limited in comparison to filamentous soil microorganisms such as fungi and actinomycetes. Indeed, several studies have indicated that the decomposition of chitin in terrestrial soils can mainly be attributed to fungi and actinomycetes. Non-filamentous soil bacterial must, however, gain some selective advantage through their possession of chitinase genes, or else these genes would have been lost. Several chitinolytic bacterial strains have been used as biocontrol agents due to their ability to lyse hyphae of fungal crop pathogens. As most fungal cell walls contain chitin, it is assumed that chinases play an important role in the lysis of fungal hyphae. The ability to lyse fungal hyphae presents the possibility that living fungal hyphae may form an additional growth substrate for chitinolytic soil bacteria. This could be an important selection pressure for the maintenance of chitinase genes, since the growth of soil microorganisms is often limited by the availability of energy substrates. We tested the mycoparasitism hypothesis by quantifying the population dynamics of chitinolytic and non-chitinolytic pseudomonads in sand microcosms in zones of extending fungal mycelium. Chitinolytic pseudomonads increased strongly in these zones whereas non-chitinolytic pseudomonads did not respond.

**S63 INITIAL CHARACTERISATION OF CAMPYLOBACTER SPECIES FROM BROILER CHICKENS IN THE WEST OF IRELAND**

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Campylobacter enteritis is the most common cause of acute bacterial diarrhea worldwide. It is essentially a food borne disease and the principle vehicle of infection is raw or undercooked meat. Poultry are by far the most important source, especially broiler chickens as they are consumed in such prodigious quantities.

Initial characterisation of Campylobacter species from 50 retail broiler chickens from various sources was carried out in our laboratory. Campylobacters were isolated using the conventional swabbing method and by the Malthus automated analyser. The Malthus analyser has been found to be more sensitive than that of conventional swabbing. PCR-DNA probe membrane based colormetric detection assays were designed and applied to confirm the identity of isolates and to discriminate Campylobacter species. Isolates were then further characterised by their antibiotic profiles, biotyping and by SDS-PAGE analysis.

To date only *Campylobacter jejuni* species have been isolated from chicken carcasses, the predominant strain being *Campylobacter jejuni* type II, *Campylobacter jejuni* type I and *Campylobacter jejuni* subspp. *doyleii* have also been isolated to a lesser extent. Antibiotic patterns varied between isolates and the majority of strains showed resistance to ampicillin. Total protein profiles were examined for correlation with biotype results.

**09.00 STRUCTURAL AND FUNCTIONAL ANALYSIS OF VIRAL RIBOSOMAL FRAMESHIFTING SIGNALS**

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Division of Virology, Department of Pathology, University of Cambridge,Tennis Court Road, Cambridge CB2 1QP UK

Several RNA viruses use programmed -1 ribosomal frameshifting to express their replicases. Most retroviruses, for example, express pol as a fusion with the upstream gag gene following a frameshift. The mRNA signals for frameshifting are composed of two elements, a slippery sequence, where the ribosome changes frame, and a stimulatory RNA structure downstream. We are studying these stimulatory RNAs by a combination of site-directed mutagenesis, phylogenetic sequence comparisons, secondary structure probing and RNA modelling, using the frameshift sites of astroviruses, coronaviruses and retroviruses as model systems. A wide-variety of structures are present, including simple and complex hairpin-loops and pseudoknots. Recently, we have obtained evidence that a triple-helical interaction occurs in some pseudoknots and is essential for frameshifting. This structural information should prove to be invaluable in terms of models of the frameshift process, the design of antiviral drugs and the creation of programs to search databases for novel frameshift sites. Such searches have revealed novel candidate frameshift sites from a variety of virus systems.

**09.15 THE NATURE OF THE INTERACTIONS BETWEEN THE HCV E1 AND E2 GLYCOPROTEINS**

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Hepatitis C virus (HCV) glycoproteins E1 and E2 are thought to constitute the envelope of the virus and they interact to form complexes. Two types of complex have been identified, a native form in which the proteins associate by non-covalent interactions and an aggregate that contains intermolecular covalent bonds. By treating cells in situ with DTT, we show that the disulphide linkages can be reduced, leading to disruption of the aggregate. However, E1 and E2 remain attached to each other. Thus, the aggregate also contains intermolecular non-covalent interactions. From analysis of proteins synthesised in the presence of DTT, this non-covalent association of E1 and E2 can occur also in the absence of disulphide bond formation. Results on those regions in the proteins which permit native complex formation and the role played by post-translational processing in E1-E2 association will also be presented.

**09.30 INHIBITION OF ECHOVIRUS ENTRY INTO RHADBOMYOSARCOMA 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 Coxsackie 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-microglubulin (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 Coxsackie B2/B3 viruses.

**09.45 RESPIRATORY SYNCYTIAL VIRUS-HOST CELL RECEPTOR INTERACTIONS**

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The mechanism of attachment of RSV to host cells is unknown and the receptor remains unidentified. Literature suggests a role for cell surface glycosaminoglycans (GAG's) in the attachment of human RSV (HRSV). We have used FACS to study the interaction of both bovine RSV (BRSV) and human RSV (HRSV) with GAG deficient cell lines. Results indicate GAG's to be involved in the adsorption of both viruses and this effect was found to be specific for heparan sulphate (HS). Several viruses are known to use HS as a primary attachment molecule but require a secondary high affinity receptor interaction for infection. In an attempt to identify a secondary attachment molecule we have modified a novel receptor cloning technique (CELICS, cloning by enzyme-linked immuno-colour screening) to allow the use of a viral attachment protein as a tool to isolate cDNA clones encoding receptor genes. CELICS is a rapid method for directly cloning genes, which eliminates multiple rounds of selective enrichment demanded by other cloning procedures. Studies using the CD4-gp120 interaction have demonstrated this modified CELICS technique is suitable for the cloning of viral receptors and our aim is to apply this to the identification of proteins involved in the secondary interaction between RSV and cells.

**10.00 GENERATION OF A BUNYAMWERA VIRUS NSS MINUS MUTANT BY REVERSE GENETICS.**

Anne Bridgen and Richard M. Elliott
Division of Virology, Institute of Biological and Life Sciences, University of Glasgow.

The smallest (S) of the three RNA segments of Bunyamwera virus encodes two proteins in overlapping reading frames: N, the nucleocapsid protein and NSs, a non-structural protein of unknown function. Using the reverse genetics technique previously developed in our laboratory we have produced a Bunyamwera virus lacking NSs. A cDNA copy of the S genome segment with the NSs gene ablated was made by overlapping polymerase chain reaction, and this construct, together with cDNAs corresponding to wild-type medium (M) and large (L) segments, were used in the rescue experiment. Virus recovered had a small plaque phenotype and was shown not to express the NSs protein. Thus the NSs gene is not essential for viral growth in tissue culture. Further biological properties of the mutant virus and possible functions of the NSs protein will be discussed.

**10.15 DEVELOPMENT OF A CHIMERIC VACCINE AGAINST PESTE DES PETITS RUMINANTS VIRUS USING REVERSE GENETICS TECHNOLOGY**

Subash Das, Michael D. Baron and Tom Barrett.
Institute for Animal Health, Pirbright Laboratory, Ash Road, Woking, Surrey GU24 ONF.

Peste des petits ruminants virus (PPRV) is a highly contagious virus infecting sheep, goats and other small ruminants. A vaccine strain of a related morbillivirus of large ruminants (rinderpest virus (RPV)) is used to control PPRV. However, since it is hoped that RPV will soon be eradicated globally, it will be important to have all animals free of RPV-specific antibodies, hence use of the RPV vaccine should cease. In order to overcome this problem, and also to make the RPV more specific to PPRV, we have used reverse genetics techniques to produce a chimeric RPV/PPRV virus in which the surface glycoprotein (haemagglutinin and fusion protein) genes of the RPV vaccine have been replaced by the corresponding genes of PPRV.

**11.00 A PRE S1/PRE S2 HBV VACCINE AS IMMUNOTHERAPY FOR HBEAG POSITIVE HEPATITIS.**

W Carman, T Tucker, E Song, D Hawarden, R Kirsch, D Lobidel, F Van Deursen, B.O'Donnel, E.Maloney, A Williams.
Institute of Virology, Glasgow, MRC Liver Research Unit, Cape Town; Dept Medicine Johannesburg and Medeva plc.

Chronic HBV is due to insufficient specific immunity. Hepagene, whichcontains novel HBsAg, pre-S1 and pre-S2 polypeptides, was employed to rectify this deficit. 22 patients (HBeAg + / HBV DNA +) were vaccinated with 8 doses in 2 courses with a 5 month interval. At 12 months follow up: of 12 patients with raised aminotransferases (ALT) pre-therapy, 6 cleared HBV DNA, 3 lost 70% -99% DNA, 8 attained normal ALT. Of 10 with normal ALT pre therapy, 2 lost at least 85% of HBV DNA. Clearance was accompanied by flares in ALT and responses were temporally associated with vaccination.

**11.15 TRACKING HERPES SIMPLEX VIRUS IN LIVE CELLS**

Gillian Elliott and Peter O'Hare,
Marie Curie Research Institute, The Chart, Oxted, Surrey RH8 0TL.

The HSV-1 tegument, located between the capsid and the envelope, is a poorly understood viral compartment. Little is known of the fate of the tegument or its individual components upon virus entry into the cell. Likewise, the location of tegument assembly at later stages of infection remains unclear. To address these issues we have now constructed an HSV recombinant in which we have replaced the gene encoding the major tegument protein VP22, with a gene expressing a GFP-VP22 fusion protein. Surprisingly, this virus has identical growth properties to parental virus, and newly synthesized GFP-22 is detectable in live cells as early as three hours post infection. Moreover, GFP-22 is incorporated into the HSV-1 virion as efficiently as VP22, resulting in particles which are visible by fluorescence microscopy. Thus, we have utilised time-lapse microscopy to follow GFP-22 in live infected cells, and will present animations of GFP-22 trafficking and assembly into virions within the infected cell.

**11.30 SPECIFIC DESTRUCTION OF KINETOCHORE PROTEIN CENP-C AND DISRUPTION OF CELL DIVISION BY HERPES SIMPLEX VIRUS IMMEDIATE-EARLY PROTEIN VMW110**

Roger D. Everett 1, William C. Earnshaw 2, John Findlay 3 and Patrick Lomonte 1
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2.Institute of Cell and Molecular Biology, University of Edinburgh, Kingís Buildings, Mayfield Road, Edinburgh EH9 3JR, Scotland, U.K.
3.Science Faculty Electron Microscope Facility, University of Edinburgh, Daniel Rutherford Building, King's Buildings, Mayfield Road, Edinburgh EH9 3JH, Scotland, UK

Examination of cells at the early stages of infection by herpes simplex virus type 1 revealed that the viral immediate-early protein Vmw110 (also known as ICP0) formed discrete punctate accumulations associated with centromeres in both mitotic and interphase cells. The RING finger domain of Vmw110 (but not the C-terminal region) was essential for its localisation at centromeres, thus distinguishing the Vmw110 sequences required for centromere association from those required for its localisation at other discrete nuclear structures known as ND10, PML nuclear bodies or PODs. We have recently shown that Vmw110 can induce the proteasome-dependent loss of several cellular proteins, including a number of probable SUMO-1 conjugated isoforms of PML, and this results in the disruption of ND10. In this study we found some striking similarities between the interactions of Vmw110 with ND10 and centromeres. Specifically, centromeric protein CENP-C was lost from centromeres during virus infection in a Vmw110- and proteasome-dependent manner, an event which caused substantial ultrastructural changes in the kinetochore. In consequence, dividing cells either became stalled in mitosis or underwent an unusual cytokinesis resulting in daughter cells with many micronuclei. These results emphasise the importance of CENP-C for mitotic progression and suggest that Vmw110 may be interfering with biochemical mechanisms which are relevant to both centromeres and ND10.

