CONTENTS

Monday 16 September 2002

0905 Regulation of toxin production

STAFFAN ARVIDSON
Microbiology and Tumorbiology Center, Karolinska Institute, Stockholm, Sweden

The production of *S. aureus* virulence determinants is controlled by several global regulators, including *agr*, *sarA*, *sarS*, *sarT*, *rot*, the alternative sigma factor, SigB, and the two-component systems *sae*, and *arl*. Several of these regulators modulate the expression of each other, forming very complex regulatory networks. Agr and sarA repress transcription of sarS and sarT. It is also well established that SarA is an activator of *agr* under certain growth conditions, and that transcription of *sarA* and *sarS* is partly SigB-dependent. Recent investigations have shown that SigB suppresses transcription of RNAIII of the agr locus.

Previous studies, have demonstrated that transcription of hla is regulated positively by *agr* and *sarA*, and negatively by sarS, sarT, and rot. However, these studies used derivatives of strain 8325-4, which is SigB deficient because of a mutation in *rsbU*. In a derivative of strains strain 8325-4 with an intact *rsbU* gene transcription of *hla* seemed to be suppressed due to elevated *sarA* expression. Using *S. aureus* strains with combinations of regulatory mutations we have tried to explain this differential effects of *sarA*, and to elucidate the regulatory network that controls alpha-haemolysin production. Different regulatory models will be discussed.

0945 Quorum sensing in the regulation of staphylococcal virulence and as a cause of interference

RICHARD P. NOVICK & JESSE S. WRIGHT
New York University School of Medicine, USA

*Ag*r is the central regulatory element governing the expression of staphylococcal accessory genes including many involved in pathogenicity. *Agr* is a two-component signal transduction module that is activated by a self-coded autoinducing peptide ligand (AIP) and is therefore a quorum-sensing (QS) system. *Agr* is conserved throughout the staphylococci but is highly variable in its specificity such that heterologous combinations of peptide and receptor generally inhibit agr expression. There are presently 4 different agr specificity groups in *S. aureus* and two or more in many other staphylococcal species. As these groupings are associated with specific biotypes, it is our view that intergroup interference could result in biological isolation and thus lead to speciation. We have performed extensive structure-function analyses of the AIPs, demonstrating the roles of critical residues, and have localized the site of receptor specificity to the distal half of the N-terminal receptor domain by exchanging segments of the receptor *(agrC)* gene. We have demonstrated AIP activity in vivo both for inhibition and activation of agr in trans and a present line of study is directed towards defining the conditions under which this interference could influence the course of an infection or survival of the organism.

1025 Characterisation and mutagenesis of the *luxS* locus in *Staphylococcus aureus*

N.C. DOHERTY[1], K. WINZER[1], M. HOLDEN[1] & P. WILLIAMS[1,2]
[1]Institute of Infections and Immunity, Queen's Medical Centre, University of Nottingham, Nottingham NG7 2UH, [2]The Pharmacy School, University of Nottingham, Nottingham NG7 2RD

In many micro-organisms, the expression of specific phenotypes is regulated by mechanisms that monitor bacterial cell density (known as quorum sensing) by the accumulation of signal molecules (autoinducers).

Quorum sensing has been shown to regulate various bacterial phenotypes, and specialised systems have been identified in Gram positive and Gram negative bacteria. The *luxS* system, however, appears to be present in both groups, as indicated by the presence of the *luxS* gene which catalyses the formation of the AI-2 autoinducer molecule. It has been suggested that this system represents a universal inter-species bacterial language. The *luxS* gene has been identified in *Staphylococcus aureus*, an important human pathogen responsible for numerous conditions ranging from food borne disease to systemic bacteraemia and endocarditis. Hospital acquired MRSA infection, recalcitrant to antimicrobial chemotherapy, is of particular concern.

The contribution of *luxS* to staphylococcal quorum sensing and pathogenesis is under active investigation in our group. The growth phase dependent expression of *luxS*, and accumulation of its cognate autoinducer molecule is described. Construction of reporter gene fusions has enabled the characterisation of the *luxS* promoter elements. Insertion-deletion mutants have been generated in various *S. aureus* backgrounds and characterisation of the *luxS*$^-$ phenotype is underway.

Assessing the contribution of the *luxS* system to staphylococcal pathogenesis will be beneficial in the elucidation of the role of *luxS* in the identification of novel therapeutic targets, essential in this era of multiple antibiotic resistance.

1115 *Staphylococcus* surface proteins

MAGNUS HÖÖK
Texas A&M University System Health Science Center, Houston, USA

Bacteria interact with their environment through the aid of surface proteins. These proteins can be associated with the bacteria in several different ways. Gram-positive bacteria, such as Staphylococci, contain a unique class of surface proteins that, through the aid of transamidase called sortase, become covalently linked to the peptidoglycan. In Staphylococci, these cell wall associated proteins include the MSCRAMMs CNA, ClfA, ClfB, FnbpA and FnbpB, as well as the Sdr Proteins. Amino acid and x-ray crystallography analysis show that these proteins represent structurally related family. Using the fibrinogen and collagen binding MSCRAMMs as examples, I will discuss the structural requirement and mechanism of MSCRAMM-ligand interaction and their role in virulence and pathogenesis.

1155 Iron acquisition by staphylococci

ALAN COCKAYNE
Institute of Infections and Immunity and The Pharmacy School, University of Nottingham

Although well characterised in many other bacteria, iron uptake systems in staphylococci are still poorly defined. Progress has been made recently in understanding siderophore-mediated iron acquisition in *S. aureus* though siderophore biosynthetic pathways have not been studied to date. Genome analysis indicates that *S. aureus* contains a large number of putative siderophore transporters but their true substrate specificity and role in iron uptake are still being determined. S. *aureus* also uses host iron binding molecules such as transferrin, lactoferrin and haemin as iron sources but the receptors involved and the mechanisms of iron removal from these compounds still require clarification. In common with iron uptake systems in other bacteria, regulation in response to iron availability is mediated by Fur but other metal-dependent regulators such as PerR also indirectly modulate expression of iron uptake systems in response to oxidative stress.

We are also studying iron uptake and its regulation in other staphylococci. Although antigenic and potentially functional homologues of *S. aureus* iron transporters have been identified in *S. epidermidis* and other CoNs, genome analysis indicates that *S. epidermidis* lacks many of the putative iron transporters found in

the *S. aureus* genomes. This finding may explain the observed differences in iron requirements of the two bacteria and could be a factor which contributes to differences in their virulence.

1235 *Staphylococcus aureus* clumping factor B (ClfB) mediates attachment to human epidermal cytokeratin, to desquamated nasal epithelial cells and to keratinocytes: implications for human host colonization

LOUISE O'BRIEN[1], E. WALSH[1], R. MASSEY[2], S. PEACOCK[2] & T.J. FOSTER[1]
[1]Moyne Institute of Preventive Medicine, Microbiology, Trinity College Dublin, College Green, Dublin 2, Ireland, [2]Nuffield Dept of Clinical Laboratory Sciences, University of Oxford, John Radcliffe Hospital, Headington, Oxford

Nasal carriage of *Staphylococcus aureus* is a major risk factor for the development of staphylococcal infection. Therefore, understanding the mechanisms by which *S. aureus* adheres to nasal epithelial cells *in vivo* may lead to alternative methods of decolonization that do not rely on sustained antimicrobial susceptibility. Here, we demonstrate for the first time that the *S. aureus* surface-expressed protein, clumping factor B (ClfB), promotes adherence to immobilised epidermal cytokeratins *in vitro*. By expressing a range of *S. aureus* adhesins on the surface of the heterologous host *Lactococcus lactis*, we demonstrated that adherence to epidermal cytokeratins was conferred by ClfB. Adherence of wild type *S. aureus* was inhibited by recombinant ClfB protein or anti-ClfB antibodies, and *S. aureus* mutants defective in ClfB adhered poorly to epidermal cytokeratins. In addition, ClfB also mediated attachment of both *S. aureus* cells and *L. lacitis* cells expressing ClfB to desquamated nasal epithelial cells and to a human keratinocyte cell line (G-HPV). ClfB appeared to interact specifically with cytokeratin 10, a molecule which was shown, by FACS analysis, to be expressed on the surface of both intact nasal epithelial cells and keratinocytes. We also confirmed that ClfB is transcribed by *S. aureus* in the human nares. We propose that ClfB is a major determinant in *S. aureus* nasal colonization.

1400 Stress resistance in *Staphylococcus aureus*

SIMON J. FOSTER
Dept of Molecular Biology and Biotechnology, University of Sheffield, Firth Court, Western Bank, Sheffield S10 2TN - Tel: 0114 2224411, E-mail: S.Foster@sheffield.ac.uk

Staphylococcus aureus is an extremely versatile pathogen being able to cause a wide range of infections and survive well outside the host. This adaptability requires stress resistance mechanisms, which allow it to be so successful. We are now beginning to understand how *S. aureus* is able to respond to environmental stresses and elicit co-ordinate regulation of overlapping regulons of genes. These integrated processes allow all aspects of the physiology of the organism to be integrated. Three regulators control oxidative stress resistance and iron and manganese homeostasis in a co-ordinated fashion. Although iron is an important element for *S. aureus* it has to be tightly managed to prevent intracellular oxidative stress. Thus iron acquisition systems and oxidative stress resistance components are co-regulated. Manganese provides a mechanism for the dismutaion of superoxide within cells independent of enzyme activities. Thus under manganese replete conditions, oxidative stress resistance components are down regulated. The complex integration of the regulatory circuits and how this effects cellular physiology will be discussed.

1440 Components involved in high-level methicillin resistance

BRIGITTE BERGER-BÄCHI
Medical Microbiology, University of Zürich, Switzerland

Methicillin resistance in staphylococci depends on the expression of the acquired penicillin-binding protein PBP2' which takes over the functions of the resident PBPs at otherwise inhibitory concentrations of beta-lactams. PBP2' needs additionally the transglycosylase activity of the resident PBP2 for this task. Typical for methicillin resistance is its heterogeneous expression. Resistance levels are strain-dependent and can vary from very low to extremely high values. Low-level resistant strains segregate in presence of methicillin a highly resistant subpopulation, which usually remains highly resistant and is the cause for beta-lactam therapy failure in MRSA infections. The general genetic mechanism and biochemical background leading to this high resistance, which often arises at a frequency well above the spontaneous mutation rate, is still not solved. The resistance levels strongly depend on the rate of peptidoglycan precursor formation and on the structure of the cell wall precursor. If these specific requirements are not met, methicillin resistance levels are lowered. Therefore, genes involved in cell wall biosynthesis and turnover, and regulators involved in their regulation, are of importance for optimal resistance formation.

1550 Vancomycin resistance in *Staphylococcus aureus*

K. HIRAMATSU
Juntendo University, Tokyo, Japan

Abstract not received

1630 Identification of drug targets

YINDUO JI
Dept of Anti-Infective Research, GlaxoSmithKline Pharmaceuticals, 1250 S. Collegeville Road, UP 1345, Collegeville PA 19426, USA

The emergence of antibiotic resistant organism and multi-drug resistance in bacterial pathogens underscores the need for development of novel classes of antibiotics. The availability of complete genome sequence data from important human pathogens provides us with a wealth fundamental information and allows us to define each gene and thus to better understand molecular pathogenesis. New techniques enable us to identify and characterize genes critical for bacterial growth and survival during infection. The combination of genome sequence data and new technologies makes it possible to systematically explore the function of each open reading frame (ORF) in a genome and identify potential novel molecular targets for drug discovery and vaccine development. This topic discusses genome-based technologies and their important applications to identification of anti-infective drug targets.

Tuesday 17 September 2002

0900 Staphylococcal biofilms – microbial factors essential for structured populations

MATHIAS HERRMANN
Institute of Microbiology and Hygiene, University of Saarland, Germany

A gradient-type of accumulative growth is an essential characteristic of staphylococcal pathogenesis: After attachment of a single cell to a biologic or artificial substrate, organisms start to proliferate forming clusters of cells which interact with surface structures and neighbouring cells, and multiple layers of microorganisms and exopolymeric substances ultimately form microbial populations termed ‚biofilms'. A number of staphylococcal factors necessary for biofilm formation has been identified such as the MSCRAMM type of bacterial adhesins promoting the initial bacterial interaction with extracellular matrix and mediating uptake of staphylococci by nonprofessional phagocytes as well as the glucosaminoglycan exopolymer identified as PIA. Additional mechanisms may contribute to physiologic population suprastructures *in vivo*. For example, a family of secreted extracellular molecules (including the recently described EMP protein) with broad binding spectrum to prokaryotic and eukaryotic cells and proteins has recently been characterized and possibly contributes to cluster stabilization at early steps of biofilm formation. A member of this family, the EAP/MAP protein, has recently been identified to possess additional functions as a potent antiinflammatory substance inhibiting neutrophil extravasation. In *S. epidermidis*, in addition to the carbohydrate PIA, a protein termed, accumulation

associated protein‘ AAP has been demonstrated to be required for intercell interaction and biofilm formation. Finally, novel aspects on potential biofilm-relevant factors originate from expression profiles of *in situ* biofilms using representational difference analyses and suggest a role of regulatory and intracellular molecules in structured staphyloccal populations. The respective role of these factors in the formation of structured staphylococcal surface populations will be discussed.

0940 *Staphylococcus epidermidis* virulence and pathogenesis

F. GÖTZ
Universitaet Tuebingen, Germany

Abstract not received

1020 Control of methicillin resistant *Staphylococcus aureus* biofilms

S.M. JONES, T.J. HUMPHREY, M.MORGAN & H.M. LAPPIN-SCOTT
Environmental Microbiology Research Group, University of Exeter, School of Biological Sciences, Exeter EX4 4PS

Methicillin resistant *Staphylococcus aureus* (MRSA) is a major cause of nosocomial infections and is notoriously difficult to eradicate from patients colonised with this bacterium. Biofilm formation by MRSA has not been investigated yet MRSA is implicated with infections of implant devices, including catheters and prosthetic heart valves. This study examined biofilm formation by MRSA using image analysis via a urinary catheter model employing artificial urine and silastic surfaces. MRSA was observed to attach rapidly to silastic (initial attachment at 2 hours, biofilm was 9 μm thick and covered 10% of the surface) and continued to increase in thickness and coverage of the surface (at 48 hours the biofilm was 69 μm thick and covered 60% of the surface).

In addition, the susceptibility of these biofilms to vancomycin and rifampicin, when administered at different time points, was also examined. Vancomycin and rifampicin, when administered at 24 hours, were both observed to slow the rate at which MRSA colonised the silastic surface. However, the MRSA biofilm was not eradicated from the surface by treatment with either antibiotic. Both vancomycin and rifampicin demonstrated a higher efficacy when administered at 8 hours. The MRSA biofilm was observed to rapidly decrease in both thickness and coverage. This study demonstrates that the antimicrobial treatments administered at earlier timepoints or even used prophlactically may be efficient at preventing implant related infections.

1110 Proinflammatory molecules

JOS VAN STRIJP
Eijkman Winkler Institute, Utrecht, The Netherlands

Invading bacteria are surrounded by formylated peptides and C5a. The human innate immune system has developed two molecules of the same receptor family to recognize these compounds as non-self. The C5aR and the formylated peptide receptor (FPR) are serpentine receptors that have a role in chemotaxis, priming and activation of neutrophils and monocytes. Staphylococci have found an answer to this innat recognition. Earlier we showed that these receptors are specifically downregulated by a activity in the supernatant fluid of growing staphylococci, while all other neutrophil receptors were not affected. This results in total inhibition of chemotaxis, priming and activation of the phagocytes. Purification of this activity and microsequencing revealed 35 amino acids of an unknown sequence so far. Using genomic sequencing we could finally reveal the whole gene, encoding a 14kd excreted protein with no homology to any known protein in the database. We call this protein CHemotaxis Inhibitory Protein of Staphylococci or CHIPS. Using homologues recombination, the CHIPS-knock-out was constructed. This KO lacks the gene, the protein and the biological activity. After transformation of the original CHIPS gene into this KO, biological activity could be restored. CHIPS acts directly on both the C5aR as well as the FPR. CHIPS is encoded on a pathogenicity Island in a Staphylococcal bacteriophage. CHIPS blocks neutrophil invasuion as well as neutrophil activation *in vitro* and *in vivo.*

1150 Invasion of host cells: investigating the Staphylococcus aureus genes involved in intracellular survival

KEN BAYLES & GREG BOHACH
Dept of Microbiology, Molecular Biology and Biochemistry, University of Idaho, Moscow, Idaho, 83844-3052, USA

The mechanism of persistence of *Staphylococcus aureus* in its host, despite adequate levels of humoral and mucosal antibodies, remains unknown. One mechanism used by some bacteria to evade humoral antibodies involves internalization of the bacteria into the host cell. We and others have demonstrated that *S. aureus* can internalize within and induce apoptosis in a variety of mammalian cells. Recent studies in my laboratory have focused primarily on the *S. aureus* genes involved in the survival of this bacterium within host cells. Three separate but complementary approaches have been used in this study. In the first approach, we are screening a Tn*917* insertion library for mutants that invade host cells but are unable to induce apoptosis. Several mutants have been identified and are currently being analyzed. The second approach utilizes microarray technology to examine global changes in *S. aureus* gene expression when the bacteria are internalized within a host cell. Preliminary experiments revealed that the cells experience an upshift in metabolic activity as indicated by increases in ribosomal protein expression and amino acid biosynthetic genes. We have recently developed an invasion protocol that allows us to synchronize the bacteria during internalization. Recovering bacterial RNA samples from a time-course experiment therefore should generate data that reflects changes in bacterial gene expression as the bacteria traverse from an extracellular environment into an intracellular niche. In the third approach, we have examined the synchronized RNA samples described above using real-time quantitative PCR assays to monitor changes in the expression of specific genes. The RNAIII gene was selected for this study since we had previously proposed that Agr-mediated quorum sensing (which is mediated by RNAIII) is important for the bacteria to escape the endosomal vacuole and to gain access to the cytoplasm. Results from these studies confirm that RNAIII transcription peaks approximately 4 hr post-infection. Whether this increase corresponds with escape from the endosome is currently being investigated.

1230 Components of *Staphylococcus aureus* expressed during human infection

S.R. CLARKE, K.J. BRUMMELL, M.R. POURMAND, M.J. HORSBURGH, P.W. McDOWELL & S.J. FOSTER
Dept of Molecular Biology and Biotechnology, University of Sheffield, Firth Court, Western Bank, Sheffield S10 2TN

The decreasing efficacy of antibiotic based therapies for staphylococcal infections, has led to the requirement of alternative strategies, such as a vaccine or therapeutic antibodies. Potential vaccine components must be expressed during human infection. Expression libraries of *Staphylococcus aureus* have been constructed, and human sera from infected patients used to identify *in vivo* expressed antigens. This has facilitated the creation of a database of *in vivo* expressed genes. We have isolated 355 clones which has yielded 115 loci. We have isolated clones of several known surface proteins, a novel haemolysin and several other novel surface proteins, some of which may be useful as vaccine components. These proteins have been overexpressed and are being used to determine the titre of their respective antibodies, in a large sample of infected individuals. The role and regulation of these proteins is being studied. One such protein, designated Ebh, is a c. 1.1 Mda surface protein, which binds human fibronectin.

1400 Lessons to be learned from models of staphylococcal infection

CURTIS G. GEMMELL
University of Glasgow

Although staphylococci represent one of the many components of the normal skin microflora of man, they rarely cause severe disease until such times as the host becomes immunocompromised. At such time the staphylococcus draws upon its wide arnamentarium of structural virulence factors and extracellular toxins in order to initiate infection. For the staphylococcus to produce such a multiplicity of factors it is suggestive that it is able to choose the most appropriate one(s) in particular situations in order to cause specific diseases. Such diseases in man range from skin infections such as impetigo contagiosa to septicaemia to endocarditis, pneumonia and osteomyelitis.

It is much more difficult to reproduce such manifestations of disease in experimental animals. Rodents are particularly resistant to staphylococcal infection and generally require high inocula together with some sort of irritant material in order to initiate infection. It has however been possible to understand more about the pathogenesis of staphylococcal infection with developments in molecular biology such as the generation of allele-replacement mutants of *S. aureus*, in which one or more genes which regulate expression of a virulence factor are absent, can be compared for their ability to induce infection. Since many of the virulence factors of *S. aureus* are controlled by *sar* and *agr*, changes in these control genes can also be shown to influence pathogenicity.

Changes in the host animal (such as gene knock-out mice) can influence susceptibility to staphylococcal infection; various host defence factors such as neutrophil killing, macrophage nitric oxide production or pro-inflammatory cytokine production play a significant part.

Coagulase-negative staphylococci are much less able to develop infections either in man or experimental animal unless given some form of selective advantage such as the presence of an indwelling catheter or prosthetic joint material. Surface adhesins have been shown to be important in the initiation of biofilm *in vivo*.

1440 Epidemiology of *S. aureus* carriage and disease: host vs bacterium

NICK DAY
Centre for Tropical Medicine, University of Oxford

Staphylococcus aureus is carried at any one time by approximately 30% of the population, yet severe disease caused by *S. aureus* infection is relatively uncommon; severe nosocomial disease occurs in around 1% of hospital inpatients, and the incidence of community-acquired disease is approximately 10/100,000. Determining the relative contributions of host, bacterial and environmental factors to the pathogenesis of severe disease requires the application of classical clinical epidemiology techniques alongside studies of bacterial population genetics, bacterial molecular pathogenesis, and human genetic susceptibility. As severe *S. aureus* disease is rare a case-control study design is particularly efficient, though cohort studies can be used in specific environments where disease is relatively common, such as in an ITU setting or in an IV drug user community.

Nasal carriage, single or multiple bacterial virulence determinants, acquired factors such as hospitalisation for concurrent disease and IV drug abuse, and host genetic polymorphisms in genes coding for the host immune response have all been shown to be important risk factors for severe *S. aureus* disease. The multifactorial nature of susceptibility to severe infection underlines the need for an integrated approach to the study of its aetiology.

1550 SCRAMM protein based therapeutics for staphylococci

J. PATTI
Inhibitex Inc, Alpharetta, Georgia, USA

Staphylococci are major human pathogens that cause a spectrum of clinical conditions that range from surgical site and catheter-related infections to pneumonia, endocarditis, ans sepsis. Due the continued increase in the number of community-acquired and nosocomial methicillin resistant *S. aureus* infections (MRSA), a need for novel alternative therapies exists. Microbial adhesion is recognized as the first crucial step in a series of events that leads to infection in humans. Staphylococci express MSCRAMM® proteins, a family of cell surface adhesins, that facilitate adherence and colonization by attaching to extracellular matrix components of host tissues or serum conditioned implanted biomaterials. Using state of the art genomic and proteomic tools, we have identified a number of human antibodies that target these MSCRAMM® proteins. The MSCRAMM® protein antibodies prevent staphylococci from attaching or recolonizing host tissues or implanted medical devices, as well as promote clearance by the immune system. We currently have three programs focused on developing MSCRAMM® protein human antibodies for the prevention and treatment of staphylococcal infections. Veronate™, a human polyclonal immunoglobulin containing elevated levels of antibodies to both *S. aureus* and coagulase-negative staphylococci MSCRAMM® proteins, is being developed for the prevention of infections in very low birth weight infants. Veronate™ is currently in a Phase I/II clinical trial. Aurexis™, the second product in the pipeline is a humanized monoclonal antibody that specifically recognizes clumping factor (ClfA), a MSCRAMM® protein expressed by almost all strains of *S. aureus*. Aurexis™ will begin Phase I clinical trials in early 2003. Finally, we are developing a nosocomial vaccine that will prevent both *S. aureus* and coagulase-negative staphylococci infections. Data supporting all three approaches will be the topic of discussion.

1630 Staphylococcal genomics

STEVEN R. GILL
The Institute for Genomic Research, Rockville, MD, USA

The availability of multiple *Staphylococcus aureus* genome sequences has stimulated the development of new genomic approaches for investigating Staphylococcal pathogenicity and physiology. While these genomic approaches have identified additional virulence factors and possible regulatory mechanisms, a more complete understanding of *S. aureus* virulence will be achieved by comparison with its 'avirulent' relative, *Staphylococcus epidermidis*. To this end, we have determined the complete genome sequence of *S. epidermidis* strain RP62A. While our initial comparative analysis has revealed many similarities between the *S. aureus* and *S. epidermidis* genomes, we have also identified several features unique to each species. With the exception of a ~500 bp region near the origin of replication, the *S. aureus* and *S. epidermidis* genomes demonstrate synteny along their entire length. Of the 2723 *S. aureus* ORFs, 2091 are shared with *S. epidermidis* and 632 are unique. Of the 2586 *S. epidermidis* ORFs, 2067 are common with *S. aureus* and 519 are unique. While *S. epidermidis* is lacking the numerous toxins, hemolysins and degradative enzymes encoded by *S. aureus*, many of the regulatory elements, such as *agr*, *sar*, and several two component regulatory systems are conserved. Two of the most intriguing regions in the *S. epidermidis* genome are a three gene operon (*cap*A,B,C) which potentially encodes a polyglutamic acid capsule similar to that found in *Bacillus anthracis* and two genes (*ppx* and *ppk*) encoding a putative polyphosphate kinase involved in energy storage. An overview of these findings and recent comparative analyses will be presented.

Wednesday 18 September 2002

0900 Biological function of the extracellular fibrinogen binding protein from *S. aureus*

JAN-INGMAR FLOCK, MARCO PALMA & OONAGH SHANNON
Dept of Microbiology, Pathology and Immunology, Karolinska Institutet, Huddinge University Hospital, F82, S-141 86 Stockholm, Sweden

Extracellular fibrinogen binding protein (Efb) from *S. aureus* (15.6 kD) contains two repeat regions at the N-terminus which are homologous to the repeats in coagulase. Efb competitively inhibits coagulase. In addition to the repeat regions, Efb binds to fibrinogen also at a site in its C-terminus. The double binding of Efb leads to formation of a precipitate when added to Fibrinogen at a 1:1 ratio. The major binding site on fibrinogen for Efb is located on the alpha-chain. Efb can also bind to activated platelets, independently of fibrinogen and blocks of platelet aggregation. A *S. aureus* knock-out mutant was constructed and found to have similar adherence properties for fibrinogen, but a reduced virulence in a *S. aureus* wound infection model in the rat. The Efb proficient wt strain led to slower healing rate. Mice were vaccinated with Efb and subjected to a mastitis infection with *S. aureus*. Bacterial recovery from the mammary glands was significantly lower for vaccinated animals. They also had fewer necrotic lesions and less histopathological signs of infection as compared with control animals.

Antibody levels against Efb in patients with acute *S. aureus* infections were significantly lower than in healthy individuals. However, during the course of infection anti Efb levels increased. Antibodies raised in the rat or sheep can block binding between Efb and fibrinogen.

Efb thus has a multifactorial effect on blood clotting in that it 1) consumes fibrinogen locally, 2) competes with coagulase and 3) inhibits platelet function. Efb is a virulence factor and it is conceivable that antibodies against it could have a clinical role or that it could be used as vaccine component against certain *S. aureus* infections.

0940 Diversity of fibronectin binding proteins amongst clinical isolates of *Staphylococcus aureus*

S.J. PEACOCK
Nuffield Dept of Clinical Laboratory Sciences, University of Oxford, John Radcliffe Hospital, Oxford OX3 9DU

Bacterial adherence is likely to play a central role in *Staphylococcus aureus* transmission, in the maintenance of carriage and in disease pathogenesis. This important human pathogen expresses a range of cell wall-associated surface adhesins which are thought to be important in such host-bacterial interactions. This talk will focus on fibronectin binding proteins (FnBP's), which are involved in the pathogenesis of experimental endocarditis and intravenous device-related infection, and in the process of uptake by a range of cell lines. Evaluation of a large population of natural *S. aureus* isolates has demonstrated diversity at both the genotypic and phenotypic levels. Two *S. aureus* FnBP's (A and B) encoded by *fnbA* and *fnbB* respectively, are present in three quarters of isolates, while the remainder are positive for *fnbA* alone. This difference is not reflected in the ability to adhere to fibronectin, and the presence of either FnBP is sufficient to confer uptake by endothelial cells *in vitro*. However, two *fnb* genes have been found to be more common in invasive versus carriage isolates, possible reasons for which will be discussed. Examining a single *S. aureus* virulence determinant in isolation is likely to represent an over-simplification of the situation *in vivo*, since most cases of severe *S. aureus* disease cannot be explained by the action of a single virulence determinant. The presence of *fnb* genes has also therefore been examined in the wider context, by defining their presence as one of 33 putative virulence determinants in isolates associated with human carriage and invasive disease. The presence of *fnb* contributed independently to virulence, as did six other bacterial factors. No single factor predominated as the major predictor of virulence, their effect appearing to be cumulative. Thus, *S. aureus* FnBP's appear to be one of a number of factors which act in combination during the infective process.

1020 A genetic approach to structure/function analysis of enterococcal aggregation substance

G. DUNNY, C. WATERS, C. WELLS & P. SCHLIEVERT
University of Minnesota Medical School, Minneapolis, MN 55455, USA

Aggregation Substances (ASs) are a group of closely related surface proteins encoded by pheromone-inducible conjugative plasmids of *Enterococcus faecalis*. Our group has analyzed the Asc10 AS protein encoded by the prgB gene of the plasmid pCF10. In addition to mediating the formation of mating aggregates by binding to the lipoteichoic acid-containing enterococcal binding substance (EBS) receptor on the recipient cell wall, AS proteins have been shown to affect several traits which increase the virulence of their host strains. We have employed random insertional mutagenesis, as well as site-directed mutagenesis of the Asc10 coding sequence in order to determine whether separate functional domains exist for the protein. In-frame insertions of a 31 amino-acid peptide using the TnlacZ-in transposon system have identified a functional bacterial aggregation domain in the N-terminal half of the protein.

Surprisingly, many of the adherence and eukayotic cell internaliztion functions mediated by Asc10 also involve the aggregation domain. In contrast, mutations of predicted eukaryotic cell adherence sequences in other regions of the protein have not shown any effects in the assays tested thus far. The results have led to the proposal of a model involving Asc10-promoted host cell interactions mediated by complexes of Asc10 and lipoteichoic acid on the surface of plasmid-containing cells. The insertion mutations have also been used to identify a target site for proteolytic cleavage of Asc10 by an enterococcal gelatinase protease.

1330 Fibronectin-binding of staphylococci and streptococci - a structural perspective

ULRICH SCHWARZ-LINEK[1], EWA PILKA[1], JOHN BRIGGS[1], HUW JENKINS, MAGNUS HÖÖK[2], IAIN D. CAMPBELL[1] & JENNIFER R. POTTS[1]
[1]Dept of Biochemistry, University of Oxford, South Parks Road, Oxford OX1 3QU, [2]Centre for Extracellular Matrix Biology, Institute of Biosciences and Technology, Texas A&M University, Houston, Texas, 77030, USA

Many pathogenic Gram-positive bacteria express cell surface proteins that bind to extracellular matrix (ECM) proteins such as fibronectin and collagen; these adhesins may play a significant role in bacterial pathogenesis. Most fibronectin-binding bacterial adhesins have a primary ligand-binding region containing 3-6 repeats of 40-50 amino acids. Fibronectin-binding proteins from *Staphylococcus aureus*, *Streptococcus pyogenes* and *Streptococcus dysgalactiae*, have been shown to bind to the N-terminal region of fibronectin, which contains five F1 modules ($^{1-5}$F1). Previously, the main binding sites for the D3 peptide from *S. aureus* FnBPA and the B3 peptide from FnbB of *S. dysgalactiae* have been located to the ^{4}F1^{5}F1 and ^{1}F1^{2}F1 module pairs of fibronectin, respectively. The structures of these two fibronectin F1 module pairs have previously been determined in this laboratory.

We have used isothermal titration calorimetry (ITC) and nuclear magnetic resonance (NMR) spectroscopy to study the interactions of peptides from *S. aureus*, *S. dysgalactiae* and *S. pyogenes* fibronectin-binding proteins with F1 module pairs from

the N-terminal domain of human fibronectin. Bacterial protein and fibronectin residues involved in the interactions have been identified and the three-dimensional structure of a complex has been determined.

1410 Don't smoke while feeding the Birds: Interaction of *Mycobacteria* with the respiratory mucosa

ANDREW M. MIDDLETON & ROBERT WILSON
Host Defence Unit and Microbiology Dept, Royal Brompton Hospital, Sydney Street, London SW3 6NP

We have investigated the colonisation and invasion by *Mycobacterium tuberculosis* (MTB) and *Mycobacterium avium* complex (MAC) of the mucosa of a human respiratory tissue organ culture model with an air interface by scanning electron microscopy (SEM). Extracellular matrix (ECM) and mucus were found to be the main sites of initial adherence for both species. Adherence to ECM was inhibited by pre-incubation of bacteria with fibronectin and by pre-incubation of tissue with mycobacterial fibronectin attachment protein (FAP) and antigen 85B protein (85B). Immunogold labelling demonstrated fibronectin in areas of epithelial damage, but FAP receptors were only exposed in ECM. Fibronectin did not inhibit adherence to mucus and fibronectin binding sites were not identified in mucus. In experiments carried out over 14 days MTB and MAC significantly increased in numbers cultured from macerated tissue. However, whereas MAC numbers also significantly increased on the mucosal surface, MTB was rarely seen. Pre-incubation of tissue with FAP and 85B significantly reduced MAC seen on, and recovered from tissue, but had no effect on MTB, compared to controls. Adherence to fibronectin may play an important role in mucosal infection by MAC, but, despite sharing similar adherence characteristics to MAC, MTB's ability to invade the mucosa is independent of its interaction with ECM.

1450 Collagen binding and sensing by streptococci

HOWARD F. JENKINSON, CATHERINE HEDDLE, MICAELA GAL, ANGELA H. NOBBS, NICHOLAS S. JAKUBOVICS & DAVID DYMOCK
Dept of Oral & Dental Science, University of Bristol, Lower Maudlin Street, Bristol BS1 2LY

Streptococci, enterococci and staphylococci are capable of adhering to collagenous substrates through the activities of cell surface anchored polypeptide adhesins. These interactions enable bacterial colonization and may promote penetration and destruction of host collagenous tissues. While the structure and properties of a major collagen-binding protein Cna in *S. aureus* are reasonably well characterized, much less is known about how streptococci interact with collagens. Members of the mutans and mitis group oral streptococci adhere to collagen type I, a major component of human dentin, and this facilitates bacterial invasion of tooth root and pulpal tissues. Adhesion to collagen type I is mediated in part by the antigen I/II (AgI/II) family of cell wall-anchored proteins that are expressed by most species of oral streptococci. In *Streptococcus gordonii*, which carries two AgI/II family proteins designated SspA and SspB, expression of the *sspA* gene is specifically up-regulated in response to collagen type I, collagen type IV, or to collagen fragments. In addition, collagen type I becomes rapidly bound to the cell surfaces of streptococci in suspension and this occurs independently of *sspA* or *sspB* gene expression. Thus collagen adhesion, fluid phase binding, and sensing may be mediated through different mechanisms. This multi-component, collagen type I recognition-response system in oral streptococci may be intimately linked with the ability of these bacteria to translocate from the oral cavity into deeper tissues.

1600 Regulation and biological functions of group A streptococcal fibronectin- and collagen- binding proteins

A. PODBIELSKI
Dept of Med Microbiology & Hosp. Hygiene, University Hospital, Rostock, Germany

Group A streptococci (GAS = *Streptococcus pyogenes*) express up to nine types of fibronectin-binding and one type of collagen-binding proteins (= MSCRAMMs) on their surface. For some of the proteins, the matrix protein binding-site has been mapped to specific modules, which may be present in the proteins together with other modules that display different functions. For few of the proteins, an involvement in eukaryotic cell adhesion and internalization as well as some importance in animal model infections has been demonstrated. The responsible genes for the fibronectin-binding proteins F1 and F2 and the collagen-binding Cpa were found to be located within a genomic region that exhibits features of a pathogenicity island. Interestingly, this island exists in several similar versions, all of which harbor version-specific genes for a signal peptidase and a sortase as well as a gene for a global, predominantly negative regulator of the RALP-family. The regulator appears to be responsible for an expressional balance between virulence factors needed for cell and tissue invasion versus other factors necessary for prolonged persistence inside of eukaryotic cells. The strain-specific combination of MSCRAMM genes, regulators, cell envelope transporters and anchoring mechanisms could be responsible for the high inter-strain variability of GAS virulence and tissue tropisms.

1640 Mechanisms, dynamics and consequences of streptococcal adherence and invasion

SUSANNE R. TALAY, KATRIN DINKLA, NELE PETERMANN, WOUTER T.M. JANSEN, MANFRED ROHDE & GUSHARAN S. CHHATWAL
Dept of Microbial Pathogenesis and Vaccine Research, GBF-National Research Centre for Biotechnology, 38124 Braunschweig, Germany

Interaction with human matrix proteins offers various possibilities to pathogenic bacteria. It is well established that binding of *S. pyogenes* to matrix components such as fibronectin, collagen and laminin is the initial step for subsequent infection. Among a variety of fibronectin binding proteins, SfbI protein is one of the most potent adhesins and invasins of *S. pyogenes*. Exhibiting a modular structure, it co-operatively interacts with human fibronectin via two distinct binding domains. Both domains trigger adherence and invasion into host cells via fibronectin-mediated integrin clustering. Host cell caveolae are recruited to the site of bacterial entry and govern the uptake process into caveosomes that is accompanied by a series of cytoskeletal rearrangements. Residing in this organelle, streptococci may persist unaffected by host defence mechanisms. A novel mechanism by which extra-cellular *S. pyogenes* colonise and impair phagocytosis is aggregation of basement membrane collagen on their surface. Two distinct bacterial factors were identified to mediate collagen aggregation, a process that leads to formation of large streptococcal aggregates and matrix deposition on the streptococcal surface. In a flow cytometry based phagocytosis assay, binding of matrix- coated bacteria to human polymorph nuclear cells was strongly impaired compared to non-coated streptococci, even in the presence of opsonising antibodies. Beside the anti-phagocytic potential, both collagen binding factors mediate tight binding to collagen type I fibres, reflecting the potential of *S. pyogenes* to adhere to non-cellular human matrix.

POSTERS:

CCS 01 Effect of human salivary IgA on the adherence of *Candida albicans* to human epithelial cells

A.R. HOLMES, M. BANDARA, P. VAN DER WIELEN & R.D. CANNON
Dept of Oral Sciences and Orthodontics, University of Otago, PO Box 647, Dunedin, New Zealand

Adhesion of the yeast *Candida albicans* to epithelial cells is an initial event in the colonisation of mucosal surfaces. In a model of *C. albicans* adherence to oral epithelium, pooled human whole saliva promoted adherence of *C. albicans* ATCC 10261 yeast cells to monolayers of three cultured human epithelial cell lines (A549, HEp-2 and HET-1A) in a dose-dependent manner. Biotinylation of salivary components demonstrated that at least four salivary polypeptides, including IgA, were bound by *C. albicans* yeast cells under assay conditions. For individual saliva samples, there was a negative correlation ($r = 0.68$ $P < 0.005$) between adherence and specific IgA titres against whole cells of *C. albicans*. The adhesion-inhibitory effect of specific anti-*C. albicans* IgA was reversed by depletion of IgA from saliva by jacalin affinity chromatography. We conclude that specific IgA in saliva inhibits *C. albicans* adherence to host cells, but that other salivary components, bound to the yeast cells, overcome this effect and promote adherence.