**11.45 THE UPTAKE AND INTRACELLULAR FATE OF ADENOVIRUS 5 PENTON COMPLEXES.**

Torkjel Matzow and G. Eric Blair
School of Biochemistry and Molecular Biology, University of Leeds, Leeds LS2 9JT, UK.

Replication deficient adenoviruses are in focus as possible gene therapy vectors, due to their high infection rate and wide host range. Their main problem, limiting the possibility for repeated treatment, is a strong immune response, triggered partly by capsid proteins in the infecting particles, and partly by leaky viral gene expression.

Virus-cell interaction, onset of endocytosis and escape of particles from early endosomes are all caused by the penton capsomer complex, consisting of fibre and penton base proteins. By basing a gene therapy vector only on the adenovirus 5 (Ad5) penton complex, it is hoped that the immune response can be minimised, while maintaining the high DNA uptake efficiency. This depends on purified penton complex being able to trigger endocytosis and penetrate endosomes on its own. Immunofluorescence microscopy shows that penton complex is endocytosed, and suggests at least some degree of endosome penetration and relocalisation of the pentons to the nuclear membrane.

**12.00 FOLLICULAR DENDRITIC CELLS SUPPORT REPLICATION OF SCRAPIE IN SPLEEN.**

Karen L. Brown*, Karen Stewart, Diane Ritchie, W Ivan Morrison†, Hugh Fraser and Moira E. Bruce
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The immune system plays a key role in the pathogenesis of scrapie and other transmissible spongiform encephalopathies (TSEs) or prion diseases". To investigate which cells in the spleen support replication we produced chimaeric mice that carry the gene for the "prion protein" (PrP) only in specific cells of the immune system. This was achieved by grafting PrP expressing ($PrP^{+/+}$) or PrP deficient ($PrP^{-/-}$) bone marrow (bm) into $PrP^{+/+}$ or $PrP^{-/-}$ immunodeficent (SCID) mice. In these mice there is a mismatch in PrP status between follicular dendritic cells (FDCs) and cells of bm origin such as lymphocytes. Using these models we obtained strong evidence that mature FDCs produce PrP in both uninfected and scrapie-infected mice. Furthermore, we have shown that replication of the ME7 scrapie strain in spleen occurs only in the presence of PrP-expressing FDCs and does not depend directly on lymphocytes.

**12.15 THE NUCLEOTIDE SEQUENCE OF YABA-LIKE DISEASE VIRUS, A YATAPOXVIRUS**

Han-Joo Lee and Geoffrey L. Smith
Sir William Dunn School of Pathology, University of Oxford, South Parks Road, Oxford, OX1 3RE

The sequence of yaba-like disease virus (YLDV) has been determined by automated DNA sequencing and assembly of random sequences by computer. The genome is 145 kb and contains a 1.9 kb inverted terminal repeat (ITR). There are 150 open reading frames(ORFs) = 65 amino acids. Within the central region of the virus genome, genes are transcribed in each direction and encode many essential enzymes or structural components that show amino acid similarity with proteins from other poxviruses. In contrast, all genes within 30 kb of each genomic terminus are transcribed towards the ends of the genome. These ORFs are more divergent in comparison with other poxviruses and some do not have counterparts in other poxviruses. Several proteins that in vaccinia virus form part of the extracellular enveloped virus (EEV) particle are absent in YLDV. Lastly, YLDV encodes many proteins likely to be immunomodulatory factors.

**P1 DEGRADATION OF CHLORINATED PHENOLIC COMPOUNDS BY A *SPHINGOMONAS* SP.**

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Environmental contamination by halogenated phenols is a serious world-wide problem. These highly toxic compounds have entered the biosphere through accidental and deliberate release as a result of their use as solvents, lubricants, insulators, pesticides and plasticisers. Chlorophenols are highly resistant to degradation due to their level of chlorination and their aromatic structure. The chlorines on the aromatic ring hinder ring fission reactions catalysed by microbial oxygenases, which are involved in the mineralisation of aromatic compounds. Microorganisms have been isolated which are capable of degrading these chemicals and amendment of contaminated soil with these organisms (bioremediation) has been shown to be a feasible method for their removal.

Following a preliminary screening programme a *Sphingomonas* sp. was selected for further study based on it's capacity to grow in the presence of high concentrations of one of the most toxic chlorophenols, Pentachlorophenol (PCP), and to degrade over 40% PCP (50mg/l) in the presence of an additional carbon source. This organism was capable of utilising other chlorophenols as a sole source of carbon and energy, with maximum growth observed with 23-dichlorophenol. This presentation discusses the factors affecting chlorophenol degradation by this bacterium.

**P2 THE HORIZONTAL GENE TRANSFER AND EXPRESSION OF STRR GENES IN A SET OF DIVERSE STREPTOMYCETE**

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All biosynthetic genes for secondary metabolites investigated so far have been found located in large gene clusters, including the genes for resistance mechanisms, regulators, transport and extracellular processing functions. The genetic material employed in the design of pathways seems to be used in a modular fashion in order to acquire a maximum variation with a minimum of genetic and enzymatic reserves.

This is illustrated by certain experimental observations. For example, the biosynthesis of the 6-deoxyhexose (6DOH) constituents is widely used in natural products making relevant genes of str cluster (strDELM) to be useful probes for the detection of a further biosynthetic gene clusters involved in the production of 6DOH constituents. This implies that antibiotic gene products with conserved enzymatic functions seem to be used at least in part in various combinations to form different pathways.

Previous studies showed a possible transfer of strA, a streptomycin resistant gene, between isolates which were diverse both
phenotypically and genetically. We investigated whether antibiotic genes move as a whole cluster or as isolated genes that confer the organism the necessary function for survival in a particular habitat (eg. resistance). This could indicate the horizontal gene transfer has a central role in the distribution and evolution of biosynthetic clusters. Our aim is to apply this hypothesis for other genes in the streptomycin cluster (eg. strR, strS, stcC). Furthermore, strR genes were amplified and sequenced from 6 soil isolates. Three of the strains were genetically different as shown by comparison of the trp B/A housekeeping gene sequence. However they shared identical strR sequences indicating horizontal gene transfer of this gene in concert with strA. RT-PCR and Western analysis showed also a differential strR expression pattern in these isolates.

**P3 EXPRESSION AND MUTAGENESIS OF DINUCLEAR IRON ACTIVE-SITE MONOOXYGENASES.**

Thomas J. Smith, John S. Lloyd, J. Colin Murrell and Howard Dalton.
Department of Biological Sciences, University of Warwick, Coventry. CV4 7AL

Soluble methane monooxygenase (sMMO) from *Methylosinus trichosporium* OB3b and alkene monooxygenase (AMO) from *Rhodococcus rhodochrous* (formerly *Nocardia corallina*) B-276 are homologous multicomponent enzymes that catalyse NAD(P)H-dependent oxygenation reactions. AMO and sMMO each have three components: (1) a dinuclear iron active-site oxygenase that is the site of substrate oxygenation; (2) a NAD(P)H-dependent reductase with a flavin nucleotide prosthetic group and an $Fe_2S_2$ cluster; (3) a small polypeptide without prosthetic groups that is also required for full activity. sMMO naturally oxidises methane to methanol. It also has a wider biotechnological potential, owing to its ability to cooxidise many other substrates, including alkanes, alkenes and aromatics. AMO catalyses stereoselective oxygenation of terminal and subterminal alkenes, yielding commercially valuable chiral epoxides, usually as the R-enantiomer with high enantiomeric excess. sMMO also oxidises alkenes to epoxides but gives racemic products. Despite considerable interest, protein engineering of sMMO and AMO was previously impossible because neither could be expressed in an active form in *Escherichia coli*. We have now obtained high-level functional expression of both enzymes by using alternative bacterial hosts. AMO was expressed by using a plasmid clone in *Streptomyces lividans*. Expression of recombinant sMMO was obtained by transconjugation of plasmid-borne sMMO genes into a strain of *M. trichosporium* in which the chromosomal copy of these genes had been inactivated by marker-exchange mutagenesis. We are now using site-directed mutagenesis to investigate the mechanisms of oxygen activation in sMMO and AMO, and the difference in stereoselectivity between them.

**P4 THE EFFECT OF HEAT TREATMENT ON THE SPORES OF *MICROMONOSPORA ECHINOSPORA.***

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Actinomycetes are commonly found in soils and are important industrial microorganisms. The genus *Micromonospora* is reported to produce representatives of practically every chemical family of antibiotics, and is commercially exploited for the production of gentamicin. Despite the industrial potential, the genus *Micromonospora* is poorly understood when compared with other actinomycete genera such as *Streptomyces*. Our work is based on gaining a greater understanding of the growth and physiology of *Micromonospora*. This particular study focuses on the effects of heat treatment on the spores of *Micromonospora echinospora*. Spore suspensions of *M. echinospora* were treated with temperatures from 50-90$^{o}$C over a range of times up to 30 minutes and the number of culturable spores was determined from viable counts on solid media. The number of culturable spores was not found to change following treatment of suspensions at 50 $^{o}$C. However, at 60 and 70 $^{o}$C a heat activation phenomenon was apparent. The activation of spores was verified by the measurement of increased oxygen uptake in heat-treated spores. This response correlates with a previous report of increased germination rates after heat treatment in *M. echinospora*. The decimal reduction times were calculated at 80 $^{o}$C ($D_{80\ oC}$ = 2.67 minutes) and at 90 $^{o}$C ($D_{90\ oC}$ = 0.45 minutes), which showed classic thermal death profiles, however some tailing of the curves was observed indicating the heterogeneity of the spore population. A possible link between heat activation and the selection of rapidly-growing members of the spore population is discussed.

**P5 METAL AND METALLOID RESISTANCE OF SOIL ISOLATES FROM AN ANTIMONY MINE SITE**

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Biomethylation of inorganic antimony by undefined mixed cultures of anaerobic bacteria and by an aerobic fungus has recently been established. In the case of the closely related element arsenic, for which biomethylation is well established, the process is thought to be a detoxification mechanism. We have investigated the possibility that aerobic bacteria resistant to antimony may also be capable of its biomethylation.

Four antimony resistant soil isolates from an antimony mine site (up to 219 µg Sb/g) were compared with regards to antimony and arsenic resistance, and capability for biomethylation. The isolates, identified as *Pseudomonas* sp., *Acinetobacter* sp., *Aeromonas* sp. and *Acinetobacter lwoffii*, were all plasmid harbouring strains. The *Acinetobacter* sp. and *Acinetobacter lwoffii* showed equivalent or greater resistance to As(III) than to Sb(III), whereas the other two isolates were more resistance to Sb(III). These data suggest differences in resistance mechanisms between strains. The capability of these soil isolates to biomethylate antimony was tested alongside species previously reported as biomethylators of arsenic, i.e. *Pseudomonas fluorescens*, *Escherichia coli*, *Corynebacterium* sp., *Flavobacterium* sp. and *Proteus vulgaris*. The approach involved hydride generation of culture supernatants and cell pellets to form volatile hydrides with subsequent analysis by gas chromatography-atomic absorption spectrometry. Methylantimony species were detected in the culture supernatant of the *Flavobacterium* sp. (known arsenic methylator) and the cell pellet of the *Aeromonas* sp. (antimony resistance isolate). This is the first report of antimony biomethylation by monoseptic cultures of aerobic bacteria. The significance of antimony biomethylation in the context of antimony and arsenic resistance is discussed.

**P6 THE REGULATION OF PRODUCTION OF POLYSACCHARIDES AND CYCLIC GLUCAN IN *XANTHOMONAS CAMPESTRIS* BY DIFFUSIBLE SIGNAL MOLECULES**

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Synthesis of extracellular enzymes and polysaccharide (xanthan, EPS) in *Xanthomonas campestris* pathovar *campestris* (*Xcc*) is co-ordinately regulated by the *rpf* cluster of genes. Two members of this gene cluster (*rpfF* and *rpfB*) are involved in a novel type of regulation mediated by a small diffusible molecule. This factor (DSF ) can restore production of extracellular enzymes and EPS to *rpfF* (but not to *rpfB*) mutants. Although DSF is probably a fatty acid derivative, it is not an *N*-acyl homoserine lactone. A transcriptional fusion was created between the promoter region of the *gum* gene cluster (which encodes the enzymes for xanthan biosynthesis in *Xcc*) and the coding region for ß-glucuronidase (GUS). The *rpfF* mutant harbouring the *gum*-gus reporter plasmid showed decreased GUS activity compared to the wild type carrying the reporter. Addition of DSF restored GUS activity to wild type levels, suggesting that regulation of xanthan biosynthesis mediated by DSF was (at least in part) at the level of transcription of the biosynthetic genes. As well as effects on EPS, mutation of *rpf* genes also leads to alterations in the patterns of periplasmic glucans; *rpfC* and *rpfF* mutants had reduced levels of two (probably cyclic) glucans with different dp. DSF could restore the wild type pattern of cyclic glucan biosynthesis to the *rpfF* strain. Membrane preparations from *Xcc* were able to incorporate radiolabelled glucose from UDP-glucose into cyclic glucan. Preparations from wild type and *rpfF* mutants had very similar specific activities. This suggests that DSF does not regulate the synthesis of cyclic glucan by regulating the level of the synthetic enzyme but rather through modulation of the existing activity and/or supply of the substrate. This may be a similar mechanism to the regulation of synthesis of cyclic glucans by osmotic stress which has been described in other bacteria.

**P7 A REGULATORY MECHANISM IS IMPLIED BY LINKAGE OF MOSAIC VARIATION AND VACA PRODUCTION IN *HELICOBACTER PYLORI***

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*Helicobacter pylori* is the major cause of gastric cancer and ulceration, and during the course of infection secretes a cytotoxin which causes vacuolation in eukaryotic cells (VacA). VacA is made as a ~140kD precursor and has 2 conserved regions, the signal (s) region and the mature (m) region. The signal region is cleaved during secretion to liberate the mature ~90kD form. The toxin is mosaic in nature, where the signal region may be 1 of 3 variants, s1a, s1B, and s2, and the mature region may be of the type m1 or m2. The most virulent strains of *Heliobacter pylori* produce s1 m1 variants of VacA. Forsyth *et al.* reported that these strains show increased transcription of *vacA*, and we have confirmed that VacA levels are similarly elevated. The secretion step is not well characterised, and whilst the toxin has homology to the IgA protease of *Neisseria gonorrhoerae* (an autotransporter), there is no evidence to suggest VacA is secreted by a similar mechanism.

A comparison of strains accounting for all the possible combinations of s and m regions was carried out using SDS-PAGE and Western blotting to access efficiency of VacA secretion. Our results are presented here with a discussion of the relationship between VacA sequence and secretion efficiency.

**P8 IDENTIFICATION OF THE AGENT OF TOMATO PITH NECROSIS, *PSEUDOMONAS CORRUGATA*, BY PCR AMPLIFICATION WITH SPECIFIC OLIGONUCLEOTIDE PRIMERS**

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*Pseudomonas corrugata* is the causal agent of tomato pith necrosis, which occurs world-wide in all tomato growing areas, causing severe crop losses. Characteristic symptoms are early chlorosis of young leaves, associated with pith necrosis and hollowing of the parenchyma tissues in the stem. Little is known about its epidemiology and transmission. The bacteria are traditionally identified by the following characteristics: absence of fluorescence on King's medium B, inability to produce levan from sucrose, oxidase-positive and nonpectolytic, ability to reduce nitrates and accumulate poly-hydroxybutyrate. They are distinguished from a closely related group, called the fluorescent Pseudomonas associated with tomato pith necrosis (FPTPN) strains by their inability to utilize sorbitol. Many workers have reported a high level of heterogeneity among strains comprising *P. corrugata*. We have used random amplified polymorphic DNA polymerase chain reaction (RAPD-PCR) to target sequences in strains of *P. corrugata*, which enabled the design of two pairs of specific PCR primers that produce one of two bands after amplification with *P. corrugata*. The results indicate that this "species" comprises at least two distinct phylogenetic groups. The specific primers enable the identification of *P. corrugata* and could provide a means of detection for this pathogen.

**P9 THE TGN IS LOST FROM CELLS INFECTED WITH AFRICAN SWINE FEVER VIRUS**

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African Swine Fever (ASF) virus causes an incurable and fatal haemorrhagic disease in domestic pigs. There are no vaccines or control measures other than slaughter of infected animals. ASF virus replicates in the cytoplasm of macrophages, and assembly takes place in discrete perinuclear plaques called viral factories. Assembly is a complex process regulated by the endoplasmic reticulum (ER). The major capsid proteins of ASF virus oligomerise on the cytoplasmic face of the ER. This leads to bending of the ER and the eventual wrapping of viral particles by ER cisternae. The functional integrity of the secretory pathway is important for macrophage function and is tightly regulated. In this study we have investigated whether the recruitment of the ER as a source of membrane envelopes for ASF virus, leads to perturbation of downstream compartments of the secretory pathway. Using a panel of organelle-specific antibodies we have shown that infection with ASF virus caused the specific disassembly of the Golgi apparatus and loss of the trans Golgi network (TGN). Consistent with these observations, the transport of cathepsin D to lysosomes, and trafficking of type one membrane proteins to the surface of cells was markedly slowed in cells infected with ASF virus. Unexpectedly, disassembly of the Golgi and loss of the TGN in response to virus infection was not blocked by cytosine arabinoside. The effect was therefore caused by the products of early viral genes, and was not a direct consequence of the recruitment of ER membranes for virus envelopment. The precise reason for disassembly of these compartments immediately after infection remains unclear. A block in the secretory pathway could be useful to ASF virus if it prevented secretion of antiviral proinflammatory cytokines from infected macrophages. Alternatively, host proteins required for maintaining the integrity of the Golgi and TGN may be used early on in infection for the recruitment of membranes into viral factories.

**P10 EFFECTS OF PH ON THE ACTIVITY OF *N*-ACYL-HOMOSERINE LACTONE DETERMINED USING AN INDUCIBLE *ESCHERICHIA COLI LUX*-BASED BIOSENSOR**

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It is fast becoming apparent that quorum-sensing amongst Gram-negative bacteria is an important method of intercellular communication required for conveying information about population density. The accumulation of the signal molecule involved, *N*-acyl-homoserine lactone (*N*-AHL), leading to increases in internal physiological concentration, allows phenotypic switching to occur which is beneficial to many bacteria. In our laboratory, analysis of the effect of *N*-AHL on phenotype currently involves creating null mutants unable to produce *N*-AHL, then reintroducing the signal molecule exogenously. However, interference of cell-cell communication could be interrupted on another level. The research described has evaluated methods of inhibiting *N*-AHL, outwith the cell environment, including competitive inhibition, introduction of the molecule to pH extremes and temperature.

**P11 DETERMINATION OF ANTIBIOTIC RESISTANCE, IN PARTICULAR TETRACYCLINE, IN CLINICAL ISOLATES IN THE WEST OF IRELAND**

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Antibiotic susceptibility patterns of 100 clinical *Campylobacter jejuni* strains isolated in Galway, Ireland for various antibiotics were determined. From these profiles, it was found that 20 isolates were resistant to various antibiotics with some isolates resistant to more than one antibiotic. Overall, antibiotic resistance among Campylobacter isolates is relatively low, with 7, 4, 2, 3 isolates resistant to ampicillin (25 mg $ml^{-1}$), naldixic acid (30 mg $ml^{-1}$), nitrofurantoin (50 mg $ml^{-1}$) and streptomycin (25 mg $ml^{-1}$) respectively. There was no clear discrimination between resistant and intermediate resistant strains for tetracycline. Minimum inhibitory concentrations (MIC) were carried out for erythromycin 0.5 - 32 mg $ml^{-1,}$ and tetracycline, 0.25 - 200 mg $ml^{-1}$. All but 5 strains had a MIC value for erythromycin $\leq$ 4 mg $ml^{-1}$. 80% of strains had a MIC value for tetracycline $\leq$ 5 mg $ml^{-1}$. All remaining strains were classified as resistant to tetracycline.

All strains were examined for the presence of plasmids. It was found that not all strains resistant to tetracycline contained a plasmid and not all strains that contained a plasmid were resistant to tetracycline. It was decided to investigate if a tetracycline gene was present in any of the strains. Results will be presented.

**P12 PROTEIN-DNA INTERACTIONS REGULATING QUORUM SENSING IN *AEROMONAS HYDROPHILA***

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The *ahyR* gene in *Aeromonas hydrophila* codes for a regulatory protein homologous to LuxR from *Vibrio fischeri.* LuxR homologues are thought to activate transcription of their target genes on binding of a ligand (an *N*-acyl-L-homoserine lactone) which accumulates in the culture medium at high cell densities. The ligand is synthesized by a homologue of LuxI from *V. fischeri* (AhyI in *A. hydrophila*). On association with the ligand, LuxR homologues are believed to bind to regions (usually containing dyad symmetry) upstream of the genes, thus stimulating transcriptional activation.