CCS 02 Identification of a matrix binding protein from *Actinobacillus actinomycetemcomitans*

LINDSAY SHARP, BRIAN HENDERSON, HANNAH DESILVA, RACHEL WILLIAMS & SEAN NAIR
Cellular Microbiology Research Group, Eastman Dental Institute, University College London, 256 Gray's Inn Road, London WC1X 8LD

Actinobacillus actinomycetemcomitans is an important dental pathogen shown to be a major causative agent of localised aggressive periodontitis. For bacterial infection to occur colonisation of the host tissue is critical and in most cases involves adherence of the bacteria to epithelial cells and the extracellular matrix. It has previously been shown that *A. actinomycetemcomitans* is able to bind to collagen and fibronectin. However, the bacterial receptors for these host proteins have not been identified. The aim of this study was to identify, using shot- gun phage display cloning, the proteins of *A. actinomycetemcomitans* that allow this bacterium to bind to the extracellular matrix. Phage display involves the expression of peptides, in this case from *A. actinomycetemcomitans*, fused with one of the coat proteins on the surface of the phage. These libraries are screened (panned) for recombinant phage which bind to immobilised target molecules. An *A. actinomycetemcomitans* phage display library was constructed and screened for clones that bound to immobilised fibronectin. After three rounds of screening, analysis of the DNA inserts of the fibronectin bound phage revealed that they all contained overlapping sequences encoding a novel 13.5 kDa outer membrane protein. Further analysis of one these recombinant phage has revealed that it also binds to collagen and plasminogen but not fibrinogen or BSA. This gene may contribute to the virulence of *A. actinomycetemcomitans* and may be a possible target for prophylactic treatment of infections caused by this bacterium.

CCS 03 Identification of a fibronectin-binding protein from *Staphylococcus epidermidis*

RACHEL J. WILLIAMS, BRIAN HENDERSON & SEAN P. NAIR
Cellular Microbiology Research Group, Eastman Dental Institute, University College London, 256 Gray's Inn Road, London WC1X 8LD

Staphylococcus epidermidis is an opportunistic pathogen which is a major cause of foreign body infections. Whilst its adhesion to biomaterial surfaces is thought to primarily occur through hydrophobic interactions, it has been shown that *S. epidermidis* can bind to implants that have been coated with host matrix components such as fibronectin. Using a *S. epidermidis* phage display library, we have selected for recombinant phage that bind to fibronectin. Further examination of the binding activity of one of the phage, pSE109FN, has revealed that it also binds to plasminogen, hyaluronic acid and heparin although to a lesser extent than to fibronectin. In contrast, phage pSE109FN does not bind to collagen, fibrinogen, laminin, vitronectin or mucin.

Analysis of the phagemid clones which bound to fibronectin revealed that most contained overlapping *S. epidermidis* DNA inserts covering approximately 1.85kb of DNA, with 364bp common to all. These inserts mapped to a region coding for a putative 34kb open reading frame within the TIGR *S. epidermidis* genome sequence database. We have termed this open reading frame *embp*. RT-PCR analysis has shown that *embp* is expressed constitutively. The Empb protein may be involved in the adhesion of *S. epidermidis* to fibronectin-coated implants and thereby contribute to its pathogenicity.

CCS 04 Multiple fibronectin binding proteins of *Streptococcus gordonii*

CLAIRE B. GILROY[1,2], RODERICK McNAB[3] & SEAN P. NAIR[1]
[1]Cellular Microbiology Research Group, [2]Dept of Microbiology, Eastman Dental Institute, University College London, 256 Gray's Inn Road, London WC1X 8LD, [3]GlaxoSmithKline, St George's Avenue, Weybridge, Surrey KT13 0DE

The oral bacterium *Streptococcus gordonii* DL1 possesses surface proteins CshA and CshB which have a number of properties including, the ability to bind to fibronectin (Fn). Inactivation of the genes that encode these proteins, generated mutants that were markedly reduced in this property. However, the mutants still bound to Fn suggesting the presence of other Fn binding proteins. Antigen I/II-like proteins have been shown to bind to Fn in a number of other oral streptococcal species. Therefore, we have investigated the possibility that the *S. gordonii* homologues of antigen I/II proteins, SspA and SspB, may also bind to Fn. Isogenic mutants of *S. gordonii* DL1, containing inactivations in the *cshA* and *cshB* genes or in the *sspA* and *sspB* genes showed reduced binding to Fn. Additionally, in these mutants, Fn binding was further reduced by preincubation with collagen. These findings suggest that SspA and/or SspB proteins bind to Fn. A third isogenic mutant, containing inactivations in the *cshA*, *cshB, sspA* and *sspB* genes also showed reduced Fn binding when preincubated with collagen. This suggests *S. gordonii* possesses at least one other Fn binding protein which is sensitive to collagen inhibition.

CCS 05 Distinct pathways are employed by osteoblasts to internalise *Staphylococcus aureus* and *Staphylococcus epidermidis*

HESHAM KHALIL, RACHEL J. WILLIAMS, SAJEDA MEGHJI, BRIAN HENDERSON & SEAN P. NAIR
Cellular Microbiology Research Group, Eastman Dental Institute, University College London, 256 Gray's Inn Road, London WC1X 8LD

Staphylococcus epidermidis is a major cause of bone infections associated with prosthetic devices. It has previously been shown that *Staphylococcus aureus*, another bone pathogen, is internalised by osteoblasts in a receptor-mediated process that depends on the bacterium's ability to bind to fibronectin. In this study we set out to determine if osteoblasts could also internalise *Staphylococcus epidermidis* and, if so, whether the host cell pathways employed were the same for these two staphylococcal species. Examination of six strains of *S. epidermidis* revealed significant differences in the ability of this organism to be internalised by osteoblasts. A recombinant protein encompassing the D1-D4 repeat region of the *S. aureus* fibronectin-binding protein B completely inhibited internalisation of *S. aureus* but failed to block internalisation of *S. epidermidis*. Similarly, a blocking antibody to the $\alpha5\beta1$ integrin inhibited internalisation of *S. aureus* but had no effect on the uptake of *S. epidermidis* by osteoblasts. Studies utilising a panel of inhibitors of host cell

endocytic pathways confirmed that osteoblasts employ distinct pathways in the uptake of *S. aureus* and *S. epidermidis*. The fact that *S. epidermidis* is internalised by osteoblasts may account for the difficulty in treating bone infections of this organism.

CCS 06 Surface-associated proteins of *Enterococcus faecalis*

K.A. HOMER, J.C. WILKINS, S. RAO & D. BEIGHTON
Dept of Oral Microbiology, Guy's King's & St. Thomas' Dental Institute, King's College London, Denmark Hill, London SE5 9RW

Enterococcus faecalis is a Gram-positive pathogen associated with a range of diseases including endocarditis, septicaemia and urinary tract infections. Some virulence determinants have been described for *E. faecalis*, including adhesins localised at the cell surface. These, and other, surface-associated proteins are likely to play a role in colonisation and disease progression and may be candidates for vaccine development. Surface-associated proteins were extracted from *E. faecalis* by treatment with detergent and these were resolved using two-dimensional gel electrophoresis. Protein identification was achieved by proteolytic digestion, analysis of peptides by mass spectrometry (MALDI-ToF-MS and LC-MS/MS) and interrogation of the partially completed genomic sequence database for *E. faecalis* (TIGR). Of 40 polypeptides excised from gels, more than 20 distinct proteins were identified, including enolase and glyceraldehyde-3-phosphate dehydrogenase. Amongst these were "anchorless" proteins that have no known mechanism for localisation to cell surface (e.g. trigger factor). Quantitative analysis of individual surface proteins demonstrated that expression was altered by environmental conditions including availability of nutrient source (e.g. presence of a high-mannose-type glycoprotein). These data describe the expression of both known and novel surface-associated proteins by *E. faecalis*. The biological function of the novel proteins at this cellular location has now to be ascertained.

CCS 07 The role of sortases in experimental *Staphylococcus aureus* infection

ING-MARIE JONSSON[1], SARKIS K. MAZMANIAN[2], OLAF SCHNEEWIND[2], TOMAS BREMELL[1] & ANDRZEJ TARKOWSKI[1]
[1]Dept of Rheumatology, University of Göteborg, Sweden, [2]Committee of Microbiology, Dept of Molecular Genetics and Cell Biology, University of Chicago, Chicago IL 60637, USA

Staphylococcal adhesion is mediated by surface proteins that are anchored to the cell wall by two subsets of sortase (SrtA and SrtB), respectively. SrtA is a constitutively expressed enzyme responsible for anchoring of all LPXTG-containing surface proteins, whereas SrtB carries out the specific iron-regulated cell wall sorting of a NPQTN signal. Our aim was to examine the role of these sortase subsets using a murine septic arthritis model. NMRI mice were inoculated i.v. with *S. aureus* strain Newman or any of its sortase single or double mutant strains. Inoculation with sortase mutants resulted in significantly better survival and significantly lower weight loss compared with the parental strain. Mice inoculated with the SrtA mutant did not express severe arthritis, while arthritis of mice inoculated with the SrtB mutant did not differ from wild-type inoculated mice. In conclusion, both subsets of sortase are virulence determinants of infection, but only sortase A is important in establishment of septic arthritis.

CCS 08 Clumping factor A protects *Staphylococcus aureus* against phagocytosis by macrophages

NIKLAS PALMQVIST, ANDREJ TARKOWSKI & ELISABET JOSEFSSON
Dept of Rheumatology, Göteborg University, Gothenburg, Sweden

Introduction Clumping factor A (ClfA), a fibrinogen-binding protein anchored to the *Staphylococcus aureus* cell wall, is of great importance for the induction and progression of septic arthritis. The mechanism(s) by which ClfA contributes to the disease is unknown. The objective of this study was to investigate if ClfA-expression affects the capacity of host to phagocytize the bacterium.
Method Peritoneal macrophages isolated from healthy mice were left to adhere to culture wells at 37°C. The macrophages were then co-incubated for 50 min with *S. aureus*-strains Newman (wild-type), DU5876 (a ClfA-negative mutant strain), DU5898 (DU5876 complemented with the clfA gene) and DU5899 (DU5876 complemented with an empty vector). After removal of viable extracellular bacteria, the macrophages were lysed and the number of viable, ingested bacteria was determined by culturing the lysate on blood agar plates.
Results Peritoneal macrophages phagocytized 2-3-fold higher numbers of the ClfA-negative *S. aureus*-strain DU5876 compared to the wild-type strain (P=0.002, N=14). Also, the complemented pair of strains showed the same pattern (P=0.008, N=8).
Conclusion The results suggest that expression of ClfA protects *S. aureus* from phagocytosis by murine macrophages.

CCS 09 Bap, a protein involved in biofilm formation in *S. aureus*, is carried in a mobile genetic element present in different coagulase-negative pathogenic species of *Staphylococcus*

MªA. TORMO, C. ÚBEDA, C. CUCARELLA, P. TROTONDA, B. AMORENA, I. LASA & J.R. PENADÉS
Dept of Chemistry, Biochemistry and Molecular Biology, Cardenal Herrera-CEU University, 46113, Moncada, Valencia, Spain - jpenades@uch.ceu.es

Recently, we have identified and characterised a surface protein of *S. aureus*, Bap (biofilm-associated protein), involved in biofilm formation (Cucarella *et al*, J Bacteriol 183:2888, 2001). By Southern-blot and PCR-analysis, we have identified and cloned the *bap* gene form several bovine coagulase-negative staphylococci species, including *S. epidermidis, S. chromogenes*, *S. xylosus* and *S. simulans*. Southern-blot analysis with an *ica*-specific gene probe revealed the absence of the classic biofilm-related *icaADBC* operon in all of these coagulase-negative staphylococci. However, as in the *bap*-positive *S. aureus* strains, all coagulase-negative staphylococcal isolates harbouring the *bap* gene were highly adherent and strong biofilm producers, suggesting that Bap is capable to form biofilm in a PIA-independent manner. Sequence comparison of these genes (including the *S. aureus bap* gene) revealed high homology between them. Moreover, analysis of the flanking region containing *bap* suggests that the *bap* gene is included in a mobile genetic element, as previously described for *S. aureus*. The extended presence of *bap*-like genes in bovine pathogenic species of S*taphylococcus* might suggest that Bap-like proteins are important determining bacterial pathogenicity.

CCS 10 Sequence and characterization of a Csh-like adhesive protein from *Streptococcus sanguis*

I. GHOSH[1], C. BLACK[1], D. ELLIOT[1], P.S. HANDLEY[2], M. WILSON[1] & R. McNAB[1]
[1]Eastman Dental Institute, University College London, [2]Dept of Biological Sciences, University of Manchester

CshA is the structural and functional component of *Streptococcus gordonii* DL1-Challis fibrils, and mediates attachment of cells to fibronectin and to other oral species. Previous work has determined that the amino-terminal non-repetitive region of CshA carries the adhesive domain(s) for these interactions. CshB, an antigenically similar protein also expressed by *S. gordonii*, is also required for oral colonization. Despite being widely distributed among mitis-group oral streptococci, sequence data are available only for CshA of *S. gordonii*. The *S. gordonii* CshA antiserum was used to screen a λZAPII library of *Streptococcus sanguis* NCTC10904 genomic DNA, and several clones were isolated and sequenced. In parallel, sequence information was obtained for CshB of *S. gordonii* from previously isolated λZAPII clones. The CshA and CshB sequences of *S. gordonii* were more similar to each other (63% identity, 74% similarity over 863 amino acid residues) than to the Csh-like sequence of *S.*

sanguis, which exhibited only 48% identity and 62% similarity to CshA of *S. gordonii*. Nevertheless, a ClustalW alignment of all three sequences revealed 5 regions of significant sequence identity. *S. sanguis* adheres to immobilized fibronectin and, like *S. gordonii* DL1, undergoes co-aggregation reactions with *Actinomyces naeslundii* PK606 and *Streptococcus oralis* 34. The mapping of sequence homology between CshA of *S. gordonii* and *S. sanguis*, and of sequence divergence between CshA and non-adhesive CshB, has revealed potential functional domains of CshA proteins from mitis group streptococci.

CCS 11 The clumping factor ClfA of *Staphylococcus aureus* inhibits opsonophagocytosis by human polymorphonuclear leukocytes

JUDY HIGGINS[1], JOS VAN STRIJP[2] & TIMOTHY J. FOSTER[1]
[1]Dept of Microbiology, Trinity College, Dublin 2, Ireland, [2]Eijkman Winkler Institute, University of Utrecht, Utrecht, The Netherlands

Phagocytosis by polymophonuclear leukocytes (PMNLs) is an important defence against invasive staphylococcal infections. The clumping factor ClfA is a surface protein that binds to the extreme C-terminus of the γ-chain of fibrinogen (Fg). ClfA is known to be a virulence factor in the rat model of endocarditis and in the murine model of septic arthritis. By studying phagocytosis of bacterial mutants we have shown that the presence of ClfA on the bacterial cell surface hinders the process of phagocytosis by human PMNLs in the presence of normal serum opsonins. Inhibition was dependent on Fg being present. ClfA was shown to act in concert with protein A, a surface protein that recognises the Fc region of IgG, and which was previously shown to inhibit opsonophagocytosis. Current studies are directed at analysing the requirement for Fg by using bacterial strains expressing a ClfA mutant defective in Fg binding.

CCS 12 SdrI, a fibronectin-binding protein of *Staphylococcus saprophyticus*

T. SAKINC & S. GATERMANN
Abteilung für Medizinische Mikrobiologie, Institut für Hygiene und Mikrobiologie, Universitätsstraße 150, D-44780 Bochum, Germany

Staphylococcus saprophyticus, an important cause of urinary tract infections in young woman, binds fibronectin, laminin and hemagglutinates sheep erythrocytes. It produces two major surface proteins, the *S. saprophyticus* surface-associated protein (Ssp) and an autolysin (Aas). Recently, a further cell-surface associated protein of *S. saprophyticus* (SdrI*)*, was cloned, that contains serine-aspartate-alanine repeats.

An isogenic SdrI knock out mutant was constructed, that showed a decreased binding to immobilised fibronectin compared with wild type *S. saprophyticus*.

Recombinant fragments of the protein were expressed and purified as a Histag fusion to analyse and localise the fibronectin binding domain.

SdrI is the first identified fibronectin-binding Sdr protein and the first surface protein of *S. saprophyticus* carrying the LPXTG motif. Therefore we propose that the SdrI Protein plays an important role during pathogenesis.

CCS 13 Characterization of the importance of the *Staphylococcus aureus* fibronectin-binding protein in adherence to platelets

CHRISTINE HEILMANN[1], SILKE ENGELBERT[2], BEATE KEHREL[2], MATHIAS HERRMANN[3] & GEORG PETERS[1]
[1]Institute of Medical Microbiology, D-48149 Münster, Germany, [2]Dept of Anaesthesiology and Surgical Intensive Care Medicine, D-48149 Münster, Germany, [3]Institute of Microbiology and Hygiene, Division of Bacteriology and Hygiene, D-66421 Homburg/Saar, Germany

The adherence of microorganisms to platelets previously immobilized on the subendocardium in "nonbacterial thrombotic endocarditis" is considered an important pathogenic step in *Staphylococcus aureus* endocarditis. Recently, we reported that the fibrinogen (Fg)-binding domains in the coagulase and Efb are involved in binding to platelets probably via Fg as a bridging molecule. In order to identify and characterize further bacterial factors involved in the adherence to platelets, we generated a new phage display library using the improved phagemid vector pG8SAET. The library was affinity panned against gel-filtered, immobilized platelets. After a third panning against immobilized platelets, a significant increase in the number of eluted phagemid particles was observed: the increase was 20-fold when 1 mM $CaCl_2$ was added in the pannings and 400-fold when no $CaCl_2$ was added. 52 E-tag positive clones resulting from panning without addition of $CaCl_2$ contained either a DNA-fragment encoding the C-terminal domain of the *S. aureus* coagulase or a DNA-fragment encoding the N-terminal domain of Efb, confirming our previous results. In addition, we found that all of 5 analysed E-tag positive clones resulting from panning in the presence of $CaCl_2$ contained a DNA fragment encoding the fibronectin (Fn)-binding domain of *S. aureus* fibronectin-binding protein A (FnBPA). FnBP-negative mutants will be characterized by a flow cytometric adherence assay for their platelet-binding capacity to determine the importance of FnBPs in *S. aureus*-platelet interaction. We conclude that the Fn-binding domain in FnBPA is involved in *S. aureus* binding to platelets.

CCS 14 Phase-variable expression of the Bap protein controls biofilm formation and the infective process of *Staphylococcus aureus*

J.R. PENADÉS, MªA. TORMO, C. ÚBEDA, C. CUCARELLA, P. TROTONDA, J. PALACIO, J. VALLE, B. AMORENA & I. LASA
Dept of Chemistry, Biochemistry and Molecular Biology, Cardenal Herrera-CEU University, 46113, Moncada, Valencia, Spain - jpenades@uch.ceu.es

In this study we describe a phase variation process affecting the expression of the Bap (biofilm-associated protein) protein in *S. aureus*. Subcultivation of the Bap-positive *S. aureus* strain V329 on Congo red agar leads to a low frequency ($5x10^{-4}$) appearance of biofilm-negative colonies. Northern-blot analysis of these colony variants with a *bap*-specific gene probe revealed absence of transcription of the *bap* gene. Moreover, the phenotype of the parent V329 strain could be restored at a similar frequency upon subcultivation of these Bap-negative variants. This phase-variable phenomenon could modify the infective process of *S. aureus*, since Bap expression induces biofilm formation that in turns blocks the surface adhesins of the bacteria therefore interfering with tissue interaction and colonisation. On the other hand, lack of expression of the Bap protein might facilitate bacterial dispersion and colonisation of new structures. In agreement with this hypothesis, the experimental infection of ovine mammary glands with a non expressing Bap *S. aureus* variant showed that, in all mammary glands with persistent infection, the bacteria was able to express Bap again. This observation demonstrates the occurrence of phase variation in *in vivo* conditions and the importance of the Bap protein in *S. aureus* pathogenesis.