In *A. hydrophila*, *ahyR* and *ahyI* comprise a divergon in which transcription of *ahyI* is positively regulated by AhyR and its cognate ligand. A region intergenic to the genes contains a palindromic sequence which is a candidate binding site for proteins of the LuxR family.

Initial attempts to overexpress AhyR have met with little success as the protein forms inclusion bodies. In this study AhyR has been overexpressed as a maltose binding protein fusion. The fusion is 80% soluble and can be semi-purified by amylose resin chromatography. Electrophoretic mobility shift assays have shown that the fusion protein can bind to the *ahyR/I* intergenic region.

**P13 QUORUM SENSING IN *AEROMONAS HYDROPHILA* BIOFILMS**

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Biofilms are a community of bacteria that form on surfaces and cause problems for industry and medicine. The high cell density nature of biofilms indicates that there may be a role for cell-cell communication in the formation of a structured bacterial film. *Aeromonas hydrophila* has a cell density-dependent signalling system (quorum sensing system) based upon a freely diffusible *N*-acylhomoserine lactone (AHL) signal molecule *N*-butanoyl-$_L$-homoserine lactone (C4-HSL). C4-HSL (synthesised by AhyI) activates gene expression (via AhyR) when it accumulates at a high cell density. A role of quorum sensing in biofilm formation by *A. hydrophila* on stainless steel is apparent. Mutations in the quorum sensing system compromise the formation of a biofilm. Furthermore, the inclusion of long chain AHLs e.g. *N*-(3-oxodecanoyl)-$_L$-homoserine lactone (3-oxo-C10-HSL), which antagonise C4-HSL, causes sloughing off in established biofilms.

**P14 A STUDY OF THE SITE SPECIFIC RECOMBINASE OF THE TETRACYCLINE RESISTANCE ENCODING CONJUGATIVE TRANSPOSON TN*5397***

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Tn*5397* is a 22 kb transposon found in *Clostridium difficile.* The central region of this element is almost identical to the well studied conjugative transposon Tn*916*, however the ends are quite different. One of the crucial features which distinguish Tn*5397* from Tn*916* is the gene coding for the site specific recombinase, TndX. The putative TndX is related to the resolvase/invertase family while the site specific recombinase of Tn*916* belongs to the integrase family. The target site choices of these two elements are also different. These suggest that these two proteins mediate different transposition processes. In this study, the gene coding for TndX was cloned and the protein expressed in *E. coli* as a fusion to a His tag. The purified TndX had been shown to be able to bind specifically to the end of the element by gel retardation assays. A 1.7 kb mini-Tn*5397* was constructed. The role of the overexpressed TndX in the excision and insertion processes of the mini-Tn*5397* are under investigation.

**P15 DEMONSTRATION OF A MOSAIC STRUCTURE IN THE CONJUGATIVE TRANSPOSON Tn*5397*: IMPLICATIONS FOR EVOLUTION AND DISSEMINATION OF VECTORS OF ANTIMICROBIAL RESISTANCE GENES**.

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Tn*5397* is a conjugative transposon originally found in a clinical isolate of *Clostridium difficile,* an important nosocomial pathogen. It confers tetracycline resistance upon its host via *tet*(M). The sequencing of this element was undertaken as part of a study aimed at understanding the molecular mechanisms of conjugative transposition of Tn*5397*. The sequence analysis showed that the central part of Tn5397 contained open reading frames that share extensive homology to the conjugative transposon Tn*916*, however, the ends were completely different. Tn5397 contained a gene ( *tndX* ) encoding a large resolvase / invertase protein. TndX belongs to a new class of site specific recombinases that include the site specific recombinase from Actinophage øC31, from *Streptomyces* and the recombinase from the mobilisable transposon Tn*4451*. In conclusion Tn*5397* has been shown to comprise of two distinct sequence blocks, this could have been formed by a single recombination event between the circular form of Tn*916* or a Tn*916*-like element and another element.

The implications for large scale rearrangements of mobile genetic elements carrying antimicrobial resistance genes are discussed.

**P16 SPECIES DIVERSITY OF AMMONIA OXIDISERS IN TERRESTRIAL HABITATS.**

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Autotrophic nitrification is the oxidation of ammonia, via nitrite, to nitrate by chemolithotrophic bacteria. It plays a major role in the global cycling of nitrogen in natural ecosystems. The major groups of micro-organisms involved in autotrophic nitrification are ammonia and nitrite oxidisers. Our studies focus on the ammonia oxidisers which are an ecologically important and physiologically specialised group. These bacteria carry out the initial, and rate determining step of nitrification; oxidation of ammonia. The strong effect of the external pH on ammonia oxidisers has been well documented; growth in liquid batch culture rarely occurs at pH values below 6.5 but autotrophic nitrification can occur in acidic soils. Acidophilic ammonia oxidisers are difficult to analyse in natural communities due to low growth rates, low biomass yield, and the limited number of distinguishing phenotypic characteristics. Developments in 16S ribosomal DNA-based techniques has enabled the study of community structure in environmental samples. Recent 16S r-DNA-based studies indicate that selection for different groups of ammonia oxidisers occurs in soils of different pH. Our hypothesis states that at pH 4.2 *Nitrosospira* cluster 2 predominate and at pH 7 *Nitrosospira* cluster 3 predominate. DNA extraction and nested PCR methods, followed by DGGE and sequence analysis are being used to determine the rate of change of community structure following manipulation of soil pH and ammonia concentrations in conjunction with traditional process measurements to assess the relationship between community structure and nitrification rates.

**P17 IDENTIFICATION OF BACTERIA IN DENTAL HISTOLOGICAL SPECIMENS USING MOLECULAR TECHNIQUES: A PRELIMINARY STUDY.**

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The aim of this study was to develop a protocol for bacterial identification from histological specimens of teeth. The material of choice was formalin fixed paraffin-embedded (FFPE) tissue from carious teeth with pulpal and periradicular lesions. Paraffin wax was removed from tissue sections (8 mm) using xylene and the DNA extracted by one of three different protocols (Puregene kit plus Wilson & Grimont protocols). 16S ribosomal RNA genes were amplified by PCR from the purified DNA using global primers. Successfully amplified samples were cloned into *E. coli* and subjected to restriction fragment length polymorphism analysis. DNA was extracted and successfully amplified only from one sample prepared by the Wilson protocol. RFLP analysis revealed three profiles, two of which were identified by gene sequencing as Streptococcus sanguis and the third as *Enterococcus faecalis*. *S. sanguis* is commonly isolated from supra-gingival plaque and carious lesions, while *E. faecalis* is often associated with root canal infections.

The results of this study firstly emphasise the importance of optimising DNA extraction protocols, and secondly provide an elegant technique for correlating specific bacterial taxa with FFPE sections of pulpal and periradicular lesions.

**P18 DETECTION OF ANTIBIOTIC RESISTANCE, ppGpp AND SIGMAS EXPRESSION IN SLOW GROWING *ESCHERICHIA COLI***

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Abstract not received

**P19 ISOLATION OF NOVEL ORGANOPHOSPHONATE METABOLOSING MICRO-ORGANISMS UNDER ANAEROBIC CONDITIONS**

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Abstract not received

**P20 THE REGULATION OF PATHOGENICITY GENE EXPRESSION IN *XANTHOMONAS CAMPESTRIS* MEDIATED BY A SMALL DIFFUSIBLE MOLECULE**

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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. This factor (DSF ) can restore production of extracellular endoglucanase and protease to *rpfF* (but not to *rpfB*) mutants. The *Xcc* system is novel in that DSF is not an *N*-acyl homoserine lactone and RpfB and RpfF are not related to LuxI/R proteins implicated in the synthesis of *N*-acyl homoserine lactones which are widely distributed in Gram-negative bacteria. To identify further genes involved in the DSF signalling system, a transcriptional fusion was created between the promoter region of the *Xcc* endoglucanase gene and the coding region for b-glucuronidase (GUS). The *rpfF* mutant harbouring the endoglucanase-GUS reporter plasmid showed increased GUS activity in response to exogenously added DSF or when grown in the presence of a DSF producing strain. A mutagenised population of *Xcc*, generated by transposon mutagenesis, was screened with the reporter strain to select mutants with alterations in DSF level. Two DSF hyperproducing strains were identified. Paradoxically both of these strains had reduced levels of extracellular enzymes and EPS and were reduced in pathogenicity. Although derived from separate transposon insertion events, both mutants carried insertions in the same gene, *rpfC*. RpfC belongs to a small sub-class of two component regulators in which the sensor and response regulator domains are fused. Immediately upstream of *rpfC* are *rpfH*, which encodes a protein with homology to the N-terminal sensor domain of RpfC, and *rpfG* which encodes a typical response regulator. These three genes are organised in an operon. 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.

**P21 MOLECULAR DIFFERENTIATION OF RENIBACTERIUM SALMONINARUM ISOLATES FROM WORLD-WIDE LOCATIONS**

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Renibacterium salmoninarum is the causative agent of bacterial kidney disease (BKD) in salmonid fish. Conventional typing methods have failed to provide a reliable means for differentiating between isolates of this slow growing, fastidious pathogen. For this purpose, we have investigated random amplified polymorphic DNA (RAPD), ribotyping, 23S and 5S rDNA sequencing, and the PCR amplification and sequencing of rRNA and tRNA intergenic spacer regions using DNA from seventy four R. salmoninarum isolates from different geographic locations and host species. Sequencing of the 23S and 5S rRNA genes confirmed the phylogenetic position of Renibacterium within the high G+C group of the Micrococcus/Arthrobacter subdivision of the actinobacteria. PCR analysis showed that the 16S-23S and 23S-5S rDNA spacer regions of all isolates are highly conserved. DNA sequencing of the spacer regions confirmed this finding although some limited sequence variation was found. Ribotyping revealed that at least two ribosomal RNA operons are present on the R.salmoninarum chromosome and these are apparently identical in size in different isolates. Consequently, the apparent homogeneity of the R salmoninarum rRNA operons rendered these techniques of limited use for isolate differentiation. RAPD and tRNA intergenic spacer regions proved to have the greatest potential for the typing of this organism.

**P22 COMPUTER SIMULATION OF THE DYNAMICS OF MICROBIAL POPULATIONS: A TEACHING APPROACH**

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The dynamics of microbial populations can often be expressed in mathematical form and these relationships invariably form part of undergraduate programmes incorporating microbiology. For many students of biology, meaningful interpretation of seemingly complex mathematical equations represents a considerable hurdle and their value to understanding the dynamics of microbial populations is often not fully appreciated. This problem can be particularly pronounced if the subject matter is taught entirely using a traditional classroom approach.