CCS 15 The biofilm-associated protein (Bap) is carried by a putative composite transposon inserted in SaPIbov2, a mobile pathogenicity island of *Staphylococcus aureus*

C. ÚBEDA, MªA. TORMO, C. CUCARELLA, P. TROTONDA, B. AMORENA, T.J. FOSTER, I. LASA & P.R. PENADÉS
Dept of Chemistry, Biochemistry and Molecular Biology, Cardenal Herrera-CEU University, 46113, Moncada, Valencia, Spain - jpenades@uch.ceu.es

Here we describe the complete sequence of SaPIbov2, a staphylococcal pathogenicity island encoding *bap*, the gene for the biofilm-associated protein. The element contains a integrase-like protein, and is bordered by 18-bp direct repeats at the left and right junctions, and the 15-nt of the integration site lies adjacent to the 3' end of the GMP synthase gene (*gmps*) in the

Staphylococcus aureus chromosome. This island has extensive regions of homology to previously described pathogenicity islands, particularly SaPIbov. However, it also has important distinctive characteristics: i) toxins present in the other pathogenicity islands have been substituted by a putative composite transposon including the *bap* gene, an ABC transporter operon and a transposase; and ii) SaPIbov2 is mobile without the presence of helper phage. In order to characterize the SaPIbov2 integrase gene (*int*), we constructed a minimal integrative module of the pathogenicity island carrying the att_L and att_R attachment sites and the functional *int* gene of the SaPIbov2. The att_L-*int*-att_R module can be excised, circularized and integrated efficiently, site specifically and RecA independently, in the bacterial att_B site present in the chromosome of *S. aureus*, confirming the existence of a functional *int* gene in SaPIbov2.

CCS 16 The surface protein Pls of methicillin-resistant *Staphylococcus aureus* is a virulence factor for arthritis

ELISABET JOSEFSSON[1], MARIA BOKAREWA[1], KATRI SAVOLAINEN[2], TIMO KORHONEN[2] & PENTTI KUUSELA[3]
[1]Dept of Rheumatology, Gothenburg University, Gothenburg, Sweden, [2]Dept of Biosciences, University of Helsinki, Finland, [3]Dept of Bacteriology and Immunology, University of Helsinki, Finland

Pls is a big surface protein, 230 kDa, which is present in a group of methicillin resistant *Staphylococcus aureus* (MRSA) strains. Pls prevents protein A mediated binding of MRSA to immobilized IgG as well as adhesion to fibronectin. Pls is suggested to be an adhesion regulator. In this study the importance of Pls for the development of septic arthritis and sepsis was investigated.

Mice were challenged intravenously with the clinical MRSA isolate 1061, a site-specific *pls* knock-out mutant, or a *pls* mutant complemented with the *pls* gene. Development of arthritis and weight was monitored for up to two weeks. In other experiments purified Pls was injected in the knee.

Mice inoculated with the *pls* mutant developed a much milder arthritis, and showed a less grave weight reduction, than mice infected with the wild-type. Also, the *pls*-mutant induced a significantly lower frequency of mortality than the wild-type. However, there was no evident inflammatory effect by the Pls molecule alone.

From these data we conclude, that Pls is a virulence factor for septic arthritis and sepsis.

CCS 17 Extracellular fibrinogen binding protein, Efb, from *Staphylococcus aureus* inhibits platelet function

OONAGH SHANNON & JAN-INGMAR FLOCK
Clinical Bacteriology, Dept of Microbiology, Pathology and Immunology, Karolinska Institute, Sweden

Extracellular fibrinogen binding protein (Efb) binds to the Aα-chain of fibrinogen at a site containing regions of functional importance in the interaction between fibrinogen and platelets. We have therefore investigated the effects of Efb on platelet function.

Efb dose dependently inhibits fibrinogen dependent platelet aggregation despite the fact that Efb significantly stimulates fibrinogen binding to platelets. In the absence of Efb, 30% of activated platelets bind fibrinogen and this increases dose dependently up to 85% ($p<0.01$, $n=4$). This stimulated fibrinogen binding cannot be inhibited by specifically blocking the normal platelet fibrinogen receptor, GPIIb/IIIa binding site with a monoclonal antibody.

We have also shown that GSTRR, which represents the N-terminal binding region of Efb, binds specifically to activated platelets. This binding also occurs in fibrinogen depleted platelet rich plasma and in the presence of a specific monoclonal antibody blocking fibrinogen binding to platelets.

We conclude that Efb binds to an as yet unidentified surface receptor on activated platelets and stimulates a novel type of fibrinogen binding. This fibrinogen is held in a non-functional conformation and platelet clot formation is therefore inhibited.

CCS 18 Phage display as a screening method for extra-cellular proteins

ANNA ROSANDER, JOAKIM BJERKETORP, LARS FRYKBERG & KARIN JACOBSSON
Dept of Microbiology, Swedish University of Agricultural Sciences, Box 7025, 750 07 Uppsala, Sweden

Extra-cellular proteins are involved in many diverse and essential cell functions and in pathogenic bacteria, they may also serve as virulence factors. Therefore, there is a need for methods that identify the genes encoding this group of proteins in a bacterial genome. Here, we present such a method based on the phage display technology. A novel gene III-based phagemid vector, pG3DSS, was constructed that lacks the signal sequence which normally orientates the encoded fusion protein to the *E. coli* cell membrane where it is assembled into the phage particle. When randomly fragmented DNA is inserted into this vector, only phagemids containing an insert encoding a signal sequence will give raise to phage particles displaying a fusion protein. These phages also display an E-tag epitope in fusion with protein III, which enables isolation of phages displaying a fusion protein, using antibodies against the epitope. From a library constructed from *Staphylococcus aureus* chromosomal DNA, genes encoding secreted as well as transmembrane proteins were isolated, including adhesins, enzymes and transport proteins.

CCS 19 *Staphylococcus aureus* adhesion to biomaterials

L.G. HARRIS[1,2,3], R.G.RICHARDS[2], S.J. FOSTER[1], S. TOSATTI[3] & M. TEXTOR[3]
[1]Dept Molecular Biology and Biotechnology, University of Sheffield, Firth Court, Sheffield S10 2TN, [2]AO Research Institute, Clavadelerstrasse, CH7270 Davos Platz, Switzerland, [3]ETH Zürich, Oberflächentechnik, Wagistrasse 2, CH-8952 Schlieren, Switzerland

Staphylococcus aureus is a major pathogen of increasing importance due to the rise in antibiotic resistance. The implantation of biomaterial into the human body, and the damage caused is known to increase the susceptibility to infection, and activates host defences, stimulating the release of inflammatory mediators. The ability of *S. aureus* to adhere to the extracellular matrix (ECM) and plasma proteins deposited on biomaterials is a significant factor in the pathogenesis of medical-device related infections. With the rise in antibiotic resistance, many are looking for compounds that can be used to coat implants, or new prophylaxis treatments, such as vaccines. PLL-PEG is a member of a family of polycationic PEG-grafted copolymers shown to chemisorb on anioic surfaces such as titanium, creating a protein resistant surface.

S. aureus 8325-4 were cultured on steel, titanium, and titanium or glass discs coated with PLL-PEG for 2, 4, 8, and 18h at 37°C. After the allocated time they were prepared for microscopy and their adhesion to the various surfaces studied. Preliminary results suggest that coating a material with PLL-PEG limits the adhesion of *S. aureus* in early growth. Western blots were used to analyse the activity of *S. aureus* on these surfaces.

CCS 20 Phenothiazinium dyes and their interactions with the *E. coli* membrane

SAIHMAH HUSSEIN, FRED HARRIS & DAVID PHOENIX
Centre for Forensic Science, University of Central Lancashire, Preston PR1 2HE

Eukaryotic studies have shown Phenothiazinium dyes and close analogues to be highly effective cytotoxic photosensitising agents with illumination leading to free radical attack on membranes, DNA and other cellular structures. Here, a number of these dyes were tested for antimicrobial action against *Escherichia coli* and each was found to possess biostatic and biocidal activity. Dye MIC's and MLC's were in the micromolar range but were reduced up to tenfold when illuminated with a light dose of 6.3 J cm^{-2}. These dyes lysed erythrocyte membranes with LD_{50}'s in the

micromolar range, but lysis levels were unaffected by illumination. Each dye induced pressure changes in lipid monolayers of up to 6 mN M^{-1}, which generally led to monolayer collapse. These results show that dyes tested are able to damage membranes by interactions apparently involving the destabilisation of membrane lipid packing and thus could be related to dye physical properties such as molecular shape. These processes appear unrelated to photosensitivity and suggest that the membrane may not be the primary killing site of these dyes.

Wednesday 18 September 2002

0900 Antibiotic resistant bacteria - what do you mean?

GUNNAR KAHLMETER
Clinical Microbiology, Central Hospital, Växjö, and The National Institute for Infectious Disease Control, Stockholm, Sweden - gunnar.kahlmeter@ltkronoberg.se

The antibiogram, i.e. the S-, I- and R-classification results of the antimicrobial susceptibility test, was developed when the antibiogram was meant to be nothing more than an instrument for directing antimicrobial chemotherapy in patients. However, due to the worldwide rapid increase of acquired antimicrobial resistance the results of susceptibility testing are used for an increasing number of purposes:

- to predict the outcome of antimicrobial chemotherapy in the single patient, i.e. as an instrument for directing antimicrobial chemotherapy,
- to predict the outcome of antimicrobial chemotherapy in future patients, i.e. for continuous evaluation of the basis for empirical therapy,
- to permit epidemiological intervention through
  - the early detection of bacteria with certain and especially unwanted or feared resistance mechanisms in hospitals (methicillinresistant *Staphylococcus aureus* (MRSA), glycopeptide non-susceptible Enterococci or Staphylococci, extended spectrum beta-lactamase (ESBL) producing Gram negative bacteria) and in the community in general (multiresistant *Mycobacterium tuberculosis*, multiresistant *Salmonella typhimurium*, penicillin and/or multiresistant *Streptococcus pneumoniae*),
  - the early detection of trends in resistance frequencies (antimicrobial resistance surveillance) and the identification of factors affecting the dynamics of such trends, i.e. consumption of antibiotics, infection control, associated resistance towards other antibiotics or other substances, crowding, etc. Knowledge obtained in this way form the basis for national and local antibiotic policies and interventions and as of late impact on national and international legislation, i.e. the inhibition of the use of some antimicrobials as growth promoters in animal husbandry.

There are a number of breakpoint committees in the world (BSAC, CA-SFM, CRG, DIN, NCCLS, NWGA, SRGA). Most committees have traditionally designed their breakpoints to predict clinical outcome in a single patient. The method for setting these breakpoints is being refined by the adoption of modern pharmacodynamic principles. However, pharmacological breakpoints often poorly measure or predict resistance development. In some instances this is because they divide bacterial wild type populations and in other instances because the breakpoint is so wide of the wild type population that resistance development will go undetected by the breakpoint. For these reasons, there is a need of developing two sets of breakpoints, a *clinical breakpoint* based on pharmacology, pharmacodynamics and above all clinical evidence of therapeutic success and a *epidemiologic (or microbiologic) breakpoint* based on the phenotypic or genotypic detection of "true" resistance.

0940 In-house genetic tests for resistance – Are they worth it?

P.M. HAWKEY, University of Birmingham

Abstract not received

1050 The future - Will it be chips with everything?

J.-P. MARCEL
Biomerieux SA, Marcy l'Etoile, France

Prediction of clinical outcome of antibiotic therapy - the goal of antibiotic susceptibility testing - is routinely performed with phenotypic methods. These totally artificial tests (media, inoculum, concentration...) are interpreted using various levels of knowledge in an attempt to identify the resistance mechanisms the bacteria may harbour.

Molecular biology techniques enable direct testing for the presence of genes coding for these resistant mechanisms: detection of *mecA* gene is routinely performed in many labs. Another step is the use of high-density probe arrays. This format can combine resistance testing, as well as species-level identification and strain typing in a single manipulation

Both wild or mutated sequences can be tested to detect various kinds of mutations such as deletions, substitutions, insertions, and even new point mutations.

Mycobacteria have been tested for *rpoB* and *katG* resistance genes. The huge quantity of information that can be tested makes this a promising tool in the development of bacteriology. This will mean a major shift from the phenotypic to the genotypic era, with new issues such as comparison with both clinical outcome and phenotypic results.

New results will be presented as well as discussion of benefits/ limits of this approach.

1140 Which quantitative disc diffusion method is best? NCCLS

MARTIN CORMICAN
Dept of Bacteriology, National University of Ireland, Galway, University Road, Galway, Ireland

In-vitro antimicrobial susceptibility testing attempts to measure the activity of an antimicrobial agent against a bacterial isolate. The measurement obtained is determined by many factors other than the properties of the antimicrobial agent and the bacterial isolate, including the culture medium, the inoculum, the conditions and duration of incubation and recording of the measurement. The NCCLS document M2-A7, includes detailed specifications in respect of the variables affecting antimicrobial susceptibility testing. NCCLS performance standards are based on evaluation of factors affecting reproducibility of measurement in antimicrobial susceptibility testing. Disc diffusion methodology is specified for a particular drug/bacterial combination only if there is evidence of a linear relationship between the zone diameter (in mm) and measurements made by dilution (MIC) methods. NCCLS performance standards are comprehensive, thoroughly reviewed and updated, and widely disseminated. M2-A7 is the *de-facto* global performance standard. Clinical application of in-vitro measurements requires interpretation. Interpretation of NCCLS disc diffusion test results is based on criteria in M100-S12 (for 2002). Interpretive criteria for zone diameters are derived from criteria for MIC methods as described in NCCLS document M23-T3. Definition of interpretative criteria is more challenging and controversial than definition of performance standards. NCCLS interpretive criteria have frequently been substantially revised as evidence showed them to be unsatisfactory. Adoption and use of NCCLS performance standards throughout Europe is appropriate to support national, European and global surveillance of resistance. Definition of European interpretive criteria may be necessary in a very small number of cases in which there is a clear consensus in Europe that the NCCLS breakpoint interpretive criteria are too high.

1210 Which quantitative disc diffusion method is best? BSAC

A. MacGOWAN, Southmead Hospital, Bristol

Abstract not received

Wednesday 18 September 2002

1900 The patent and the patent process

C. BOERMA
Unilever Research, Sharnbrook
Abstract not received

1920 What the university does in patent exploitation

J. DERBYSHIRE
Director of Intellectual Property, Loughborough University
Abstract not received

1940 When your job takes you to court

K. MAY
R&D Director, Inverness Medical
Abstract not received

2000 Sources of Finance

BRUCE SAVAGE
BioQuest Consulting Ltd, Trelawn House, East Street, Bodicote, Banbury, Oxfordshire OX15 4EB
All high-tech companies with an R & D programme require funding by way of equity; that is by selling shares to investors. Bank finance is not suitable because these types of companies are not trading. Before reviewing sources of equity it is worth remembering government/EU grants such as the SMART award scheme.

Investors need to see a good business plan that sets out clearly and with the absolute minimum of details on the science, what it unique and about your technology, patent protection, details of the market and how the idea will be commercialised, and then how much money is required and what you intend to spend it on. An interesting point about investors is that you need to give them an idea in the business plan, as to how they will get their investment back by way of a trade sale or by floatation.

Sources of investment capital are: business angels, venture capital trusts, and venture capitalists. Each of these will be reviewed in the presentation with their pros and cons. Details will be given as to how to make contact with these groups and details will be given as to their particular suitability relevant to the stage of development of the company. Finally routes to a public market will be reviewed and when in a company's life these are relevant.

2020 Commercialisation: a case study

N. BUTLER
Oxford Biosensors, Yarnton
Abstract not received

2040 The Ultimate Laboratory Notebook

P. MUCCI
Inventor & Intellectual Property Specialist for the DTI Innovation Unit, Durley, Southampton
The systematic recording of theoretical and experimental work is the backbone of good research. It was once sufficient for researchers to keep simple notebooks and produce a few graphs and tables. Now they must combine the discipline of the laboratory with a wider understanding of the world beyond science if the knowledge they transfer is to have any real value. In particular, a basic understanding of IPR in all its forms has become essential where research may lead to commercial exploitation.

A two year study by the author and consultations with researchers, businesses, legal professionals and higher education has led to the Innovation Logbook. It combines a professional logbook for the chronological recording of original work with guidelines for securing all forms of IPR currently valid in the UK including rights that extend throughout the EU. The design and layout of the book draws strongly on UK and USA experience, with the latter having special importance due to their 'first to invent' laws for patents. The book also includes guidelines for good laboratory practice and a contact list for free help and advice.

Thursday 19 September 2002
This session is dedicated to the memory of David Wynn-Williams who died tragically on 24 March 2002.

0900 H. LAPPIN-SCOTT (University of Exeter)
Tribute to David Wnyn-Williams

0910 Geothermal origins of Life
J. BAROSS
University of Washington, USA
Abstract not received

0955 Origins of microbial photosynthesis
R.E. BLANKENSHIP
Arizona State University, USA
Abstract not received

1135 Primitive RNA genomes
J. HISCOX
University of Reading
Abstract not received

1155 Photosynthetic signals for Astrobiology
JOHN A. RAVEN
Division of Environmental and Applied Biology, School of Life Sciences, University of Dundee, Dundee DD1 4HN
The signals from photosynthesis on a planet that could potentially be detected from space are all spectroscopic. Photosynthesis of all kinds involves pigments that absorb incident radiation and perform photochemistry. On Earth the photosynthetic pigment that is most readily sensed remotely is chlorophyll in land plants with a long wavelength cut-off at 700nm, followed by chlorophylls in phytoplankton in the top few metres of the ocean. Photosynthesis on an Earth-life planet (ELP) might not necessarily used pigments with the same long wavelength cut-off as on Earth. In any case, seasonality of pigmentation could help to distinguish photosynthetic pigments from abiological pigments on an ELP.

The other categories of spectroscopically detectable signals from photosynthesis are major biogeochemical consequences of changes in the substrate(s) and/or product(s) of photosynthesis. Here the possibility of detection is more constrained than with the pigments since it is more dependent on the chemistry of substrates and products that vary considerably among photosynthetic organisms on Earth. The biogeochemically most significant photosynthetic variant on Earth consumes carbon dioxide and water and produces reduced carbon and oxygen. Oxygen is only produced in significant amounts by photosynthesis, and could be detected spectroscopically as the photochemical derivative ozone or, perhaps, as oxygen itself. Seasonal variations in atmospheric carbon dioxide on Earth as a result of the changes in the ratio of photosynthesis to respiration are unlikely to be detectable on an ELP.

These suggestions for the detection of photosynthesis are, of course, subject to a number of caveats. The exact geochemistry and geophysics of the ELP and the spectral distribution of radiation from the star that the ELP is orbiting can influence the likelihood of evolution of oxygen-producing photosynthesis and the kind of photosynthetic pigments. If photosynthesis were constrained to occur solely below a few metres depth in an ocean then pigments would not be detectable. Furthermore the low radiant energy input would restrict the biogeochemical impact of photosynthesis relative to other biochemical and geological processes and makes any kind of remote sensing of photosynthesis more difficult.

1240 Archaeal DNA polymerases – characterisation, application & *in vivo* function
C.D.O. COOPER[1,2], A.E. LAW[1,2], S.C. BAKER[3], A. LOUWRIER[4], C.J. KNOWLES[1] & M.J. BAILEY[2]
[1]Oxford Centre for Environmental Biotechnology, Dept. Engineering Science, University of Oxford, [2]NERC Centre for Ecology & Hydrology, Oxford, [3]School of Biological & Chemical Sciences, Birkbeck College, London, [4]ABgene Ltd., Epsom, Surrey
DNA is frequently subjected to the damaging effects of a range of chemical and environmental assaults, particularly in the harsh habitat conditions of hyperthermophilic archaea. DNA polymerases help to overcome these effects by maintaining accurate DNA replication and repair activities. A number of archaeal DNA polymerases have been isolated and characterised for their commercial applicability to PCR. However, their in vivo functions are poorly characterised, i.e. classifying those DNA polymerases involved in replication and those in repair. Multiple archaeal model systems have increased our understanding, but little is known of archaeal DNA polymerase transcriptional regulation, protein-protein interactions or structural dynamics. We isolated a family B1-type DNA polymerase (dpo1/polB1) from Acidianus brierleyi. It exhibited sequence similarity to other crenarchaeotal DNA polymerases but contained a number of novel motifs. dpo1 was transcribed as a monocistronic RNA from a Box A promoter upstream of a coincident GTG transcriptional/translational start codon, and expression at the RNA and protein level was followed. PolB1 exhibited 3'(5' exonuclease activity and DNA synthesis activity was optimal at 65(C, but it required additives such as trehalose or KCl to maintain in vitro structural stability. Further understanding of archaeal DNA replication may provide insight into the relationship between the eukaryal and eubacterial superkingdoms, and may help answer questions about the evolution of DNA replication.

1400 Microbial UV tolerance and early evolution
FERRAN GARCIA-PICHEL
Arizona State University
Several lines of paleoenvironmental evidence, including stellar evolution and atmospheric modeling, suggest that severe changes in the amount and wavelengths of solar ultraviolet radiation reaching the ground have occurred during Earth's history. Today, one of the mechanisms used by microbes to thrive under high UV fluxes is the synthesis of UV suncreeens, typical in prokaryotes like cyanobacteria and eukaryotes like fungi. A study of microbial phylogeny in combination with knowledge on the biosynthetic pathways and comparative biochemistry of sunscreen secondary metabolites (scytonemin and mycosporines for cyanobacteria; melanines and mycosporines for fungi), reveals patterns that are consistent with the proposed long-term history of UV exposure, and suggest that a particular period existed, some 0.5-1.5 thousand million years ago, when UV exposure reached a maximum in intensity and biological impact. This must have been much more intense that ever before or after, including any possible future increases due to anthropogenic ozone depletion.

1445 Soda lakes, salt and microbial longevity
W.D. GRANT
Dept of Microbiology and Immunology, University of Leicester, Leicester LEI 9HN
It is reasonable to assume that the early geochemical evolution of Mars and Earth may have been similar and that models of the early Earth's oceans can be used to discuss the Martian hydrosphere. One such model proposes an alkaline soda ocean and the soda ocean scenario has been developed by analogy with modern environments provided by present day soda lakes. Soda lakes and soda deserts represent the major type of naturally

occurring highly alkaline environment on Earth. These environments are characterised by the presence of large amounts of Na_2CO_3, generating pH values between 9.5 and 12.0. These lakes are usually hypersaline since Cl^- ions also dominate in solution as evaporative concentration progresses and the salinity of these lakes range from around 5 % (w/v) to saturation. The best studied examples of soda lakes are those present in the East African Rift Valley. These lakes are remarkably productive with cyanobacteria1 primary production driving the ecosystem. A systematic examination of chosen examples of many strains brought into laboratory culture reveals a remarkable diversity of prokaryotes with most isolates being obligately alkaliphilic and either halotolerant or halophilic. The lakes are of biotechnological interest as potential sources of alkaliphilic enzymes. Two alkaliphilic cellulases derived from bacteria isolated from these lakes are currently marketed for detergent and fabric processing use.

The hypersaline sites harbour alkaliphilic halobacteria (haloarchaea). Observations of the interaction between halobacteria and the precipitation of halite (NaCl), initially in alkaline salt ponds, stimulated an interest in the microbial ecology of subterranean ancient halite. There is a considerable literature stretching back half a century that suggests that bacteria can be isolated from within ancient halite crystals, raising the intriguing possibility that these isolates were entrapped at the point of halite precipitation and have thus survived over geological time. There is no doubt that ancient halite deposits contain dense populations of halophilic and halotolerant prokaryotes, particularly halobacteria, but it is difficult to prove that these are other than recent inhabitants and that the halite is original – for example 16S rRNA gene sequence comparisons have repeatedly failed to show consistent differences between ancient halite isolates and those from salt lakes (presumed modern!). Nevertheless, the possibility of long term survival of bacteria and/or biomarkers such as DNA should not be summarily dismissed - there has been a recent controversial report of the recovery of 16S rRNA gene fragments from ancient halite that has been precisely dated using laser ablation mass spectrometry.

I will review work on soda lakes, leading to the ancient halite story.

1530 Under pressure: the story of a bacterial response

K.A.G. KARATZAS[1], J.A. WOUTERS[1], C.G. GAHAN[2], C. HILL[2], T. ABEE[1] & M.H.J. BENNIK[1]
[1]Wageningen Centre of Food Sciences (WCFS), Diedenweg 20, 6703 GW, Wageningen, The Netherlands, [2] National Food Biotechnology Center and Dept of Microbiology, University College Cork, Ireland

Our research has been aimed at understanding the mechanism underlying High Hydrostatic Pressure (HHP) tolerance of bacteria. HHP has detrimental effects on cellular processes, resulting from perturbation of membranes and membrane-associated processes, to disruption of macromolecular quaternary structures. HHP treatment is currently used in the food industry because it allows for inactivation of undesireable microorganisms, while other food constituents, such as colour and certain nutrients, are not affected.

We isolated a strain of the foodborne pathogen *L. monocytogenes* that shows ~1000-fold higher survival rates than the wt upon exposure to 350 MPa. This strain shows altered morphological characteristics, such as, elongation of cells, lack of flagella, cross-resistance to heat, acid, H_2O_2, and importantly, attenuated virulence. Using protein 2D-gel electrophoresis, we identified differences in expression levels of flagellin and the serine protease ClpP, a Class III heat shock protein. Upon sequencing genes involved in the regulation of these proteins, we could conclusively link the observed phenotype of the mutant to a single codon deletion in a key regulator. This codon deletion seems to occur relatively frequently in wt populations of *L. monocytogenes*, suggesting a role in adaptation to not only High Hydrostatic Pressure, but also other environmental stresses.

1550 *N*-dimensional hyper-space environments

JULIAN WIMPENNY
Cardiff School of Biosciences, Cardiff University, Cardiff CF1 3TL

Concepts of hyperspace described at a meeting devoted to Astrobiology will almost certainly be misconstrued! The term, as used here, was applied originally to ecology by G. Evelyn Hutchinson in the early fifties who described the effects of all the environmental factors that affect the life of a species as an *n*-dimensional hypervolume located within an *n*-dimensional axis system, where *n* is the number of factors under consideration. I have described the envelope within which an organism can grow as its 'habitat domain'. An important question concerns the habitat domain of living things on earth. Of all environmental factors the most important and best documented are temperature, pH and salinity with a fourth, pressure, also of great importance. The first three are discussed in this paper. In terms of *optimal* values, for pH versus temperature the growth domain is mushroom shaped with most organisms, including those found at highest and lowest temperatures, growing best at neutral pH values. Acidophiles and alkaliphiles appear over a temperature range of 20 to 80 ^oC. No psychrophiles or thermophiles are found with optima below pH 4. When salinity is plotted against temperature NaCl tolerance increases as a function of temperature with maximum salt tolerance between 40 and 50 ^oC. At temperatures from about 50 ^oC to over 100 ^oC and below 20 ^oC only occasional organisms can tolerate more than 5% w/v NaCl. When pH is related to salinity the majority of organisms show salt tolerance between pH 6 and 10. The envelopes for maximum or minimum values are greater than for the optima though the profiles are similar. In regions where no growth is observed the question arises as to whether the relevant habitats exist, alternatively if growth, for physico-chemical reasons, is not possible under these conditions. Clearly pressure is an important environmental variable since many hyper-thermophiles can only survive at temperatures above 100 ^oC at elevated pressures. Unfortunately too few published data values exist to add this factor as a fourth dimension

Data for temperature, pH and salinity have been incorporated into a three dimensional envelope which characterises for these factors, the domain for life on this planet.

1630 Biosensors for remote microbial physiology

A. STEELE
Carnegie Institute, Washington, USA

Abstract not received

1715 Assessing bias in environmental profiling using DNA analysis

RUTH CORDERO PETERS[1], NOZOMI YTOW[2] & DAVE ROBERTS[3]
[1]School of Biomedical & Life Sciences, University of Surrey, Guildford, Surrey, GU2 7XH, [2]Institute of Biological Sciences, University of Tsukuba, Tsukuba 305-8572, Japan - E-mail: nozomi@biol.tsukuba.ac.jp, [3]Dept Zoology, The Natural History Museum, Cromwell Road, London SW7 5BD - E-mail: dmr@nhm.ac.uk

The use of nucleic acid extraction, amplification and analysis to determine a profile of diversity in environmental samples has become the method of choice in microbial ecology because it avoids the biases inherent in traditional cultivation techniques and gives access to information about strains which are reluctant to grow in culture. DNA was extracted from freshwater sediment through a series of washes using the common phenol-cholorform methodology. Up to 12 washes could be completed in one day through to lyophilisation and high molecular weight (genomic) DNA was recovered in significant quantities from all washes. PCR amplification with universal primers to eukaryotic small-subunit ribosomal DNA yielded no amplified product, but universal primers to all domains gave good product. The results were examined by DGGE analysis using eukaryote-specific primers and revealed a changing pattern of bands across the washes. Use of the hydroxyapatite extraction method resulted in

more bands being visible and the difference between washes being far less dramatic.

These results suggest that lysis methods probably have slight taxonomic bias through the wash cycle but that DNA-binding material is co-isolated with the DNA and inhibits the PCR reaction, either in a random or in a non-specific stochastic manner. These results suggest that the hydroxyapatite method should be the routine method of choice. Further, these results suggest that failure to find target sequences should not be taken to indicate their absence, specifically in the context of extreme environments.

Friday 20 September 2002

0900 Survival of microbes in meteorites 2

M. BURCHELL
University of Kent, Canterbury
Abstract not received

0945 Microbial life in subglacial lake environments

J.C. ELLIS-EVANS[1], J.C. PRISCU[2] & S. BULAT[3]
[1]British Antartic Survey, Cambridge, [2]Dept of Land Resources and Environmental Sciences, Montana State University, Bozeman, Montana, USA, [3]Dept of Molecular and Radiation Biophysics, St. Petersburg Nuclear Physics Institute RAS, Russia
The discovery of up to 100 large water bodies beneath the East Antarctic ice sheet has prompted questions as to whether these lakes contain life and what form this life might take. The lakes lie beneath 3.0-4.5 km of glacial ice. Some may be up to 30 million years old and the largest, Lake Vostok, may occupy a pre-glaciation lake basin more than 35 million years old. Ice core dating suggests the lakes have probably been isolated from the atmosphere for around 1 million years. Technological constraints and the need to avoid contamination of these pristine environments has prevented penetration and sampling of these lakes to date. However detailed geophysical surveying, sophisticated 3-D modelling and recent access to accreted ice derived from subglacial Lake Vostok have provided intriguing information on the likely envelope of environmental conditions for subglacial life. This presentation presents some of the latest data and speculates on the likely composition of a lake microflora.

1005 The physiological and genetic characterisation of a hydrocarbon degrading consortium from the Great Salt Lake

JAMES ASCOTT, TERRY McGENITY, SARA BURTON, JONATHAN GETLIFF & HILARY LAPPIN-SCOTT
University of Exeter
This research considers the use of extreme halophiles as a possible solution for degrading hydrocarbons at high salt concentrations and bioremediating oily drill cuttings. During the last 30 years of North Sea oil operations it is estimated that between 1 – 1.5 million tonnes of drill cuttings have been produced. What to do with the rock cuttings generated while drilling poses a significant problem for the oil industry as they can have significant effects on the receiving environment.

The drill cuttings are complex mixtures of materials consisting of rock cuttings from the drilled hole coated with drilling fluid. The drilling fluids or "muds" used for this study are known as invert emulsion fluids. Such fluids comprise a brine internal phase within a continuous external oil phase. The oil phase can be based upon a range of different hydrocarbon chemistries, however the chemicals, which we are particularly interested in degrading, are the straight chain alkanes or linear paraffins.

Strains of bacteria enriched from soil and marine environments which are able to these degrade hydrocarbons are commonplace. However, because of the high salinity of this waste it is not always possible to use such bacteria unless the salt concentration is reduced. Extreme halophiles, which require salt concentrations of 20 % (w/v) maybe a suitable choice for bioremediating such wastes. A water sample from the Great Salt Lake (USA) was enriched via continuous subculturing and a consortium of extreme halophiles capable of degrading medium chain alkanes has been established (GSL 1). Investigations using gas chromatography have shown that this consortium is able to degrade C12 to C15 length alkanes. Preliminary screening studies are now underway to assess the degradation potential of other extremphile consortia. The GSL 1 consortium consists of four culturable isolates of which three have been putatively identified as members of the archeal order *Halbacteriales* using 16S rDNA sequence analysis. Physiological and chemotaxonmic investigations such as lipid analysis are currently underway to fully characterise these isolates.

1100 Planetary protection

S. SANCISI-FREY & J.M. PILLINGER
The Open University, Milton Keynes
Abstract not received

1120 Future life on Mars

MARTYN J. FOGG
Probability Research Group, c/o 44 Hogarth Court, Fountain Drive, London SE19 1UY - mfogg@globalnet.co.uk
Should Mars be devoid of indigenous life, there is a question as to whether terrestrial life might be implanted there, a biosphere created and, in the fullness of time, the planet rendered fit for human habitation. Presently, such a possibility remains speculative, but has nevertheless attracted the interest of various scientists and writers over the past thirty years. Thinking about terraforming Mars has proved edifying in providing novel perspectives in planetology, restoration ecology and environmental ethics, as well as being used as a teaching tool for school science and on a number of undergraduate astrobiology courses.

Whilst the surface of Mars is uninhabitable, all the elements required to support life appear to be present. Moreover, the geomorphology of the planet indicates that it may have had a more benign, biocompatible, climate in the distant past. Some studies using simple Mars climate models have suggested that a comparatively modest programme of planetary engineering might release enough moisture and gases to warm Mars to the point where anaerobic ecosystems might be introduced. The realism of this however, and the extent to which terraforming could be taken further, await a much better knowledge and understanding of Mars that will only emerge following future decades of robotic and eventually human exploration.

1145 Microbial survival in Hot Springs

L. ROTHSCHILD
NASA / Ames Research Center, California, USA
Abstract not received

POSTERS:

EM 01 Rock-inhabiting fungi: formation of biogenic micro-fabrics in limestone minerals

E.P. BURFORD[1], S.HILLIER[2] & G.M. GADD[1]
[1]Division of Environmental and Applied Biology, Biological Sciences Institute, School of Life Sciences, University of Dundee, Dundee DD1 4HN, [2]Macaulay Land Use Research Institute, Craigiebuckler, Aberdeen AB15 8QH
Fungi contribute to the dissolution of rocks and mineral aggregates in soil through the excretion of H^+, organic acids and other ligands, or through redox attack of mineral constituents such as Mn and Fe. Such interactions between fungi and minerals are of fundamental importance to biogeochemical cycles including those of C, N, S and P. It is possible that fungi also play a role in mineral formation through precipitation of secondary minerals, e.g. oxalates, and through nucleation of crystalline material onto cell walls. This would result in the

formation of biogenic micro-fabrics within minerals although direct experimental evidence for this is lacking.

Our research demonstrates that fungi can play an important role in the transformation of micro-fabrics in limestone ($CaCO_3$) and dolomite ($CaMg(CO_3)_2$). Scanning electron microscopy and X-ray microprobe analysis of rehydrated limestone has provided direct evidence of mineralized fungal filaments with the crystal coatings exhibiting different crystalline lattices and elemental composition from the initial limestone. Powder X-ray diffraction analysis indicated that these were secondary carbonates. Other experiments using fungal cultures grown in microcosms amended with calcium carbonate showed that fungi precipitate secondary crystals exhibiting a range of morphologies. These were a mixture of calcite ($CaCO_3$) and whewellite (calcium oxalate monohydrate, $CaC_2O_4.H_2O$).

Our results directly confirm that living fungi are capable of secondary calcite formation and point to a wider biogeochemical role for fungi in extreme environments than has previously been appreciated.

Euan Burford gratefully acknowledges receipt of a Natural Environment Research Council postgraduate research studentship.

EM 02 Cloning and characterization of the genes of beta-1,3-glucan hydrolyzing enzymes from a marine bacterium, *Pseudomonas* sp. PE2, which degraded the cell walls of *Pythium porphyrae*

ETSUSHI KITAMURA & YUTO KAMEI
Marine and Highland Bioscience Center, Saga University, Saga 847-0021, Japan

A marine bacterium, *Pseudomonas* sp. PE2 isolated from the Ariake Sea, Japan, degraded the cell walls of *Pythium porphyrae*, a causative fungus of red rot disease of *Porphyra yezoensis*. The complete genes *gluA* and *gluB* were cloned into ZAP phage from the *Pseudomonas* sp. PE2, by using beta-1,3-glucan. The *Escherichia coli* clone harboring the *gluA* gene showed the degrading activity against beta-1,3-glucan while the *E. coli* clone harboring the *gluB* gene degraded both beta-1,3-glucan and beta-1,3;1,4-glucan. The *gluA* gene encoding beta-1,3-glucanase A (GLUA) consisted of an open reading frame of 2,259 nucleotides and encoded 752 amino acids. Based on the homology search in the GenBank and SWISS-PROT databases, the N-terminal region of GLUA was 40.8% sequence similarity to the catalytic domain of laminarinase from *Rhodothermus marinus*, and possessed a significant active site motif of glycosyl hydrolase family 16. The *gluB* gene encoding beta-1,3(4)-glucanase B (GLUB) consisted of an open reading frame of 1,693 nucleotides and encoded 565 amino acids. The N-terminal region of GLUB was composed of the repeated carbohydrate-binding module family 6. The C-terminal region had 49.8% sequence similarity to the catalytic domain of laminarinase from *Thermotoga maritima*, and possessed the same active site motif as GLUA.

EM 03 Antifungal mechanism of an anti-*Pythium* protein (SAP) from a marine bacterium, *Streptomyces* sp. AP77 specific to *Pythium porphyrae*, a causative agent of red rot disease in *Porphyra* spp

JUNG-HEE WOO & YUTO KAMEI
Marine and Highland Bioscience Center, Saga University, Saga 847-0021, Japan

An antifungal protein specific to *Pythium porphyrae* was found in a marine bacterium, *Streptomyces* sp. AP77 (Woo et al; Appl. Environ. Microbiol., 2002). This study was conducted to prove such an antifungal mechanism of an anti-*Pythium* protein (SAP). Though we first examined the effect of SAP on the *P. porphyrae* cell walls, SAP did not decompose the six structural polysaccharides in *Pythium* cell walls. However, the significant inhibition of hyphal growth was detected in *Pythium* cells treated with 50 μg/ml of SAP by MTT assay. Then, the protoplasmic leakage was observed in *P. porphyrae* hyphae treated with the SAP for 1 hour, followed by hyphal swelling and disintegration, using SYTOX Green, and SAP permeabilized the membrane of *P. porphyrae* in dose-dependent manner. Treating *P. porphyrae* cells with SAP in the presence of carbonyl cyanide *m*-chlorophenylhydrazone (CCCP), a membrane- depolarizing agent, significantly reduced the membrane permeabilization to SYTOX Green. The similar effect was observed when *P. porphyrae* cells was treated with SAP in the presence of $MgCl_2$. In contrast, KCl significantly increased the membrane permeabilization to SYTOX Green in *P. porphyrae* cells. These results suggested that anti-*Pythium* mechanism of SAP was related to the alteration of membrane permeabilization in *P. porphyrae*.

EM 04 Sulphate-reducing bacterial biofilms in the linked redox-cycling of sulphur and selenium under anaerobic conditions

S. HOCKIN & G.M. GADD
Division of Environmental and Applied Biology, Biological Sciences Institute, School of Life Sciences, University of Dundee, Dundee DD1 4HN - s.hockin@dundee.ac.uk

Biological sulphur cycling comprises both assimilation as sulphide and dissimilatory oxidation-reduction transformations. The dissimilatory cycle includes broad-scale cycling between sulphate and sulphide by sulphate-reducing and sulphide-oxidising bacteria and also small-scale cycling through elemental sulphur by syntrophic associations of sulphide oxidisers and sulphur-reducers. Here we report that sulphate-reducing bacterial biofilms mediate the formation of elemental sulphur in the presence of the selenium oxyanion selenite (SeO_3^{2-}). The indirect, enzymatically-mediated coprecipitation of significant quantities of sulphur and selenium is proposed as a generalised ability among SRB, arising from dissimilatory sulphide biogenesis, and can take place under low redox conditions and in the dark. The biological cycling of selenium is receiving increasing attention, due to its importance as an essential trace element and also the potential for selenium pollution. SRB can enzymatically reduce small amounts of selenium to selenide and, by a separate mechanism, to elemental selenium. However, the ability for SRB to participate in selenium cycling under low-redox environmental conditions, where selenium oxyanions are scarce, has been questioned. Biofilm-growing SRB may nevertheless remain active well into the oxic zone. Furthermore the reaction described here can take place away from the site of SRB activity. We conclude that biogenic sulphide may thus be responsible for the precipitation of elemental selenium from more oxidised environments, where concentrations of selenium oxyanions are greatest.