The computer-aided learning (CAL) software described here was designed to enhance student understanding of the dynamics of microbial populations through the use of interactive computer simulations. The software combines the linear authoring capability of *Authorware Professional* with the dynamic simulation capability of *PowerSim*. A structured front-end provides background and activities for each simulation, as well as a computer-based library. Students address the activities in the simulation part of the software by exploring, in a relatively unstructured manner, the influence of change of parameter values on model variables. Simulations of the Monod relationship, inhibitory growth substrate, exponential growth, batch culture and chemostat culture are incorporated. The software is essentially modular in design and new simulations can be added with relative ease. Positive student-feedback and evidence of enhanced understanding following use of the software in undergraduate programmes has been gained, which suggest that the use of simulation to explore mathematical relationships can represent a powerful approach to learning for students of biology.

**P23 MONITORING PHENAZINE AND N-ACYL-L-HOMOSERINE LACTONE PRODUCTION BY *PSEUDOMONAS AUREOFACIENS* WITH LUXAB REPORTER SYSTEM**

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Phenazines antibiotic compounds are involved in the suppression of many fungal plant pathogens (e.g. *Gaeumannomyces graminis*, *Pythium spp*. and *Fusarium oxysporum*). Production of this antibiotic is positively regulated by N-acyl-L-homoserine lactones (AHLs) signal molecules in a cell-density dependent manner in *Pseudomonas aureofaciens* 30-84 and P. fluorescent 2-79. Phenazine-1-carboxylic acid (PCA) is also important for the survival of bacteria in the rhizosphere. Its production is affected by nutritional growth conditions, AHLs and other regulators such as gacA. It is, however, not clear how the production is regulated in the rhizosphere and further work is needed to determine the precise relationship between cell density, AHL stability, local concentration and the switch on of phenazine production. We are studying the extent to which AHLs influence phenazine production by *Pseudomonas aureofaciens* in vitro in liquid culture and on biofilms, and in situ on root soil-free and in *Phaseolus vulgaris* rhizosphere. A range of bioassays were developed to measure phenazine and AHLs production by the wild-type strain PGS12. In order to study the influence of AHL at the molecular level, a luxAB-reporter strain, P. aureofaciens B10, was constructed by transposon mutagenesis. This reporter strain allowed an estimation of the phenazine gene cluster promoter activity. The influence of population density together with AHL diffusion was studied preferentially on agar while the importance of nutritional factors was related to promoter activity in liquid culture.

**P24 *IN SITU* PHYSIOLOGY OF FILAMENTOUS SULPHUR BACTERIA PRESENT IN ACTIVATED SLUDGE**

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The identity of the various filamentous bacteria present in activated sludge and concomitantly a better understanding of their *in situ* physiology are essential to design strategies to control and limit their growth. The overgrowth of those bacteria (bulking) reduces the settling properties of the sludge, which is a major operational problem in wastewater treatment plants (WTPs). Activated sludge samples from an industrial WTP in Denmark (Grindsted) were investigated. The filaments present in the Grindsted samples were morphologically identified using the Eikelboom classification system, which includes phase contrast microscopy, Gram and Neisser staining. Fluorescence *in situ* hybridisation (FISH) with 16S and 23S rRNA-targeted nucleic acid probes specific for Type 021N and *Thiothrix*, and for the gamma subclass of *Proteobacteria* were used. Viability and respiratory activity of the filaments were detected by reduction of CTC (5-cyano-2,3-ditolyl tetrazolium chloride) and microautoradiography (MAR). The formation of S-granules by oxidation of thiosulphate under aerobic conditions was monitored using phase contrast microscopy. MAR was combined with FISH using confocal laser scanning microscopy (CLSM) to determine the *in situ* uptake of different organic compounds like $^{14}$C-acetate and $^{14}$C-$HCO_3^-$ on a single cell level, and assess the growth of the filamentous bacteria under heterotrophic, mixotrophic and autotrophic conditions. The *in situ* results were compared to the known physiology of pure cultures of Type 021N and *Thiothrix*. The combination MAR-FISH proved to be a robust technique to evaluate the *in situ* activity of filamentous sulphur bacteria, and the results clearly revealed the diversity of these bacteria in activated sludge.

**P25 THE IDENTIFICATION AND CHARACTERISATION OF A SECOND QUORUM SENSING LOCUS IN *YERSINIA PESTIS***

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Quorum sensing is the ability of bacteria to regulate gene expression in a population 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. *Yersinia pestis*, the causative organism of bubonic plague, expresses a complex array of virulence factors, the regulation of which is poorly understood. In a previous study we identified a quorum sensing system and characterised the *lux* homologues, designated *ypeR* and *ypeI*, and the AHLs produced. We now report the identification of a second quorum sensing system in *Y. pestis*. The regulatory genes, designated *yepR* and *yepI*, were cloned and sequenced and the AHLs produced by the synthase gene were characterised. Mutants of *Y. pestis* defective in selected quorum sensing genes were produced and the effects of these mutations were studied.

**P26 COMPARATIVE ANALYSIS OF THE PARTICULATE METHANE MONOOXYGENASE (PMO) GENE CLUSTERS FROM METHYLOSINUS TRICHOSPORIUM OB3B AND METHYLOCYSTIS SP. STRAIN** M,Gilbert, B.; McDonald, I.R.; Finch R., Murrell, J.C.

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The particulate methane monooxygenase (pMMO) gene cluster has previously been sequenced from *Methylococcus capsulatus* (Bath) (Semrau et al. 1995). It consists of three genes, pmoC, pmoA, and pmoB, coding for the 26, 27 and 45 kDa polypeptides of the pMMO complex. The genes are present in at least two nearly identical copies, the function of which is still unclear. In this study, we have cloned and sequenced the pmo gene cluster of two type II serine pathway methanotrophs, *Methylosinus trichosporium* OB3b and Methylocystis sp. strain M. They belong to the a subclass of the class Proteobacteria and possess both
the soluble and the particulate (membrane-bound) methane monooxygenase (sMMO and pMMO, respectively). The pmo genes showed high degrees of identity between these type II organisms (80 to 90 % sequence identity) and to the corresponding genes from Methylococcus capsulatus (Bath) (60 to 76 % sequence identity). The hydrophobicity plots further stress the high similarity between the derived amino acid sequences. Southern blots of both *Methylosinus trichosporium* OB3b and *Methylocystis sp*. strain M suggest the presence of at least two pmo gene clusters. This study extends the database of methane monooxygenase sequences in type II methanotrophs and will be important for the design of functional gene probes for environmental and biotechnological applications. Reference: Semrau et al. (1995) J. Bacteriol. 177, 3071-3079

**P27 QUORUM SENSING, FLUID SHEAR AND POPULATION DYNAMICS IN PSEUDOMONAS AERUGINOSA BIOFILMS**

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Bacterial pheromones, in the form of N-acyl homoserine lactones (HSLs), are thought to play a signicficant role in the formation and maintenance of Gram-negative bacterial biofilms. In this study we have examined the role of cell-cell signalling in cellular detachment from biofilms as a function of fluid dynamic shear. Pseudomonas aeruginosa PA01 (HSL wild type) biofilm productivity (cells detached/cm2/min) was assessed at different shear rates and comparisons in productivity made to the mutants PAN067 and PA0-R1, defective in the C-4 butanoyll HSL and C-12 dodecanoy HSL respectively. Fluid dynamic shear was achieved by growing cultures in a stainless steel rig composed of four cylindrical sections rotated within four concentric chambers. Each chamber is independently supplied with fresh medium and can be accessed for sampling. At any given angular velocity the fluid dynamic forces experienced by the stationary and rotating surfaces are proportional to their radius. Our results suggest that whilst biofilm productivity might be similar at different shear forces, the nature of the biofilm and steady state population is affected by shear. Moreover, both the C-4 and C-12 HSL moderate biofilm formation and detachment. The greatest effect of shear was observed with PAN067, cells tending to detach readily as sloughs. By comparison, there was little effect of shear on PA0-R1 biofilm stability, cell detachment occurring through the continual erosion of cells. The data strongly suggests that HSL signals do moderate biofilm physiology, possibly through the deposition of different exopolymers, but that the behaviour of biofilm communities at different interfaces are regulated by the relative concentrations of a multiplicity of signals.

**P28 INDUCTION OF DNA OVER-REPLICATION IN COPPER-STRESSED *SACCHAROMYCES CEREVISIAE***

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A critical feature of eukaryotic cell cycle control is that one round of DNA synthesis must alternate with one round of cell division. Abrogation of this control can lead to unscheduled DNA replication and polyploidy. This paper describes copper-induced DNA over-replication. The eukaryotic model *Saccharomyces cerevisiae* cultured in supplemented medium exhibited DNA over-replication during exposure to >2 mM Cu. Over-replication was rapid and led to cells with greater than 4C DNA within 15 min. Above the thresh-hold concentration of 2 mM Cu, the rate and extent of DNA over-replication did not increase with Cu concentration. Cells arrested at various cell-cycle stages using alpha-factor, hydroxyurea or nocodazole all accumulated DNA rapidly following addition of Cu. Levels of Cdc28p, the principal cell cycle regulatory kinase, did not change during Cu treatment as revealed by immunoblotting. We are presently assessing effects on Cdc28 activity. This novel DNA over-replication model may prove valuable in characterizing the underlying mechanisms of stress-induced genomic instability.

**P29 REGULATION OF ATYPICAL CLASS II CELL-CYCLE DEPENDENT PROMOTERS OF *CAULOBACTER CRESCENTUS***

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The aquatic Gram-negative bacterium *Caulobacter crescentus* undergoes obligatory asymmetric cell division resulting in two distinct cell morphotypes with different fates. The swarmer cell has a single polar flagellum and is chemotactically competent. DNA replication is suppressed in the swarmer cell and is only activated once it differentiates into a stalked cell, concomitantly losing the polar flagellum and chemoreceptors. The stalked cell is non-motile and competent for DNA replication, thus allowing cell growth and cell division to give rise to a stalked cell and a swarmer cell.

Ordered transcription of flagellar and chemotaxis genes is a well characterized feature in *Caulobacter* and a useful tool to probe regulatory signals and proteins which mediate cell cycle dependent gene expression in this model system. Two cell cycle regulated genes have been selected for study, the chemoreceptor gene *mcpA* (methyl-accepting chemotaxis protein A) and a gene transcribed divergent to *mcpA*, *cagA* (chemotaxis associated gene A). Flagellar biogenesis involves the ordered transcription of >50 genes, which can be classed according to their position in a hierarchy. Epistasis experiments have shown that *mcpA* and *cagA* are atypical class II flagellar hierarchy promoters. Transcriptional start sites for *mcpA* and *cagA* have been characterized. The requirement of DNA replication for *mcpA* and *cagA* transcription has been demonstrated. Trancription of *cagA* is cell cycle dependent with transcription in the swarmer cell. CtrA (the master two-component regulator shown to regulate other class II genes) is required for transcription of *mcpA* and *cagA*. Since the promoter sequences for *mcpA* and *cagA* do not contain consensus target sequence(s) for CtrA binding, current experiments aim to resolve whether CtrA binds to these promoters. These results will be discussed in the context of attempts to identify genes which regulate the expression of *cagA* and *mcpA*. This will include reverse genetic analysis of several ECF-type sigma factors identified from the genome sequencing project.