The authors gratefully acknowledge receipt of a BBSRC Industrial Postgraduate Research Studentship (SH) and research support from BNFL plc.

EM 05 Xenobiotic degrading communities: an insertional gene inactivation approach to monitor community dynamics and horizontal gene transfer

T.J. ASPRAY, I.C. BLOMFIELD & R.G. BURNS
Research School of Biosciences, University of Kent at Canterbury, Canterbury, Kent CT2 7NJ

The genes involved in the degradation of a number of xenobiotic compounds are carried on large (~80-100 kb), broad-host range, self-transmissible plasmids. One such plasmid, pJP4, found in the host strain *Ralstonia eutropha* JMP134 carries genes (*tfdA-F*) for the partial degradation of 2,4-dichlorophenoxyacetic acid (2,4-D). An insertional gene inactivation approach involving homologous recombination is being used to knockout specific degradative genes on pJP4. A fragment (~2000 bp) of the *tfdB* gene was amplified and cloned into a pUC18 based suicide vector carrying the counter-selectable *sacB* gene. Plasmid pJP4 was then plate mated into *Escherichia coli* strain XL1-Blue, as *R. eutropha* JMP134 was found to be poorly transformable. The *sacB* gene and *tfdB* fragment is being cloned into a derivative of pSC101 carrying a temperature sensitive (Ts) replicon. The Ts based system can be used to construct mutants of pJP4 in *E. coli.* Cassettes carrying constitutively expressed green fluorescent protein (*gfp*) and its derivatives have been constructed and will be used to inactivate the *tfd* genes. A flow cell system will be

used to monitor the movement of plasmids expressing autofluorescent proteins as part of a 2,4-D degrading biofilm community. The approach should aid future bioremediation strategies and increase our understanding of microbial community dynamics.

EM 06 Influence of the rhizosphere on the biodegradation of the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) in soil

LIZ J. SHAW & RICHARD G. BURNS
Research School of Biosciences, University of Kent, Canterbury, Kent CT2 7NJ

To understand how plant roots enhance biodegradation (rhizoremediation), we quantified the influence of the *Lolium* and *Trifolium* rhizospheres on the degradation of ^{14}C-2,4-D in previously uncontaminated soil and related differences in mineralisation rates to soil chemistry and microbiological properties. Culturable numbers were significantly ($p<0.05$) enhanced in planted (e.g. 4.0×10^7 cfu g^{-1} for 25 day-old *Trifolium*) compared to non-planted controls (5.8×10^6 g^{-1}) whereas the MPN of 2,4-D degraders was low (<100 g^{-1}) and not related to planting. For 2,4-D mineralisation, the most pronounced effect was for *Trifolium*; the maximum rate was significantly ($p<0.05$) enhanced (*Trifolium*= 10.0 h^{-1}, non-planted = 7.6 h^{-1}). The rhizosphere effect on 2,4-D mineralisation could not be mimicked by elevating microbial numbers by addition of a non-selective carbon source.

Ribosomal intergenic spacer analysis revealed no difference in the composition of 2,4-D-degrading cultures enriched from non-planted and rhizosphere soils. Enrichment cultures did not possess the canonical *tfd* genes. These cultures are being tested for the presence of the *cad*A gene (encodes a novel 2,4-D oxygenase; Kitagawa *et al*. 2002 *J. Bacteriol*. 184, 509). The hypothesis that specific compounds produced by plant roots stimulate 2,4-D degradation by acting as inducers will be developed.

EM 07 Bacterial degradation of arsenobetaine to dimethylarsinate *via* dimethylarsinoylacetate

ALISDAIR W. RITCHIE & RICHARD O. JENKINS
School of Molecular Science, De Montfort University, The Gateway, Leicester LE1 9BH

Arsenobetaine (trimethylarsonioacetate) is considered a benign form of arsenic, which through human consumption of seafood is the major arsenical compound in the diets of most people. The ability of mixed-cultures of marine microrganisms to convert arsenobetaine is well documented. The aim of the present work was to test the possibility that mixed community functioning is an obligate requirement to arsenobetaine degradation.

Microorganisms from *Mytilus edulis* (marine mussel) degraded arsenobetaine in undefined mixed-culture, with the formation of trimethylarsine oxide, dimethylarsinate and methylarsonate. Two pure culture isolates (identified to genus level as *Flavobacterium* sp and *Pseudomonas* sp) were shown by HPLC-inductively coupled plasma-mass spectrometry (ICP-MS) analysis to degrade arsenobetaine by initial cleavage of a methyl-arsenic bond to form dimethylarsinoylacetate, with subsequent cleavage of the carboxymethyl-arsenic bond to yield dimethylarsinate. Arsenobetaine biodegradation by monoseptic-cultures was biphasic, with dimethylarsinoylacetate accumulating in culture supernatants during the culture growth phase and its removal accompanying dimethylarsinate formation during a carbon-limited stationary phase. *Flavobacterium* sp also converted exogenously supplied dimethylarsinoylacetate to dimethylarsinate only under carbon-limited conditions. The work establishes the capability of particular bacteria to cleave both types of arsenic-carbon bonds of arsenobetaine and demonstrates that mixed community functioning is not an obligate requirement for arsenobetaine biodegradation.

EM 08 Biological treatment of corn processing wastewater with converting its organic compounds to "SCP"

S. HAGH NAZARY
Dept Food Science & Technology, Mashhad University, Iran - Haghnazary2@yahoo.co.uk

In one of the cornstarch producing plants in Iran, approximately 23 tons of organic materials are left out daily in wastewater. This wastewater, in addition to its illegal contamination for discharging into city effluent system would cause microbial growth due to its nutritive materials leading to spread of microbial contamination in process line and products in the factory.

In order to solve the above mentioned problems, also to get the benefit of nutritive substances of wastewater in the said plant for the production of a valuable byproduct so-called " SCP " to be used as protein supplement in animal, poultry and fish feed; the wastewater was treated by biological methods regarding the reduction of maximum chemical oxygen demand (COD) in lab scales.

After elimination of suspended solid from the wastewater by centrifugation, the purified wastewater was fermented under various conditions as given below:

- PH: "3.5", "4.5", and "4 without controlling"
- Temperatures "25" and "35" degree centigrade
- Fermentation with "Mixed-cultured" and "Mono- cultured " of wastewater microflora
- Addition / no addition of %0.7 glucose
- Fermentation in incubation shaker unit with controlled heating was done
- Samples were taken in 4 hour intervals, the amount of COD reduction and produced biomass in different samples were measured during 72 hours.

The best conditions for fermentation found to be at " PH=4 with no control" the temperature of "35 degree centigrade " using "Mixed-cultured " and "without addition of glucose". The results of this research can be used at the high aerobic condition in the large-scale fermentor, so that its effect will be distinguished.

EM 09 Production of SCP by biological treatment of corn processing wastewater

S. HAGH NAZARY
Dept Food Science & Technology, Mashhad University, Mashhad Sity, Iran - Haghnazary2@yahoo.co.uk

The most portions of Iranian water sources are consumed in agricultural section and related industries. Also vast areas of this country are confronted with water deficiency .In the food factories, especially starch production units, which water has the main role for processing and carrying raw materials, therefore large amount of water is consumed. Produced wastewater has much organic compounds, which resulted from dissolving the milled raw materials in it. When exiting from factory, its characteristics don't correspond to the related standard and also it contaminates the environment and products.

For solving above mentioned problems, wastewater's compounds can be converted into useful substance (SCP), by aerobic biological treatment, in order to feeding animals, poultry and fish, and also recovering purified water for agricultural irrigation.

At the first stage of experiment, centrifuged wastewater sample were incubated under optimum condition found in previous research of this writer in a ten-liter fermentor for better aeration. After 48 hours of incubation, the amount of COD reduction was %90.58 and produced dried matter after 48 hours and 124 hours of fermentation amounted to 2.55 gr./l and 5.09 gr./l respectively.

At the second stage, for increasing the production of biomass, the amount of nitrogen and phosphorous in wastewater were adjusted by the urea and ammonium phosphate sources. This sample was fermented under conditions similar to the first stage with respect to aeration, temperature, stirring speed and pH condition, in 10-liter fermentor.

The amount of COD reduction was % 71.17 and dried material after 72 hours and 116 hours by considering evaporation during the fermentation amounted to 5.85 gr./l and 10.57 gr./l respectively.

To increase the efficiency of the aerobic biological treatment system and increase the production of biomass, it is recommended to use continuous fermentation system in a reactor under the optimum condition obtained by this investigation.

Thursday 19 September 2002

0900 Coupling forces from actin polymerization to membrane trafficking machinery in *Saccharomyces cerevisiae*

DAVID G. DRUBIN
University of California, Berkeley, CA 94720-3202, USA - drubin@uclink4.berkeley.edu

Several lines of evidence have suggested that forces from actin polymerization are used in specific steps of membrane trafficking that might include vesicle budding, fission, and movement of vesicles away from the donor membrane. We have been investigating the cytoskeletal machinery that is responsible for these functions. Abp1 and Pan1 are two actin-binding proteins that are also implicated in endocytic trafficking. Both are components of yeast cortical actin patches. We recently showed that both are activators of the Arp2/3 complex, a major regulator of actin filament nucleation. Further studies show that for Pan1, linkage between the Arp2/3 complex and the endocytic machinery is regulated by the serine/threonine protein kinases Ark1 and Prk1. These kinases appear to negatively regulate Pan1 function as part of a dynamic endocytic pathway. Further studies are aimed at better understanding regulation and dynamics of this system using biochemical and microscopic analysis. A current interest is also how the cytoskeletal machinery associates with membranes.

0940 Spatial and temporal regulation of Cdc42 in yeast

Y. SHIMADA, A.-C. BUTTY, M.-P. GULLI, P. WIGET & M. PETER
ISREC/ETHZ, Ch. des Boveresses 155, 1066 Epalinges, Switzerland

Rho-type GTPases regulate cytoskeletal polarization, cell migration and chemotaxis in eukaryotic cells. In *S. cerevisiae*, activation of Cdc42 by its guanine nucleotide exchange factor Cdc24 triggers polarization of the actin cytoskeleton at bud emergence and in response to mating pheromones. The adaptor protein Bem1 localizes to sites of polarized growth where it interacts with Cdc42, Cdc24 and the PAK-like kinase Cla4. We have isolated Bem1-mutants (Bem1-m), which are specifically defective for binding to Cdc24. The mutations map within a conserved motif termed BCM, which is necessary and sufficient to interact with the OPR-motif of Cdc24. Although the Bem1-m mutant proteins localizes normally, *bem1-m* cells are unable to maintain Cdc24 at sites of polarized growth. As a consequence, they are defective for many forms of apical growth including formation of elongated buds and mating projections. Localization of Bem1 to the incipient bud site requires activated Cdc42, and conversely expression of Cdc42-GTP is sufficient to localize Bem1 to the plasma membrane. Thus, our results suggest that Bem1 functions in a positive feedback loop: local activation of Cdc24 at the polarization site produces Cdc42-GTP, which recruits Bem1. In turn, Bem1 stabilizes activated Cdc24 at the site of polarization, leading to apical growth.

1020 Biochemical and cytological studies of a bacterial actin homologue

J. ERRINGTON
University of Oxford

Abstract not received

1130 Spindle orientation and the timing of sister chromatid separation in fission yeast

Y. GACHET[1], S. TOURNIER[2], J.B.A. MILLAR[2] & J.S. HYAMS[1]
[1]Dept of Biology, University College London, London WC1E 6BT, [2]National Institute for Medical Research, London NW7 1AA

We recently described a spindle orientation checkpoint (SOC) in *S. pombe* that delays entry into anaphase when actin is disorganised [Nature 412, 352 2001]. To further characterise the SOC we filmed living cells expressing tubulin-gfp. Spindle orientation was dependent upon the balanced interaction of astral microtubules with the medial cell cortex. In Lat B, astral microtubules made unproductive cortical interactions, spindles failed to orient and sister chromatid segregation (anaphase A) and spindle elongation (anaphase B) were delayed. Mutants affecting the integrity of the cytokinetic actomyosin ring (CAR) (*cps8*, *myo52Δ*) or astral microtubules dynamics (*cdc11*) showed defective spindle orientation and an inherent activation of the SOC. These results show that fission yeast possesses an active spindle orientation mechanism and that the CAR, in addition to its well characterised role in cytokinesis at the end of anaphase, has an additional, pre-anaphase role in spindle orientation. How a misaligned spindle generates a signal that delays sister chromatid segregation will be discussed.

1210 Fission yeast kin1p is a cortically associated protein kinase that regulates the actin cytoskeleton organization

X. LE GOFF, C. ALLIX & M. PHILIPPE
CNRS UMR 6061 "Génétique et Développement", Université de Rennes I, Faculté de Médecine - 2, Av. du Pr. Léon Bernard - CS34317 - 35043 Rennes Cedex - FRANCE

We are investigating the role of the fission yeast kin1p, a member of the conserved serine/threonine protein kinase KIN1/PAR-1/MARK family implicated in cell cycle, microtubule stability and cell polarity in eukaryotes. The fission yeast *Schizosaccharomyces pombe* is an excellent model system to study cell polarity. Interphase *S. pombe* cells display a polarized actin cytoskeleton at the cell ends where growth resumes. In interphase kin1Δ cells (a kin1 null allele strain), the actin cytoskeleton is delocalized all around the cell cortex. Kin1p interphase function is predominantly required for the G2 bipolar actin organization. In early mitosis, a medial F-actin contractile ring assembles in wild type cells. Kin1Δ mitotic cells misplaced F-actin ring assembly. The position of the F-actin ring defines the site of septum synthesis at the end of mitosis and hence the site of cell division. Offset actin ring assembly in kin1Δ cells resulted in an asymmetric cell division leading to two unequally sized daughter cells. Immunolocalization experiments showed that kin1p is cortically associated but its distribution changes according to the cell cycle. Kin1p is localized in actively growing areas (cell ends) in interphase and at the division site during cytokinesis. Overproduction of kin1p resulted in a loss of cell polarity through actin cytoskeleton disorganization. Our data and previous studies on members of the KIN1/PAR-1/MARK family suggest a role of these kinases in controlling cell polarity at the cortex.

1225 In *Candida albicans*, Gin4p is required for the formation of a septin ring within the hyphal germ tube but not for the septin ring at its base

STEVEN BATES[1†], RAY WIGHTMAN[1], HELEN CRAMPIN[1], CHERYL GALE[2], JUDITH BERMAN[2], MARYAM GERAMI-NEJAD[2] & PETER SUDBERY[1]
[1]Dept of Molecular Biology and Biotechnology, University of Sheffield, Western Bank, Sheffield S10 2TN - Tel 0114 2226186 - email: P.Sudbery@shef.ac.uk, [2]Genetics and Cell Biology, University of Minnesota, 250 Biological Sciences Centre, St Paul MN 55108, USA - †Current address: Oxford GlycoSciences, 10 The Quadrant, Abingdon, Oxfordshire OX14 3YS

During the formation of hyphae in the human fungal pathogen *Candida albicans*, a septin ring forms at the base of the

developing germ tube and a second brighter and more organised ring formed within the growing germ tube. Gin4p was found to be required for the formation of the septin ring within the hypha but not for the septin ring at the base of hyphae, suggesting that a developing hyphal germ tube may not be a modified bud but may be more analogous to a mating projection in *S.cerevisiae*. Despite the lack of septin rings, nuclear migration and mitosis occurred normally in Gin4p-depleted hyphae. Thus septin rings within the germ tube are not required for hyphal growth and do not determine the site of mitosis. The behaviour in time lapse videos of Myo1p, Cdc3p, Spa2 and Abp1 was studied using YFP fusions. Myop localises to the site of septation in yeast hyphae and pseudohyphae, at the time of mitosis rather than G1. This is in contrast to *S.cerevisiae* where Myo1 localises to the bud neck in G1.

1400 Characterisation of the *S. pombe* SIN protein cdc11p

ANDREA KRAPP, ELENA CANO & VIESTURS SIMANIS
Swiss Institute for Experimental Cancer Research (ISREC)
1066 Epalinges, Switzerland

The signal for the onset of septum formation in the fission yeast *Schizosaccharomyces pombe* is transduced by the septation initiation network (SIN). Many of the components of the SIN are located on the spindle pole body during mitosis, from where it is presumed that the signal for septum formation is delivered. Cdc11 mutants are defective in SIN signalling, but its role in the pathway has remained enigmatic. Cdc11p most closely resembles *S. cerevisiae* Nud1p, and is essential for septum formation. Cdc11p is a phosphoprotein, which becomes hyper-phosphorylated during anaphase. It localises to the spindle pole body at all stages of the cell cycle, in a sid4p dependent manner, and cdc11p is required for localisation of all the known SIN components, except sid4p, to the SPB. Cdc11p and sid4p can be co-immunoprecipitated from cell extracts. Finally, like its *S. cerevisiae* orthologue Nud1p, cdc11p is involved in proper organisation of astral microtubules during mitosis. Our data suggest that cdc11p acts as a bridge between sid4p and the other SIN proteins, mediating their association with the spindle pole body. We are now investigating how cdc11p is regulated. In particular, we are investigating which kinases are responsible for phosphorylating the protein, and the role played by the various domains of cdc11p. We will present the results of these studies.

1440 The roles of the fission yeast myosins in cytokinesis

DANIEL P. MULVIHILL & JEREMY S. HYAMS
Dept of Biology, University College London, London WC1E 6BT

Myosins are evolutionarily conserved motor proteins which associate with actin, and which participate in a variety of diverse cellular functions. The genome of the fission yeast *S. pombe* contains 5 myosins from 3 separate classes (I, II, & V). All 5 myosins localise to the division site, either as components of the cytokinetic actomyosin ring (CAR) (Myo2, Myp2, Myo51), or in the formation of the septum (Myo1, Myo52). We have been investigating the roles of each of these myosins. Myo2, a type II myosin, is the only myosin essential for growth and is a key component of the CAR. We have used a strain in which the sole genomic copy of the *myo2*$^+$ gene has been tagged with gfp cDNA to examine the timing of CAR formation and contraction in the absence each of the other myosins. We will present data to demonstrate that whilst the speed of ring contraction does not vary in the absence of the CAR components Myp2 (type II) and Myo51 (type V), it appears less stable. However in the absence of Myo52 (type V) the CAR contracted more slowly and erratically, indicating that CAR contraction is tightly coupled to septum formation.

1600 Triggering cell division in fission yeast: the spindle pole and protein kinases

IAIN HAGAN, ALAN BRIDGE, ESTHER JOLLEY, FIONA H. MACIVER, JANNI PETERSEN, AGNES GRALLERT, DANIEL MULVIHILL & KAYOKO TANAKA
Paterson Institute for Cancer Research, Manchester

Commitment to mitosis is regulated by the protein kinase MPF. MPF is inhibited by phosphorylation on its catalytic sub-unit by protein kinases related to Wee1. Removal of this phosphate by Cdc25 promotes entry into mitosis. Full scale commitment to mitosis involves the promotion of Cdc25 activity and downregulation of Wee1 in a positive feedback loop that is triggered by active MPF and involves the protein kinase polo. Fission yeast *cdc25.22* mutants can divide only if either *wee1*$^+$ or *cut12*$^+$ are also mutated. *cut12*$^+$ encodes an essential spindle pole body component. Recessive *cut12* mutants block spindle formation while the dominant *cut12.s11* mutant permits division of cells which lack Cdc25. The polo kinase Plo1 is prematurely recruited to the interphase SPB of *cut12.s11* mutants. This recruitment, and the suppression of *cdc25.22* by *cut12.s11* is dependent upon the function of the NIMA related kinase Fin1. Recessive temperature sensitive *fin1* mutants block spindle formation while increasing *fin1*$^+$ transcription promotes the association of Plo1 with interphase SPBs of wild type cells and raises the restrictive temperature of both *cut12.1* and *cdc25.22* mutants. These data suggest that the localisation of key protein kinases to the SPB plays a critical role in driving commitment to mitosis.

1640 Mitotic exit is regulated by multiple pathways

E. SCHIEBEL
The Beatson Institute for Cancer Research, Glasgow

Abstract not received

1720 The centrosome, a cell individuation organ

MICHEL BORNENS
CNRS-Institut Curie, Paris, France

In agreement with the early view from Boveri according which the centrosome (CTR) would coordinate nuclear and cytoplasmic divisions, the injection of a CTR in a Xenopus oocyte suffices for orchestrating cell cleavage and allowing parthenogenetic development. The centrosome-microtubule system (CTR-Mts) is thus a major agent of cell morphogenesis. The presence of a centriole pair in the CTR from animal cells is instrumental in organizing centrosomal components into a structurally stable organelle. As an approach to better analyze CTR behavior and duplication, several vertebrate stable cell lines expressing GFP-labeled centrin have been generated. The fusion protein concentrates in the lumen of both centrioles, making them clearly visible in the living cell. Time-lapse fluorescence microscopy has revealed that the centriole pair inherited after mitosis splits during or just after telophase. At this time the mother centriole remains near the cell center while the daughter migrates extensively throughout the cytoplasm. While both centrioles possess associated γ-tubulin, and nucleate similar number of Mts in Mt-repolymerization experiments, only the mother centriole, where the anchoring protein ninein is concentrated, is located at the focus of the Mt array during G1 and S. We will show that this pattern has important implications for cell integration during cell migration.

A critical step in the cell division cycle is when two recently divided cells have resumed spreading and locomotory activity but are still connected by a cytoplasmic bridge. Investigating the behavior of the CTR at that moment revealed a transient post-anaphase repositioning of the mother centriole which apparently controls the release of central Mts from the midbody and the completion of cell division. Moreover, absence of the CTR leads to cytokinesis defects. Together with other results in yeasts and animal cells, our data point to a conserved CTR-dependent pathway integrating spatial controls into the decision of completing cell division and of starting a new cell division cycle. We will present recent results relevant to this pathway.

Altogether, the data show that the asymmetric organisation of the centriole pair, which could reflect the constraints on the duplication mechanism, allows a specific and maturation-dependent role for each centriole during cell locomotion or cell division. They shed new light on how activities of the CTR and of the cell cortex are coupled during cell division and cell locomotion. We will argue that they suggest a critical role of the CTR for cell individuation.

Friday 20 September 2002

0900 New functions for gamma-tubulin

NATALIE PRIGOZHINA, CASEY JOWDY, C. ELIZABETH OAKLEY & BERL R. OAKLEY
Dept of Molecular Genetics, Ohio State University, 484 W. 12th Ave., Columbus, Ohio, 43210, USA

γ-tubulin plays an important role in microtubule nucleation, but recent results from several labs suggest that it plays an equally important, but as yet incompletely defined, role in cell cycle regulation. We have previously created several conditionally lethal γ-tubulin mutations in *Aspergillus nidulans* by alanine-scanning mutagenesis. We now report a detailed analysis of the phenotype of one of these mutations, *mipA*D159. Immunofluorescence microscopy of synchronized material reveals that, at restrictive temperatures, spindle formation is similar to wild-type controls. Nuclei remain in mitosis slightly longer than wild-type controls, but most nuclei re-enter interphase without dividing. In many nuclei, chromosomes are strung-out along the spindle indicating an inhibition of anaphase A. In time-lapse observations of chromosomal movement with a histone H1-GFP fusion, a variety of defects was seen, including inhibition of anaphase A, premature mitotic exit, non-disjunction, severely stretched chromosomes and abnormal nuclear movement after mitosis. Chromosomal movement was evident in all nuclei, indicating that microtubules attached to, and exerted force on, the chromosomes. These data suggest that mipAD159 causes failure of multiple checkpoints and suggest that γ -tubulin plays an important role in the regulation of mitotic progression.

0940 Eta-tubulin and basal body duplication

F. RUIZ[1], P. DUPUIS-WILLIAMS[2], C. KLOTZ[1], F. FORQUIGNON[1], J. BEISSON[1] & F. KOLL
[1]Centre de Génétique Moléculaire, CNRS, 91198, Gif-sur Yvette, France, [2]Ecole Supérieure de Physique et Chimie Industrielles, 10 rue Vauquelin, 75005 Paris, France

The thermosensitive alllelic mutations *sm19-1* and sm19-2 of *Paramecium* cause defective basal body duplication at the non-permissive temperature without affecting progression of the cell cycles. Complementation cloning of the *SM19* gene identified a new tubulin, eta-tubulin (Ruiz et al, Curr Biol 2000, 10: 1451-1454), showing low homology with either of the other 5 tubulins - α to ε - characterized in *Paramecium*.. By screening of suppressors of the *sm19-1* mutation and looking for genetic interactions with mutations affecting microtubules, we have shown that *sm19-1* interacts with mutations in two different β-tubulin genes and with microtubules. By a refined cytological analysis of the pattern of basal body duplication, which proceeds in two successive "waves" during division, we established that the mutations only block the first wave involving ca 60% of the total basal body complement (ca 4400 basal bodies per cell) and leaves the second wave unaffected. It remains to ascertain which microtubular structure, basal bodies or other, η-tubulin binds. Eta-tubulin can thus be envisaged as a microtubule-associated receptor of a temporally restricted mitotic signal specific of basal bodies.

1020 The cytoskeleton and trypanosome morphogenesis

K. GULL
University of Manchester

The flagellate protozoan *Trypanosoma brucei* is characterised by a shape and form imposed by an internal microtubule cytoskeleton. The trypanosome exhibits a cell polarity defined by a flagellum attached to the cell body which transects a left-handed helix from posterior to anterior. This left-handed helix is mirrored internally by a unique set of mirotubules and filaments constituting the flagellum attachment zone along with a subpellicular corset of microtubules.

We have used genomic, RNA interference and proteomic/mass spec approaches to study the involvement of proteins in flagellum ontogeny and cell morphogenesis.

These approaches have revealed new cytoskeletal constituents such as unusual tubulins, flagellum constituents and microtubule associated proteins. In addition we have shown that cell polarity and form is defined, in part, by epigenetic phenomena such as cytotaxis. In this case a unique flagellum connector links the tip of the new flagellum to the lateral aspect of the old – so enabling new cellular structure to acquire structural position and polarity from the existing cell.

We will illustrate the structural organisation of the cytoskeletal elements, their protein constituents and functions and dependency relationships.

1130 The hen and the egg problem: Myosin and actin polymerisation

THIERRY SOLDATI, EVA SCHWARZ & CLAUDIA KISTLER
Dept of Molecular Cell Research, Max-Planck Institute for Medical Research, Heidelberg, Germany and Dept of Biological Sciences, Imperial College of Science, Technology and Medicine, London

The components of the molecular machineries responsible for directed cell migration are evolutionarily conserved and their molecular and cellular dissection in *Dictyostelium* is directly relevant to higher organisms. Like some other myosin I, *Dictyostelium* MyoK localizes to dynamic regions of the actin cortex. Despite the potential functional redundancy between the seven myosin I in *Dictyostelium*, *myoK* null cells are impaired in chemotactic motility and phagocytosis. MyoK is unique as it virtually lacks a tail domain but carries in a surface loop of its motor domain a 150 residues insertion rich in Gly, Pro and Arg, the GPR-loop. Most strikingly, the GPR-loop shares 40 to 50 % identity with the Pro-rich domain of WASp family members. The GPR-loop binds F-actin in a salt-dependent fashion but also contains at least four Pro-rich motifs (pPPø) shown to bind the profilin-actin complex. In addition, it contains an SH3 binding motif (RxxPxxP) that binds Abp1, a recruiter and activator of the actin nucleator Arp2/3, and possibly another complex containing the actin-binding protein EF1A. Also, MyoK is the first myosin targeted to the plasma membrane by C-terminal farnesylation. Our working model conceives MyoK as an integrator of force production, actin polymerisation and regulation by signaling cascades.

1210 Regulation of fission yeast polarity by microtubules, tea1p and mod5p

K. SAWIN, P. LOURENCO & H. SNAITH
Wellcome Trust Centre for Cell Biology, Edinburgh University

Fission yeast microtubules regulate patterns of polarised growth. Here we describe mechanisms and molecules involved in this regulation. We show that microtubules are not required for the maintenance of polarised growth. However, if polarity is transiently disturbed, the position of polarity re-establishment in the cell will reflect the intracellular microtubule distribution. In addition, the communication of positional information from cytoplasmic microtubules to the cortical cell polarity machinery requires the protein tea1p, which thus provides a critical link between the microtubules and the actin cytoskeleton. We have also carried out a screen to identify additional regulators of polarised growth. This identified a novel protein, mod5p, which regulates tea1p. In wild-type cells, tea1p is transported by microtubules to cell ends, where it anchors to the cortex. By contrast, although tea1p is transported along microtubules in mod5- mutants, it fails to anchor. Mod5p is found at cell ends, dependent on C-terminal prenylation. We suggest that mod5 is a

membrane receptor or unloading factor for tea1p; preliminary evidence suggests that they may interact physically, although this interaction may be weak and/or transient. Interestingly, Mod5p is mislocalised in tea1- mutants, suggesting that high-fidelity polarised growth may involve a positive feedback loop between tea1p and mod5p.

1225 ε -and ζ-tubulin in *Trypanosoma brucei*

SUE VAUGHAN, PAUL McKEAN, ANDREA BAINES & KEITH GULL
University of Manchester, Division of Biochemistry, Oxford Road, Manchester M13 9PT

The cortical microtubule cytoskeleton of the protozoan parasite *Trypanosoma brucei* remains intact during the cell cycle. A precisely ordered series of cytoskeletal events produces two daughter cells and central to this process is the duplication and inheritance of basal bodies. Microtubules, composed of heterodimers of α/β-tubulin are nucleated by γ-tubulin at microtubule organising centres, such as basal bodies. Seven families of tubulin have been reported and many of the most recently identified family members have been implicated in basal body duplication/inheritance. We identified ε -and ζ-tubulin in *T. brucei* and RNA interference experiments of ε-tubulin resulted in cells that failed to form a new flagellum during the cell cycle. In addition, cells also failed to correctly position the flagellar pocket and incorrect positioning of the cleavage furrow led to divisions resulting in different sized daughter cells. By the use of anti-peptide antibodies, *T. brucei* ζ-tubulin was localised between the mature and immature basal bodies. Analysis of temporal localisation during the cell cycle showed ζ-tubulin to be a new molecular marker in basal body duplication in *T. brucei*.

1400 The nuclear migration protein NUDF mediates dynein binding to microtubules

N. RONALD MORRIS, BERND HOFFMANN & STEPHANE GROSS
UMDNJ – Robt Wood Johnson Medical School, 675 Hoes Lane, Piscataway, NJ, USA

Our laboratory has used the fungus *Aspergillus nidulans* to study the function of cytoplasmic dynein, dynactin, and other proteins required for dynein function. NUDF, a protein required for nuclear migration through the cytoplasm closely resembles LIS1, a protein required for neuronal migration to the human cerebral, and evidence suggests that both affect dynein function *in vivo*. As dynein mediates vesicle migration in fungi, we prepared *Aspergillus* vesicles and asked whether NUDF depletion affects dynein-mediated vesicle function *in vitro* and if so, how? NUDF depletion caused a defect in dynein-mediated vesicle binding and migration on microtubules that was not reversed by addition of pure NUDF, but was reversed by a supernatant fraction from either *Aspergillus* or rat neurons plus NUDF. The active factor in the neuronal supernatant was identified as CLIP-170. A similar 170 kDa protein was found in the *Aspergillus* supernatant. NUDF depletion decreased the amount of *Aspergillus* and neuronal CLIP-170 associated with dynein-mediated vesicles. CLIP-170 could be restored to its normal level by addition of the purified 170 kDa proteins, but only in the presence of NUDF. These data suggest that NUDF acts as a linker between CLIP-170 and dynein/dynactin to enhance dynein-mediated vesicle binding to and migration on microtubules.

1440 Intraflagellar transport and disease

JOEL ROSENBAUM[1], GREG PAZOUR[2], GEORGE WITMAN[2], JOSEPH BESHARSE[3], DOUG COLE[4] & MAUREEN BARR[5]
[1]Yale University, [2]University of Massachussetts Medical Center, [3]Wisoconsin Medical School, [4]University of Idaho, [5]University of Wisconsin-Madison, USA

Intraflagellar Transport (IFT), a motility process unrelated to flagellar beating was first observed in the flagella of the bi-flagellate alga *Chlamydomonas*. Particles between the flagellar axoneme and cell membrane can be observed by DIC microscopy to be moving continuously to the flagellar tip at ca. 2.0 u/sec, and from tip to base at 3.5 u/sec. The movements are powered by kinesin-II and cytoplasmic dynein 1B, respectively. IFT is absolutely essential for the assembly and maintenance of all eukaryotic cilia and flagella, whether they are motile (9 +2) or immotile primary (9+0) cilia. This includes the primary cilia which can be found on G-0 cells in most tissues of vertebrates. The functions of these primary cilia are largely unknown, other than the retinal rod and cone outer segments which are clearly involved in phototransduction. New studies, including several on the sensory cilia of the worm *C. elegans*, now indicate that the primary cilia are almost certainly involved in sensory perception (chemo, mechano, photo) and certain channels and receptors have been localized to them, eg. the somatostatin-3 receptors in brain cells, and certain ion channels. By analyzing the genes involved in IFT it has now been shown that mutations in either the motors or IFT polypeptide genes, which cause ciliary assembly/maintyenance defects, are related to certain human diseases. Among these diseases are polycystic kidney disease (PKD), in which the polycystins (cation channels) have been shown to be positioned on the primary clia of kidney tubule cells; defects in kidney primary cilia formation which in turn do not permit proper targeting of these polycystin channels to the primary cilia, result in PKD. Likewise, the assembly and maintenance of the rod and cone outer segments, which are derived from primary cilia, is dependent on IFT, and mutations in the IFT process result in rod/cone degeneration and blindness. The condition known as situs inversus, in which the organs of vertebrates are positioned on the wrong side of the body midline, can be traced to the proper assembly and function of the nodal cilia on the early embryo: failure of these cilia to form because of defects in IFT results in situs inversus. The general cell/molecular biology of IFT will be reviewed along with a description of how studies on the bi-flagellate alga *Chlamydomonas* led to the various disease insights.

POSTERS:

EUK 01 Proteomic analysis of the Gcn4p-mediated response in *Saccharomyces cerevisiae* and *Candida albicans*

L. SELWAY[1], D. STEAD[1], J. WALKER[1], Z. YIN[1], G. TRIPATHI[1], T. MCLNERNEY[2], P. CASH[1] & A.J.P. BROWN[1]
[1]Aberdeen Fungal Group, Institute of Medical Sciences, University of Aberdeen, Foresterhill, Aberdeen AB25 2ZD, [2]Nonlinear Dynamics Ltd, Tyne House, 26 Side, Newcastle upon Tyne NE1 3JA

In *Saccharomyces cerevisiae* transcription of genes on all amino acid biosynthetic pathways is stimulated in response to starvation for a single amino acid. This transcriptional activation is mediated by *GCN4*. The synthesis of the transcriptional activator Gcn4p is regulated by the availability of amino acids. When cells are grown under conditions of amino acid limitation, an elevation in the cellular amount of Gcn4p is accomplished through an increased translation of *GCN4* mRNA. We have isolated a functional homologue of *GCN4* from the pathogenic fungus *Candida albicans*. In this study we have compared the Gcn4p-mediated response in these two fungi at the proteome level. This study showed that amino-acid starvation regulated a wide range of functions in both *Saccharomyces cerevisiae* and *Candida albicans*. In *S. cerevisiae*, we established the identity of 9 proteins that were repressed and 23 proteins that were induced significantly (of which 22 are involved in amino-acid metabolism). In *C. albicans*, we established the identity of 23 proteins that were repressed and 31 proteins that were induced significantly (of which 6 are involved in amino-acid metabolism).The data suggest that a common amino-acid starvation response is shared in *S. cerevisiae* and *C. albicans*. P.S. *This is a demonstration project of COGEME [Consortium for the Functional Genomics of Microbial Eukaryotes]*.

EUK 02 Genome analysis provided by COGEME (Consortium for the Functional Genomics of Microbial Eukaryotes)

Z. YIN[1], R. BEYNON[3], A. BRASS[5], A.J.P. BROWN[1], P. CASH[1], M. CORNELL[5], S. GASKELL[4], A. HAYES[5], N.W. PATON[5], I. RIBA-GARCIA[4], L. SELWAY[1], D. STEAD[1], N. TALBOT[2], C. TAYLOR[5], J. WALKER[1] & S.G. OLIVER[5]
[1]University of Aberdeen, [2]University of Exeter, [3]University of Liverpool, [4]UMIST, [5]University of Manchester

COGEME is funded by the UK Biotechnology and Biological Sciences Research Council under the Investigating Gene Function Initiative. COGEME is led by Professor Steve Oliver (Manchester University), and is run by a Steering Committee chaired by Professor Mick Tuite (University of Kent, Canterbury).

COGEME is establishing national state-of-the-art facilities for the analysis of the transcriptome and proteome of *Saccharomyces cerevisiae*. In addition to their primary role in serving the UK yeast research community, COGEME is providing facilities of genome analysis for a number of important organisms among the yeasts and fungi. These will include both plant and human pathogens.

COGEME consists of:

- Transcriptome Research Facility (TRF); Steve Oliver, Manchester University
- Proteome Research Facility 1 (PRF1); Al Brown & Phil Cash, Aberdeen University
- Proteome Research Facility 2 (PRF2); Simon Gaskell, UMIST
- Bioinformatics Centre; Norman Paton, Manchester University

The TRF is using gene arrays to generate transcript profiles under the conditions of interest. PRF1 is using 2-D gel analysis followed by MALDI mass spectroscopy to identify proteins whose levels change under the conditions of interest. PRF2 complements the activities of PRF1 by using novel chromatographic separations and advanced MS analyses to enhance protein identification. The Bioinformatics Centre is performing data management and analysis.

EUK 03 The *Cre-lox* system: an approach for gene disruption in *Candida albicans*

PAUL DENNISON, MARK RAMSDALE & ALISTAIR BROWN
Aberdeen Fungal Group, Institute of Medical Sciences, University of Aberdeen, Foresterhill, Aberdeen AB25 2ZD

Our lab is developing a new gene disruption system for the diploid pathogenic yeast *Candida albicans* based on the *Cre-lox* system used in *Saccharomyces cerevisiae*. The system utilises two auxotrophic markers from *C. albicans*, HIS1 and URA3. The addition of flanking 34 bp *loxP* elements, creating LHL2 (*loxP*-HIS-*loxP*) and LUL2 (*loxP*-URA-*loxP*), enables the markers to be recycled. The cassettes are targeted to a specific ORF by the PCR-mediated addition of 5′ and 3′ homologous sequences. Genotypes of selected ura^+, his^+ transformants are confirmed by PCR diagnosis. An artificial codon-optimised *Cre* recombinase has been constructed, which catalyses site-specific recombination between the *loxP* elements; excising the marker and leaving one *loxP* element integrated at the target locus. The benefits of the system will be: (a) control over marker recycling (b) high efficiency of the *Cre* recombinase and (c) the reduced use of an expensive mutagenic medium (5-FOA). There is also a reduced chance of reintegration of cassettes at previous integration sites due to the small size (34 bp) of the remaining *loxP* element, thereby allowing multiple gene knockouts.