**P30 CHARACTERISATION OF STRESS RESPONSES IN *CAMPYLOBACTER JEJUNI***

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Campylobacters cannot multiply in the environment and the organism's ability to survive and remain infective in the external environment remains unclear. An important element in the mechanism of survival is the ability of the bacterium to adapt to the adverse conditions associated with natural environments such as oxidative stress, nutrient-limitation, elevated temperature (heat shock), and pH changes. These stressors are known to induce distinctly similar sets of chaperonin/stress proteins, including the GroES and GroEL chaperonin proteins (products of the bicistronic *groESL* operon in *Escherichia coli*), and members of the highly conserved heat shock HSP 60 family which occur ubiquitously. Both proteins have been correlated with a strong cross-resistance against heat shock and other stress-induced damages.

In this study, the survival characteristics of *C. jejuni* to a range of stressful conditions was examined via direct viable plate counts. Western blot analysis of whole cell protein extracts revealed the effect of various stresses on the induction of a 62KDa GroEL protein homologue. In addition, characterisation of the *C. jejuni groESL* operon was carried out. An internal *C. jejuni groEL* gene fragment was amplified via PCR and used as a gene probe in order to isolate and sequence the *groELS* genes.

**P31 CHARACTERISATION OF THE ROLE OF A *Helicobacter pylori* PHOSPHOLIPASE IN THE COLONISATION OF THE GASTRIC MUCOSA**

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Phospholipase activity is considered to play a role in the pathogenicity of *Helicobacter pylori*. Furthermore some drugs that are effective against *H. pylori* infection are phospholipase inhibitors. Scrutiny of the *H. pylori* 26695 genome sequence revealed the presence of a putative protein with homology to outer membrane phospholipase A (PldA). The aim of this study was to investigate the role of this putative PldA in the pathogenicity of *H. pylori*. An isogenic *pldA* mutant was constructed and analysed for *in vitro* phospholipase $A_2$ and haemolytic activity. The *pldA* mutant showed minimal phospholipase $A_2$ activity and a marked reduction in haemolytic activity compared to the wild-type strain. Adherence of the mutant to human gastric adenocarcinoma cells and the ability to colonise mice were also investigated. The mutant was unable to colonise mice at 2 and 8 weeks, but did induce a significant immune response. In contrast, the ability of the mutant to adhere to AGS cells was unaffected, indicating that adhesion to and colonisation of the gastric mucosa are separate stages in *H. pylori* infection. These results suggest a role for PldA in both the colonisation of the gastric mucosa and tissue damage following colonisation.

**P32 THE ROLE OF PhoPQ, A TWO-COMPONENT REGULATORY SYSTEM, IN STRESS RESPONSES OF *Yersinia pseudotuberculosis***

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The two-component regulatory system PhoPQ has been shown in *Salmonella typhimurium* to play an important role in virulence and survival in the hostile host environment. Given the similar environments encountered by *Y. pseudotuberculosis* during infection, the role of PhoPQ in the virulence of this pathogen was investigated. Using degenerate primers, based on published sequence data from *E. coli* and *S. typhimurium*, PhoP homologues were identified in all three *Yersinia* species The *phoP* gene from *Y. pseudotuberculosis* YPIII pIBI was cloned and sequenced, revealing a gene encoding a protein with 79% identity and 90% similarity to *S. typhimurium* PhoP. Further confirmation of the identification of a *phoP* homologue was shown by the complementation of a *S. typhimurium phoP* mutant with the cloned *Y. pseudotuberculosis phoP* gene. Using inverse PCR mutagenesis, a defined deletion and *Bgl*II site were introduced into a cloned Y. *pseudotuberculosis phoP* internal gene fragment. The mutated gene fragment was sub-cloned into a suicide vector pCVD442 and used to construct a defined *Y. pseudotuberculosis phoP* mutant. Stress response experiments have shown that the *phoP* mutant has a reduced ability to survive acid stress compared to the wild-type, as reported for *S. typhimurium*. Oxidative and heat stress experiments and protein profile analysis of both wild-type and *phoP* mutant strains under varying external conditions using 1D and 2D SDS-PAGE are currently in progress.

**P33 ANALYSIS OF FLAGELLAR BIOSYNTHESIS IN *Helicobacter pylori*.**

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*Helicobacter pylori*, a motile bacterium isolated primarily from the human stomach, is associated with gastritis, gastric and duodenal ulcer and gastric carcinoma. Experiments in animals using isogenic mutants have shown that both the FlaA and FlaB flagellins are required for full motility and for establishing persistent infection. The *flaA* and *flaB* genes are unlinked on the chromosome and are preceded by different promoters suggesting that they may be differentially regulated. Recently FlgR, a transcriptional activator, has been shown to control via $s^{54}$-dependent promoters five operons containing genes for flagellar biosynthesis. Scrutiny of the *H. pylori* 26695 genome sequence has suggested there are over 40 genes with a role in flagellar biosynthesis. For seven of these putative flagellar genes, isogenic mutants were constructed, all of which were shown to be non-motile. Further examination by electron microscopy revealed that all these mutants were aflagellate. Northern slot hybridisation (to detect flagellin-specific mRNA) and immunoblotting (to detect flagellin proteins) have shown differences in transcription, translation and secretion of FlaA and FlaB between both individual mutants and when compared to the wild-type strain.

**P34 MULTIPLE *neu*B GENES IN *Campylobacter jejuni***

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N-acetyl neuraminic acid (NANA) is a common constituent of *C. jejuni* lipooligosaccharide (LOS). Such structures often mimic gangliosides and are thought to be involved in the Guillain-Barre and Miller-Fisher syndromes following *C. jejuni* infection. Our analysis of the recently completed genome sequence of *C. jejuni* NCTC 11168 identified three putative N-acetyl neuraminic acid synthetase genes termed *neu*B1, *neu*B2 and *neu*B3. LOS from *C. jejuni* NCTC 11168 binds cholera toxin (CT) indicating the presence of NANA in a LOS structure mimicing the oligosaccharide portion of ganglioside $GM_1$. Isogenic mutants were created in all three *neu*B genes and for one such mutant (*neu*B3) LOS was shown to have an increased mobility in SDS -PAGE gels and also failed to bind CT consistent with loss of a sialic acid residue from the structure. Heterologous expression of the *neu*B3 gene in *E. coli* demonstrated that NeuB3 was capable of *in vitro* NANA biosynthesis. The distribution of the *neu*B3 gene in *C. jejuni* strains with well characterised LOS was examined. Mutagenesis of *neu*B1 and *neu*B2 did not affect LOS, but *neu*B1 mutants were aflagellate and non-motile whereas no phenotype was evident for *neu*B2 mutants.

**P35** CHEMOTACTIC SIGNAL TRANSDUCTION IN *HELICOBACTER PYLORI*

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The publication of the complete genome sequence of *Helicobacter pylori* 26695 revealed several homologues of chemotaxis genes previously characterised in other species of bacteria. These included a copy of the ternary complex forming protein, *cheW* (*Escherichia coli*), three copies of the bi-fuctional *Bacillus subtillus* homologue *cheV* (annotated as HP0019, HP0393,and HP0616), and the autophosphorylating histidine kinase protein, *cheA*. We have previously sequenced the *cheA* homologue and shown it to be a bifunctional protein consisiting of both *cheA* and *cheY* domains, termed *cheF*. It is homologous to the bifunctional protein, *frzE* of *Myxococcus xanthus*. In addition to these homologues, Bierer *et. al*. have previously characterised the response reulator, *cheY*. The HP0393, *cheF* and *cheW* may be arranged in an operon. The *cheY*, HP0616 and HP0019 are at other unlinked sites on the chromosome. We have constructed isogenic insertional inactivation mutants in the above homologues using a chloramphenicol resistance cassette and analysed the swarming phenotype on soft agar. The *cheY*, *cheW*, *cheF* and HP0019 genes are essential for chemotaxis as mutants do not swarm. The HP0393 and H0616 displayed functional redundancy as single mutants exhibited only a slight reduction in swarming as compared to wild type levels. The above genes have been cloned into the expression vector pTM30 and the proteins overexpressed. The *cheY* and *cheF* have been purified. The autophosphorylating reaction of the *cheF* and the subsequent transfer of phosphate to the *cheY* domain and the *cheY* is currently under study. The role of the three *cheV*'s is unknown but in the absence of a *cheZ* homologue, they may serve as phosphate sinks to control flagellar switching.

**P36 IRON ACQUISITION SYSTEMS AND THEIR REGULATION IN *HELICOBACTER PYLORI***

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The genome sequence of the gastric pathogen *Helicobacter pylori* suggests that the organism has a well developed system for scavenging iron from its environment. The control of iron acquisition systems in many bacteria is managed, at least in part, by the global ferric uptake regulator (Fur) protein. A single copy of a *fur* homologue has also been identified in the genome sequence of *H. pylori* strain 26695. In order to study the role of Fur in *H. pylori*, we have generated a *fur* mutant by insertion of a chloramphenicol resistance cassette into the predicted coding sequence of Fur in strain 26695. In $Fe^{55}$ uptake assays of cells grown under iron replete conditions, the rate of $^{55}Fe$ uptake was 4.5-fold higher in the *fur* mutant than in the wild-type strain. The whole cell iron content of the *fur* mutant was also found to be significantly higher than that of the wild type strain in cells grown in both low and high-iron medium. The *fur* mutant was also found to be more resistant to manganese, and more sensitive to the redox-cycling agent paraquat than its wild-type parent. Thus, Fur plays a major role in the regulation of iron uptake in *H. pylori*. In addition, SDS-Page gels of fractionated cells grown in low- and high-iron medium have revealed both Fur-dependent and Fur-independent, iron regulated gene expression in *H. pylori*.

**P37 THE FERROUS IRON TRANSPORT OF *HELICOBACTER PYLORI***

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The complete genome sequence of the gastric pathogen, *Helicobacter pylori*, reveals that the organism possesses multiple iron (Fe)-scavenging systems including a ferrous Fe transport system encoded by a *feo*B homologue. In order to acertain the physiological function of FeoB in the high affinity acquisition of Fe, a chromosomal *feo*B null mutant was consructed by insertion of a chloramphenicol resistance determinant into the predicted coding sequence of FeoB. Analysis of whole cells for elemental Fe revealed considerably lower levels of Fe in the *feo*B mutant. Inactivation of *feo*B resulted in a failure to transport $^{55}$ferrous Fe and $^{55}$ferric dicitrate suggesting that both ferrous Fe and ferric dicitrate uptake are mediated by the FeoB homologue. Restoration of *feo*B on a shuttle vector restored $^{55}$ferrous Fe and $^{55}$ferric dicitrate uptake. Transport of ferrous Fe in wild type cells is saturable giving an apparent Ks value of 0.42μM. The ferrous Fe chelator, ferrozine, inhibits transport of both $^{55}$ferrous Fe *and* $^{55}$ferric dicitrate. Sensitivity to inhibition by DCCD, FCCP and vanadate, suggests that the ferrous Fe transport via FeoB is driven by ATP hydrolysis. The lack of competition by other divalent cations for the *feo*B transporter suggests that it is highly specific for ferrous Fe. Finally, we demonstrate that FeoB is a prerequisite for infectivity of the mouse model of *H.pylori* infection suggesting that *H.pylori* has access to a ferrous Fe source *in vivo*, which is scavenged by the FeoB protein.

**P38** NAD(P)H OXIDATION AND METRONIDAZOLE RESISTANCE IN *HELICOBACTER PYLORI*

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Metronidazole is commonly used to treat *Helicobacter pylori* infection, but resistance to this drug is becoming widespread. Metronidazole has to be reduced to an active form for cytotoxicity and recent studies have shown that a gene, *rdxA*, encoding a reductase enzyme is largely responsible for this in *Helicobacter pylori.* However, although mutations in *rdxA* have been shown to result in metronidazole resistance, other studies have also reported a decrease in NADH oxidase activity in metronidazole resistant strains. In this study, isogenic pairs of metronidazole sensitive and resistant strains have been analysed by non-denaturing PAGE followed by activity stains for NADPH or NADH dehydrogenase activities. We have found that the metronidazole resistant strains show a pleiotropic loss of many proteins which possess NADH dehydrogenase activity. We are currently identifying these enzymes and investigating the link between metronidazole resistance and the regulation of their expression.

**P39 FLAVODOXIN AND ITS ROLE IN ELECTRON TRANSPORT REACTIONS IN *HELICOBACTER PYLORI***

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Flavodoxin and/or ferredoxin are likely to be the *in vivo* electron acceptors for the crucial enzymes pyruvate and 2-oxoglutarate:acceptor oxidoreductases (POR and OOR respectively). The ability of POR to indirectly reduce *H.pylori* flavodoxin has been previously demonstrated, as has the ability of POR to reduce NADP, however our current work suggests that POR/OOR reduced flavodoxin may not be the *in vivo* electron donor to NAD(P).Thus, another intermediate electron acceptor, e.g. a ferredoxin, has to be involved, since we have previously shown that purified POR and OOR are not capable of using NAD(P) directly.

*H.pylori* flavodoxin (FldA, encoded by the *fldA* gene) has been cloned, overexpressed, and purified to homogeneity by low pressure ion-exchange chromatography on DEAE-sepharose, followed by a high pressure uno-Q anion exchange step, and finally by gel filtration. This purified flavodoxin, contained in anaerobic cuvettes under oxygen-free nitrogen, was used to perform a series of spectrophotometric assays, using anaerobic *H.pylori* cell free extract, added from a side arm, as a source of POR and OOR, and adding the approriate substrates by injection from anaerobic stock solutions. These assays confirmed our previous findings, however it was not possible to demonstrate the coupling of reduced purified flavodoxin to NAD(P). Disruption of *fldA* appears to be lethal. *H.pylori* ferredoxin (Hp 0277) has also been cloned for overexpression.

**P40 *YERSINIA PESTIS* F1 ANTIGEN ASSEMBLY - PROPERTIES OF A PROTOTYPE PERIPLASMIC CHAPERONE OF THE FGL SUBFAMILY.**

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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 can be considered a prototype of 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 not for maintenance of the finally folded structure. 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. Possible implications of these results to subunit synthesis and assembly are considered.

**P41 A PUTATIVE PENICILLIN BINDING PROTEIN INVOLVED IN THE PENICILLIN RESISTANCE OF *STREPTOMYCES COELICOLOR.***

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ß-lactam antibiotics act by inhibiting the enzymes that are involved in the final stages of peptidoglycan biosynthesis, the penicillin binding proteins (PBP's). Bacterial resistance to such compounds in Gram-positive organisms can be due to: alteration of the affinity of the PBP towards penicillin; appearance of an additional PBP (as demonstrated in methicillin resistant *Staphylococcus aureus*) or b-lactamase activity. In Gram-negative bacteria, antibiotic resistance can also arise due to an alteration in the permeability of the outer membrane.

Our work focuses on the PBP's expressed in *Streptomyces coelicolor* and their function in b-lactam antibiotic resistance. One PBP appears to be expressed consistently throughout growth, and has been found to be located on both the cell membrane and wall. This PBP can be readily washed away from the cells with mild salt solutions. This protein species appears to bind penicillin extensively, being seen as one of the strongest signals in the PBP assays. The importance of this protein in acting as a protective coating during treatment with b-lactam antibiotics will be discussed.

**P42 THE MOLECULAR ECOLOGY OF METHANOGENIC ARCAHE AND SULPHATE REDUCING BACTERIA FROM TWO CONTRASTING ANTARTIC ENVIRONMENTS**

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Abstract not received

**P43 MICROBIAL COMMUNITY DEVELOPMENT IN POLYCYCLIC AROMATIC HYDROCARBON DEGRADING BIOFILMS**

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Two polycyclic aromatic hydrocarbons (PAH), naphthalene and phenanthrene, were used as sole carbon sources to enrich and grow PAH-degrading biofilms in a continuous–flow slide culture system. The biofilms were positively stained with SYTO 13 and visualised using scanning confocal laser microscopy and image analysis. The resulting spatial relationships between bacteria within the community, established after three weeks, were unique to PAH grown biofilms, and were not maintained when biofilms were grown on an equivalent carbon concentration of a more labile substrate (tryptic soy broth). Degradation rates, species richness and substrate utilisation profiles of the biofilm community were compared to consortia isolated by standard enrichment techniques. The distinctive structure and function of the PAH grown biofilms indicate that syntrophic interactions may optimise PAH degradation.

**P44 COAGGREGATION OF IDENTIFIED BACTERIA FROM A BOREHOLE WATER SOURCE**

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Cell to cell adhesion or coaggregation occurs as a result of specific interactions between different species of oral bacteria and has recently been shown to occur between aquatic bacteria. In this study we identified, by partial 16S rRNA gene sequencing, 19 culturable heterotrophic bacteria from a borehole water source and have shown that they all have an ability to coaggegate. Using a visual coaggregation assay, it was observed that the expression of coaggregation was dependent upon the duration of growth in batch culture at 25°C. Most coaggregating strains belonged to the genera *Blastomonas* and *Sphingomonas*. The ability to coaggregate was strain specific, with *Blastomonas natatoria* 2.1, *Sphingomonas* sp. 2.15 and *Afipia* sp. 2.2 being the most promiscuous coaggregators. Both intra- and inter- generic coaggregation reactions occurred between strains. Preliminary work on selected strains using heat, protease and simple sugars suggest that *B. natatoria* 2.1, 2.3, 2.6, 2.8 and *Micrococcus luteus* 2.13 coaggregate by specific lectin-carbohydrate interactions.

**P45 HOMOSERINE LACTONES IN NATURAL MICROBIAL ECOSYSTEMS**

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N-acylated homoserine lactones (AHL) act as autoinducers for a broad variety of physiological inductions under conditions of high cell density (quorum sensing) and are found in a wide range of Gram-negative organisms [1]. They induce the transcription of organismic specific genes, as e.g. bioluminescence in *Vibrio fischeri* or the synthesis of exoenzymes in *Pseudomonas aeruginosa*. High concentrations of microorganisms similar to stationary phase conditions occur also in nature, e.g. in microbial biofilms or mats and blooms. In these systems homoserine lactone(s) are detected by using a bioassay [2}. *Chromobacterium violaceum* CVO26 is blocked in the synthesis of AHL but produces violacein after addition AHL. The sensitivity of the test system to synthetic AHL is 500 ng. When pieces of microbial mats, a drop of cyanobacterial bloom material or washing solution from plant leaves are incubated on petri dishes inoculated with the *Chromobacterium violaceum* mutant violet rings around the sample are induced. This shows that AHL possibly act as signal molecules in natural microbial habitats with high cell concentrations.

[1] S. Swift *et al.* 1998 Molecular Microbiology (S. Busby *et al.*, eds.) Springer, Berlin, pp. 185-207.
[2] R. Bachofen and A. Schenk 1998 Microbial Res 153, 61-63.

**P46 BACTERIAL DIVERSITY OF A MEROMICTIC ALPINE LAKE AS REVEALED BY SEQUENCING OF CLONED 16S rDNA FRAGMENTS AND TEMPORAL TEMPERATURE GRADIENT GEL ELECTROPHORESIS (TTGE)**

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In the chemocline of the meromictic Lake Cadagno in the Swiss Alps an extremely dense layer of mainly phototrophic bacteria develops annually during the summer season. Its diversity has been investigated using techniques of the molecular biology.

The total bacterial 16S rDNA was amplified by PCR and subcloned into plasmid pBluescript. DNA of 47 randomly selected clones was analyzed by restriction fragment length polymorphism (RFLP) using the restriction enzymes *Hae*lll and *Hin*fl. This approach allowed to distinguish 28 different patterns; 22 of which were unique to single clones. Comparative sequence analysis of the clones with different RFLP profiles revealed the presence of different groups of the domain *Bacteria*. Our results also confirm that *Chromatiaceae* species predominate in the chemocline bacterial community in the lake.

In addition, a 530 bp fragment of the cloned 16S rDNA (*Escherichia coli* position 8 to 536) was used for direct analysis by sequence-dependent separation in temporal temperature gradient gel electrophoresis (TTGE). Compared with the TTGE pattern of the environmental sample, several bands of the clones showed the same gel position as bands of the environmental sample.

Bosshard, P. P., Stettler, R. and Bachofen R. 1998 Documenta 1st Ital. Idrobiol., 63, 53-56.

## P47 THE EFFECT OF INCREASING LEVELS OF NisB ON BIOSYNTHESIS OF BACTERIOCIN NISIN

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The antimicrobial agent nisin is a ribosomally synthesised and posttranslationally modified peptide which is produced by food-grade organism Lactococcus lactis and used safely for decades as a food preservative. It is a 34-residue peptide, containing the unsaturated amino acids dehydroalanine and dehydrobutyrine and five ring structures due to the presence of lanthionine and b-methyllanthionine residues which are introduced by post-translational modification. It has been proposed that posttranslation modification of precursor nisin requires the products of nisB (for dehydration of Serine and Threonine residues) and nisC genes (addition of sulphur from neighbouring Cysteine residues). However, there has been no direct evidence for this and the enzymology of the maturation process has still to be elucidated. In an attempt to understand, the role of NisB protein in this maturation process, we have cloned the nisB gene from a nisin-producing strain of L. lactis and its activity was confirmed by complementation of a strain defective in nisB. Overexpression of the nisB gene generated elevated levels of the NisB protein as detected by SDS-PAGE electrophoresis and Western analysis using polyclonal antisera raised against NisB peptide. The effect of overexpressing this gene in some engineered nisin producing strains was monitored in an investigation of the role of NisB in the dehydration reaction of prenisin maturation.

## P48 MUTATIONAL ANALYSIS OF NISK: A HISTIDINE KINASE PROTEIN INVOLVED IN THE TWO COMPONENT REGULATORY SYSTEM CONTROLLING BIOSYNTHESIS OF THE LANTIBIOTIC NISIN

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Nisin is a ribosomally synthesised and posttranslationally modified peptide produced by *Lactococcus lactis* which has practical application in the food industry. It is the most prominent member of the group of antimicrobial peptides termed lantibiotics. Expression of the biosynthesis genes is strongly regulated by two proteins which belong to a family of environmental regulators. This comprises a membrane located histidine kinase protein (NisK) and a cytoplasmic response regulator protein (NisR). Nisin acts as an extracelluar signal for this two component regulatory system and the presence of the modified peptide in the external media triggers gene expression via a signal transduction pathway. An engineered *L. lactis* strain encoding a shortened *nisA* gene displayed no antimicrobial activity and was also sensitive to nisin. This indicated that expression of the *nis* genes was not being initiated and that the truncated peptide, although retaining antimicrobial activity, was unable to induce its own biosynthesis. Derivatives have been isolated that have overcome the block in the induction process and which exhibit a $Nis^+$, $Imm^+$ phenotype. Further characterisation of two independent derivatives revealed spontaneous changes within the *nisK* gene resulting in different point mutations within the cytoplasmic domain of NisK. The effect of these variant sensor proteins on nisin autoregulation in different hosts has been analyzed using nisin immunity assays and *gusA* gene fusions.

**P49 FUNCTIONAL ANALYSIS OF A MANGANESE-DEPENDENT STREPTOCOCCAL GENE REGULATOR**

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Streptococci and enterococci are major causative agents of bacterial infective endocarditis and it has recently been suggested that the ability of these bacteria to uptake manganese is linked to their virulence. Two members of the LraI family of cell surface-associated lipoproteins, ScaA in *Streptococcus gordonii* and PsaA in *Streptococcus pneumoniae*, have been shown to be components of a conserved ABC-type transporter involved in manganese uptake. ScaA production is increased >10 fold when cells are cultured in medium containing low manganese ion concentration (<0.5 mM), but the mechanism of regulation of *scaA* gene expression is unknown. The best studied family of metalloregulatory proteins in Gram-positive bacteria is a group of homologues of the *Corynebacterium diphtheriae* DtxR protein, members of which are present in several oral **streptococci and *Enterococcus faecalis*.** To determine whether the *S. gordonii* DtxR-homologue was involved in regulation of ScaA production, an isogenic knockout mutant was constructed. Mutant cells constitutively expressed high levels of ScaA, independently of the extracellular manganese ion concentration, indicating that the product of the *rmtA* (regulator of expression of metal ion transporter) gene normally functions as a manganese-dependent repressor of ScaA expression. Inactivation of *rmtA* did not affect expression of cell surface protein adhesins SspA and SspB, nor production of a major manganese-cofactored cytoplasmic enzyme, superoxide dismutase.

**P50 ANALYSIS OF A CONSERVED STREPTOCOCCAL LANTIBIOTIC GENE CLUSTER**

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*Streptococcus salivarius* is a predominant member of the normal oral microflora. Approximately 45% of *S. salivarius* strains have been shown to produce a lantibiotic, salivaricin A (SalA) that is inhibitory to a variety of other streptococci including *Streptococcus pyogenes* (group A streptococcus). Surprisingly, the structural gene for SalA was found also to be present in over 95% of *S. pyogenes* isolates examined. We proposed therefore that SalA may have an alternative function, possibly as a signaling factor helping to stabilize oral streptococcal populations. A group of 9 genes comprising at least one operon with *salA* as the first gene has now been identified within the genome of *S. pyogenes* M type 1 strain. The structure of the gene cluster resembles those of other class I lantibiotic gene clusters, and contains open reading frames coding for peptide modification and transport proteins, a histidine kinase and putative response regulator. It is likely therefore that SalA production is linked with the activity of a two component regulatory system. Preliminary evidence indicates that this system may sense the presence of extracellular SalA peptide produced by streptococci, up-regulating *salA* expression and modulating the expression of other genes, one or more of which may be necessary for immunity to lantibiotic inhibition of growth.

**P51 FlpA PROTEIN, A FIBRONECTIN-BINDING ENIGMA IN STREPTOCOCCI**

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Attachment of oral streptococci to the host is a primary stage in pathogenesis. Numerous bacteria have been shown to bind fibronectin, and several fibronectin-binding proteins have been characterised in streptococci. Most of these proteins contain amino acid sequence motifs typical of Gram-positive bacterial surface proteins, including a cell wall anchorage region. Several recently-identified streptococcal fibronectin-binding proteins lack these motifs although they seem to be surface located. One of these proteins is FBP54 from *Streptococcus pyogenes*, a homologue of which has been identified in *Streptococcus gordonii* and designated FlpA. The gene encoding FlpA is adjacent to, and downstream of, *cshA* that encodes a wall-anchored fibronectin-binding protein. We hypothesised that FlpA might act in concert with CshA to promote fibronectin-binding by *S. gordonii*. Isogenic mutants were constructed by allelic replacement or by insertion duplication in which FlpA production was disrupted. Mutants producing truncated FlpA ($FlpA^t$), or abrogated in FlpA ($FlpA^-$), were reduced somewhat in their abilities to adhere to human fibronectin compared with wild-type strain DL1 (Challis) cells. However, Western immunoblot analysis of cell wall protein extracts indicated that CshA might be over-expressed in the $FlpA^-$ mutant and thus compensate for loss of FlpA-associated function. A double mutant was therefore generated in which both *cshA* and *flpA* genes were inactivated. Unexpectedly, this mutant did not demonstrate a level of binding to fibronectin below that of the *cshA* mutant. The results do not resolve yet the function of FlpA in cell adhesion, but provide further evidence that multiple molecular interactions contribute to *S. gordonii* adhesion to fibronectin.

**P52 CHARACTERIsATION OF ADJACENT PROLINE IMINOPEPTIDASE AND ASPARTASE GENES FROM *EIKENELLA CORRODENS.***

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*Eikenella corrodens*, a Gram-negative, asaccharolytic, facultatively anaerobic rod, colonises the oral cavity and upper respiratory tract. There is conflicting evidence for the role of *E. corrodens* in periodontal disease; however, there is no doubt that it is an opportunistic human pathogen in extraoral infections such as abscesses, bacteraemias, osteomyelitis, meningitis and endocarditis, where it is normally part of a mixed microflora but may be the sole isolate. *E. corrodens* metabolises proline, glutamate, serine and glutamine resulting in high rates of nitrate production. Proline iminopeptidase (Pip) activity has been shown in bacterial cells and supernatants and may be crucial for cell survival through a role in energy attainment, modification of the local environment or protection against host immune mechanisms. We have identified ten Pip-expressing clones from an *E. corrodens* genomic library the smallest of which, pTLS11, was selected for further study. The gene expressing Pip was localised on a 3-kb *Eco*RI fragment subcloned into pUC18, and subsequent sequence analysis revealed an open reading frame 936 bp that had predicted amino acid sequence identity of 67% to the cloned Pip of *Neisseria gonorrhoeae*. The *E. corrodens* Pip has a predicted molecular mass of 35 kDa. 200 bp downstream from *pip* is an open reading frame of 1392 bp encoding a protein with predicted molecular mass of 50.3 kDa. Sequence comparisons revealed 89% identity to a putative aspartase inferred from the genome sequence of *N. meningitidis*. pTLS11 complemented an *E. coli* aspartase mutant confirming the enzymatic function of the protein. Interestingly, the *E. corrodens* aspartase is highly similar to the *Haemophilus influenzae* aspartase (75% identity), that was originally identified on the basis of ability to bind to plasminogen. At present, we do not know if the *E. corrodens* aspartase has a similar function. Southern hybridisations indicate that the genes encoding Pip and aspartase are each single copy, and are conserved within the genomes of a diverse panel of *E. corrodens* isolates.

**P53 A MODEL OF ESCHERICHIA COLI MAGNIFIED TWO MILLION TIMES - ITS USE IN THE TEACHING OF METABOLIC PATHWAYS AND THEIR INTEGRATION INTO THE ECONOMY OF THE BACTERIUM**

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At this magnification, molecules appear as discrete, tangible units. Metabolic pathways can therefore be constructed with three-dimensional metabolites, enzymes and coenzymes. The basal unit at 2 x $10^6$ is a 1 to 3mm coloured glass bead representing a fairly small molecule such as a monosaccharide or amino-acid. Some medium-sized molecules such as ATP, CoA, etc are built up from the small units. Large molecules such as DNA and proteins may be built up similarly and some examples are shown but these are more practically represented by scale models in other materials. Colour-coding of various kinds is used throughout.

Set out on the adjacent table is the part of the model so far made. An external medium containing glucose, lactose and mineral salts is represented and bounded by a length of cell wall. Glycolysis, citric acid cycle, pentose phosphate pathway, oxidative phosphorylation, syntheses of amino-acids and of mRNA are shown in relation to one another. Energy from the sugar substrates is shown channelled into synthesis of ATP and into a flagellar motor. Part of the nucleoid is shown, including a *lac* operon with its mRNA, tRNA, and amino-acyl t-RNA synthetases. Construction of an R plasmid is underway. The model, named the Millennium Bug, being made for the Millennium.

**P54 CAPSULE PRODUCTION BY *BURKHOLDERIA PSEUDOMALLEI* AND *BURKHOLDERIA MALLEI*, AND THE IDENTIFICATION OF A GENE INVOLVED IN CAPSULE BIOSYNTHESIS**

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*Burkholderia pseudomallei* occurs in tropical and subtropical climates and is the causative agent of melioidosis. Melioidosis used to be considered a relatively rare disease, but with improved diagnostic techniques and an increase in global travel, melioidosis is becoming more common and being isolated from more varied environments. *Burkholderia pseudomallei* is closely related to *Burkholderia mallei,* the aetiological agent of glanders. No genetic tests currently exist that differentiate between *Burkholderia mallei* and *Burkholderia pseudomallei*. Both pathogens can cause either chronic or acute infections associated with high fatality rates. Very little is known about the virulence factors produced by either of these organisms.

Capsular polysaccharide has been shown to be an important virulence determinant in many bacterial pathogens. Previous work on the capsule of *Burkholderia pseudomallei* has focused on its characterization and the immune response of the host to the capsule. Here we use molecular genetic techniques to investigate the genetics of capsule production of *Burkholderia pseudomallei*. Using transmission electron microscopy we have shown that *Burkholderia mallei* and some strains of *Burkholderia pseudomallei* posses a capsule and the capsular layer is thicker in *Burholderia mallei*. Subsequently we attempted to identify genes involved in the synthesis of capsular material. A gene fragment has been amplified by polymerase chain reaction and identified as having 77% identity to the *BexC* gene of *Haemophilius influenzae* The *BexC* gene is part of a locus thought to be involved in capsular export in *Haemophilius influenzae.*

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