EUK 04 Regulation of a hypha-specific gene in *Candida albicans*

ABIGAIL MAVOR, JILL WISHART, SUSAN MACASKILL, MELISSA STRAFFON, MUNIR MURAD & ALISTAIR BROWN
Aberdeen Fungal Group, Institute of Medical Sciences, University of Aberdeen, Foresterhill, Aberdeen AB25 2ZD

Morphogenesis in *Candida albicans* is influenced by numerous environmental conditions including temperature, ambient pH and serum. Several hypha-specific genes have now been identified in *C. albicans*, which are activated specifically during hyphal development irrespective of the environmental stimulus. These include *ECE1, HWP1, HYR1, ALS3* and *ALS8*. The *ALS8* gene encodes an adhesin in the Agglutinin-Like Sequence family. Using the *Renilla reniformis* luciferase reporter, we are investigating the *cis* and *trans*-acting factors that regulate *ALS8*. *ALS8* expression is subject to both positive and negative regulation. *ALS8* is repressed by Nrg1 and Tup1. These *trans*-acting factors act via two Nrg1 Response Elements [(A/C)(A/C/G)C_3T] in the *ALS8* promoter. *ALS8* activation is dependent upon Efg1. A 150 bp region of the *ALS8* promoter has been shown to mediate this hypha-specific gene activation. Interestingly, this region mediates activation both by serum and pH signals. We are currently mapping the *cis*-acting elements that are responsible for this positive regulation of *ALS8* and developing a model describing the mechanisms by which the developmental regulation of *ALS8* is executed in *C. albicans*.

EUK 05 *CaMSN2* and *CaMSN4*: roles in stress response and morphogenesis

MELISSA STRAFFON, SUSAN MACASKILL, SUSAN NICHOLLS & ALISTAIR BROWN
Aberdeen Fungal Group, Institute of Medical Sciences, University of Aberdeen, Aberdeen AB25 2ZD

A number of environmental conditions trigger the morphogenetic switch from yeast phase to hyphal development in *Candida albicans*. Many of these conditions involve placing cells under stress (eg thermal, nutritional, oxidative or pH stress). Hence, stress responses and morphogenesis in *C. albicans* appear to be intimately linked. For this reason we are examining stress responses in this fungus. In *Saccharomyces cerevisiae*, the general stress response is mediated *via* the (partially redundant) transcription factors Msn2p and Msn4p, which act *via* Stress Response Elements (STRE). A *S. cerevisiae msn2*, *msn4* strain shows sensitivity to a variety of severe stress conditions. Two genes containing sequence similarity to the *S. cerevisiae MSN2/4* zinc finger DNA-binding region have been identified in the *C. albicans* genome, and tentatively named *CaMSN2* and *CaMSN4*. *C. albicans msn2*/*msn2*, *msn4*/*msn4* single and double mutants have been constructed. Preliminary results indicated that the mutants show no significant phenotypic difference from the wild type when placed under a variety of stress conditions. Hyphal development also does not appear to be significantly affected under the conditions tested. The promoter regions of many *C. albicans* hyphal-specific genes contain STRE-like sequences. A *lacZ*-reporter construct is being used to examine whether *CaMSN2* and/or *CaMSN4* are able to regulate transcription *via* STRE-like elements, and whether this regulation is induced under stress conditions that promote hyphal development.

EUK 06 Cellular morphogenesis in trypanosomes: structural characterisation of the flagella connector

LAURA BRIGGS, JACKIE DAVIDGE, PAUL McKEAN, FLÁVIA F. MOREIRA-LEITE, ANDREA BAINES, SUE VAUGHAN & KEITH GULL
University of Manchester, School of Biological Sciences, Oxford Road, Manchester M13 9PT

In the procyclic form of *Trypanosoma brucei*, a single flagellum exits the flagellar pocket at the posterior end of the cell and is attached along the length of the cell body. The flagellum follows a left-handed helical pattern from posterior to the anterior end of the cell. During cell division, the tip of the new flagellum is connected to the side of the old flagellum. This attachment is

mediated by a structure we have termed the flagella connector which is involved in templating structural information from the old to the new cell, a concept known as cytotaxis. Transmission electron microscopy reveals five main sub-domains within the flagella connector. The flagella connector has the form of a unique junctional complex linking the tip of the new flagellum axoneme, through two flagella membranes to the lateral aspect of the old flagellum axoneme. Specific sub-domains can be detected by immunogold electron microscopy. Use of specific antibodies also revealed the timing and mechanisms of appearance and disappearance of the structure of the cell cycle.

EUK 07 Fission yeast mod5p regulates localisation and dynamics of tea1p

HILARY SNAITH, PAULA LOURENÇO & KENNETH SAWIN
Wellcome Trust Centre for Cell Biology, University of Edinburgh, Mayfield Road, Edinburgh EH9 3JR

Fission yeast grow in a bipolar fashion. A key regulator of this process is the tea1p protein. tea1p is transported along microtubules to both ends of the cell, where it is involved in establishing a zone of polarised growth. From an insertional mutagenesis screen to identify new genes regulating morphology in *S. pombe* we isolated *mod5+* (*morphology defective*), which is required for normal cell shape and for the proper localisation of tea1p. mod5p is a novel protein containing a C-terminal prenylation motif. In Δ*mod5* cells, tea1p is transported to the cell ends, but instead of decorating the cell ends uniformly, tea1p remains bound to the tips of the microtubules. Interaction of mod5p with tea1p is also demonstrated during re-establishment of polarised growth following recovery from stationary phase. Normally, approximately 10% of Δ*mod5* cells form branches during this recovery. However if Δ*mod5* cells are returned to growth in the presence of microtubule poisons, the percentage of branched cells dramatically rises to approximately 75%, phenocopying Δ*tea1* cells. Thus without microtubules, the absence of mod5p prevents even small amounts of tea1p from localising to cell ends, thereby causing branching. Furthermore, mod5p itself is localised specifically at cell ends, dependent on the presence of its C-terminal prenylation signal. These results are consistent with a model in which tea1p is transported to cell ends by microtubules, is subsequently retained there in a process mediated by mod5p.

Tuesday 17 September 2002

0930 Provision of recombinant proteins for research – an industrialists view of the expression systems available

IAN TAYLOR
AstraZeneca, Enabling Science & Technology, Alderley Park, Macclesfield, Cheshire SK104TG

The modern drug discovery process exploits recombinant protein production either directly as Biotherapeutic agents in their own right or as a tool to find small molecule drug leads. This paper will concentrate on the latter, describing how the choice of expression system can influence the success of delivering biologically active protein for screens or structural chemistry studies. It will focus on expression from E. coli and how either adapting the fermentation process or using an "in house" vector can overcome shortcomings in commercial vectors. Reference will be made to the various uses of recombinant protein in drug discovery, the differences in quality required and how these needs are met in this multidisciplinary environment.

1010 Molecular genetics in the industrial production of plasmid DNA

AMANDA M.E. WEISS, ROCKY M. CRANENBURGH, JULIAN A.J. HANAK
Cobra Biomanufacturing, Stephenson Building, The Science Park, Keele, Staffs ST5 5SP

The large-scale production of recombinant biotherapeutics provides significant process and regulatory challenges to the biotechnology industry in order to meet the regulatory agencies stringent requirements in a cost effective manner. The use of antibiotic resistance genes is rapidly becoming unacceptable in many areas of biotechnology, particularly in DNA vaccine and gene therapy applications. Plasmid-borne antibiotic resistance genes cause considerable metabolic burden to the host bacterial cell. The resistance gene product or residual antibiotic contamination can induce an immune response or cytotoxicity in patients. Risk of antibiotic resistance genes spreading from GMO's to environmental pathogens is also of concern. We have constructed novel *Escherichia coli* strains that facilitate the antibiotic-free selection and stable maintenance of recombinant plasmids in complex media.

Another difficulty associated with the manufacture of plasmid DNA is the removal of the host cell-related impurity RNA following cell lysis. We have constructed a modified *Escherichia coli* JM107 plasmid host containing a bovine pancreatic ribonuclease expression cassette integrated into the host chromosome at the *dif* locus. The RNaseA protein is stable throughout incubation at high pH and acts to hydrolyse host cell RNA following neutralization of alkaline lysis. This provides a suitable methodology for the removal of RNA, whilst avoiding addition of exogenous animal sourced RNase and its associated regulatory requirements.

1120 Contract fermentation process development – How to culture somebody else's ideas

GUSTAV SILFVERSPARRE & LISELOTTE LARSSON
BioGaia Fermentation AB, P.O.Box 965, St. Lars väg 47, S-220 09 Lund, Sweden

Pharmaceutical products normally must go through approximately 7-8 years of toxicological and clinical testing before an approval. During the 1990's the average drug development time from synthesis to approval was approximately 14 years. Since "time to market" is very important to maximise patent protection and competitiveness the time allocated for the development phase is often very short. Additionally, the difficulties associated with a complete characterisation of biopharmaceuticals puts high demands on the "batch to batch" reproducibility for the process. To meet the regulatory demands the process needs to be well characterised and documented already during early clinical phases and this further decreases the time available for and the "degrees of freedom" during process development.

To meet these constraints, the development of the fermentation, recovery and purification processes must partly overlap in time and process robustness is hence often of higher value than optimal yields. The development of general proprietary technologies to be used in the manufacturing process adds value for customers and increases the company competence, that together with experience, routines and flexibility are key parameters for successful contract process development.

1200 Scale up of filamentous fermentations

STUART MICHAEL STOCKS
Novozymes A/S, Krogshoejvej 36, 8XA1.10, DK-2880 Bagsværd, Denmark

Novozymes A/S is the largest producer of technical enzymes world wide. The company makes extensive use of GM and non GM filamentous micro organisms in gassed agitated submerged fermentation processes. Examples include both fungi (*Aspergillus oryzae, Aspergillus niger, Fusarium venenatum*) and bacteria (e.g. *Streptomyces murinus*) producing enzymes for use in a spectrum of applications in the process industries including detergents, food, feed, and paper. Filamentous organisms are selected because they are naturally adapted to secrete their products into the medium, greatly reducing the effort required in recovery of the enzymes. In comparison to the health care sector, the regulatory aspects of bringing new products to market, or optimising existing production processes, are less demanding. This leads to a highly dynamic R & D effort with a "first time right" concept to scale-up in ambitiously small time frames. Due to the filamentous morphology and consequent high viscosity of the fermentation broth, the main challenges in the transfer of a process from laboratory scale to production scale are related to seed production and oxygen mass transfer.

In addition to classical approaches to seed scale up and mass transfer, examples of counter intuitive but beneficial behaviour can often be exploited.

1345 Process evolution from laboratory to clinical production: Scale-up issues and process integration

BRENDAN FISH
Cambridge Antibody Technology Ltd., Franklin Building, Granta Park, Great Abington, Cambridge CB1 6GD

The presentation will provide an overview of CAT's core technologies and describe how this technology is used to create human monoclonal antibodies. The strategies and issues associated with purification development at CAT will also be considered.

- Areas to be discussed in the presentation include:
- An overview of CAT's core technologies
- Generation of human monoclonal antibodies
- Development of scaleable purification methods
- Purification development strategy employed at CAT
- Tailoring generic processes and example results with this approach.

1425 Integrated molecular design and bioprocess engineering

A.P.J. MIDDELBERG, J.C. MILLER, G. MORREALE & H. LANCKRIET
Dept of Chemical Engineering, University of Cambridge, Pembroke St, CB2 3RA

There is a disconnection between molecular biology and engineering that can, on occasion, result in a grossly sub-optimal bioprocess. Decisions in molecular biology are often made for cloning convenience without regard to their ultimate impact on bio-separation. In this work we outline an alternative and superior approach whereby the molecular design specifies, and is specified by, bio-separation considerations. Through integrated and iterative design we are able to specify a globally optimal solution for specific polypeptide products. In this work we illustrate this philosophy by considering the production of two specific peptide sequences. Both peptides have interesting surfactant or self-assembly characteristics, and very different properties. The peptides are expressed in *E. coli*, with specific cleavage and purification handles engineered in at the cloning stage. By careful molecular design we are able to ensure that the product has, at each stage of the bio-separation process, a unique and practical separation metric (e.g. an affinity handle or a unique isoelectric point). These molecular decisions are made to ensure that economic unit operations can be used for bio-separation (e.g. ion-exchange chromatography). The results demonstrate the power of carefully optimising the molecular design with respect to global process considerations.

1530 Technology transfer – from development to successful manufacturing

BRUCE R. WILLIAMS
Avecia Biotechnology, P.O. Box 2, Belasis Avenue, Billingham TS23 1YN

The benefits of having a systematic methodology for transferring technology between two parties are widely recognised in industry and regulatory bodies such as the Food and Drug Administration (FDA). Formal technology transfer of mature products between two manufacturing sites is common. However, there are also considerable business benefits to be gained from applying the technology transfer methodology at the development stages of the product lifecycle, not least in providing a framework for the different technical disciplines and business functions to interact. This is particularly the case for biopharmaceutical processes due to their complexity.

The tools for a successful technology transfer are simple – having an agreed procedure, using checklists to prompt key issues to be addressed, and producing a clearly documented package of information which is approved by the parties involved in the transfer – and most importantly, good project management.

1610 Process Engineering – Scale-down Influences

C.J.A. DAVIS
Bovis Lend Lease Pharmaceutical, Alexandra Court, Carrs Rd, Cheadle, Cheshire SK8 2JY

In developing a process the input of Process Engineers can be invaluable. Process Engineers understand how a series of unit operations can be integrated into an economically viable process that will be safe and will meet the regulatory constraints of the target market, of particular importance within the pharmaceutical sector. The input of process engineering early in the development phase can ensure the process that is developed will, so far as is practical, be a scale-down mimic of the final production scale. In addition the Process Engineer can examine the process economics early in the development phase to assess the production costs and, if necessary, target process development effort to increase economic efficiency. These scale-down factors will benefit the project by ensuring a robust and reliable process is developed and thereby reduce risk and reduce time to market.

POSTERS:

FB 01 Over-production and biochemical characterization of three *Bacillus* lipases

VINCE Y.Y. LI, HARDY W.L. CHAN, GARY W.L. CHAN & WALLACE B.L. LIM
Dept of Zoology, University of Hong Kong, Pokfulam, Hong Kong, China

Genetically engineered *Bacillus* strains that can secrete large amount of *Bacillus* lipases into the extracellular culture medium were constructed, including a thermophilic lipase (LipBT) cloned from a *B. thermoleovorans* strain (ATCC no. 43506) and the lipase A (LipA) and lipase B (LipB) from the *B. subtilis* strain 168. The lipases were purified from the culture medium by ammonium sulphate precipitation followed by phenyl-sepharose column chromatography. Their apparent MWs in SDS-PAGE gel were 21.5kDa, 22kDa and 43kDa for Lip A, Lip B and Lip BT. All three lipase exhibited good enzyme activities at alkaline pH, and the optimal pH lied at pH 9-10. While LipA and LipB were not stable at temperature higher than 50^{o}C, the thermophilic LipBT was most active at 60^{o}C. LipBT also exhibited higher lipolytic activities than LipA and LipB towards p-nitrophenyl esters and triacylglycerols with longer carbon chains. The biochemical properties of LipBT, including high temperature stability, alkaline pH optimum and good enzymatic activities towards substrates with longer carbon chains, may permit its application in detergent industry.

FB 02 Interaction of a thermo-inducible repressor and a putative transcriptional regulatory protein of the *Bacillus subtilis* phage ϕ105 with operator DNA

Y.M. CHAN & B.L. LIM
Dept of Zoology, University of Hong Kong, Pokfulam, Hong Kong, China

A heat –inducible expression system based on *Bacillus subtilis* phage ϕ105 has been developed previously, in which the induction of foreign gene expression was suggested to be regulated by a 144 a.a. cts-52 mutant repressor. Adjacent to the gene of this repressor, a putative open reading frame, designated ORF4, was found to share 37% homology with the repressor. A DNA-binding αhelix-βturn-αhelix(HTH) motif that resembles a λ repressor-like protein is identified in the N-terminal region (residues 18-37) of the repressor as well as the 90a.a. polypeptide of ORF4 (residues 22-41). In order to elucidate the details of the control mechanism of the heat-inducible expression, both the repressor and the ORF4 protein were overexpressed and purified in *Escherichia coli.* Surface plasmon resonance (SPR) analysis reveals that ORF4 binds to the same six operator sites as the repressor but with great difference in the binding affinities. Our data suggest that ORF4 might be a counter protein to the phage repressor in the modulation of the two divergent-oriented promoter P_M and P_R within the *Eco*RI-F immunity region (*imm*F).

In the future, influence of temperature on the DNA-operators binding affinity will be examined using fluorescence polarization system. The mRNA levels of these two proteins will also be monitored during the course of thermo-induction.

FB 03 Improvement of textile effluent treatment for colour removal using immobilised bacteria on polymer supports

THUY DO[1], JINSONG SHEN[1], GORDON CAWOOD[2] & RICHARD JENKINS[3]
[1]Dept of Textile Design and Production, [2]Dept of Biological Sciences, De Montfort University, The Gateway, Leicester LE1 9BH, [3]Quantum Clothing Ltd Stevensons, Derby

A laboratory-scale continuous bioreactor was used to evaluate the potential of immobilised bacteria for degrading azo dyes in dyehouse effluent streams. Polymer beads and foams were used as immobilisation supports for the bacteria. Basic parameters such as dilution rate, pH, temperature and effluent composition mimicked those of an industrial-scale biotreatment process. The culture systems were assessed for colour removal throughout the

lab-scale study. In simulated dye effluent experiment, biomass was slowly washed out of the bioreactor and colour absorbencies increased to 62% after three days. The addition of a co-substrate such as hydrolysed starch maintained high levels of biomass and low colour absorbencies (less than 2%). Results show a potential for improvement of an existing industrial effluent treatment plant for removal of colour, through introduction of an immobilisation support for bacteria and addition of nutrients to support biomass growth in the biological treatment tank.

FB 04 Application of multi-parameter flow cytometry for improved control of recombinant *E. coli* fermentation processes

G. LEWIS[1], I.W. TAYLOR[2] & C.J. HEWITT[1]
[1]Center of Formulation Engineering, University of Birmingham - GXL897@bham.ac.uk, [2]AstraZeneca, Alderly Park, Macclesfield - Ian.W.Taylor@astrazeneca.com

The combination of genetic engineering and microbial cultivation methods have ensured that *E. coli* has become a powerful tool for the production of high value recombinant proteins for research applications such as structural, kinetic and drug screening studies or for the production of commercially important pharmaceutical products. The expression of Eukaryotic proteins in *E. coli* is not without problems, since the organism lacks the key cellular machinery required for post translational modifications such as glycosylation or phosporylation which can be essential for the biological activity of many complex proteins. Further more the over expression of some but not all heterologous proteins in the cytoplasm of *E. coli* is often accompanied by their miss-folding and segregation into insoluble refractive aggregates known as inclusion bodies. Although inclusion body formation can greatly simplify protein purification, there is no guarantee that the *in vitro* separation and refolding will yield large amounts of biologically active product. This investigation shows how multi-parameter flow cytometry can be used to determine when inclusion bodies form and the physiological changes they cause. The *E. coli* strain used is capable of producing the recombinant protein such that it makes up 25-40% of the total cell protein; however a high percentage of the recombinant protein is produced as insoluble inclusion bodies. Batch fermentations were done at 500ml in shake flask and at laboratory scale (4L) bioreactor. Using varying induction times it has been shown that flow cytometry can be used to determine inclusion bodies 1 hour after induction by the intrinsic light scattering and the resulting physiological changes 2-4 hours after induction, as well as changes in the plasmid stability during the latter stages in fermentation.

FB 05 Oil utilisation during oxytetracycline fermentation by *Streptomyces rimosus*

PANOS PAPAPANAGIOTOU, C.J. HEWITT & A.W. NIENOW
University of Birmingham, School of Chemical Engineering, Centre for Bioprocess Engineering, B15 2TT

This is a project industrially sponsored by Cognis (Deutschland) and Pfizer Ltd (UK). Previous project on the utilisation of lipids during oxytetracycline fermentation by *S.rimosus* showed that when the oil feed is supplied in the form of a microemulsion, leads to lower residual oil amounts at the end of the process. In addition, the antibiotic yield increases towards the end of fermentation when compared to the control process.

The current project concentrates on the utilisation of several oil feeds with varrying oil droplet sizes, therefore, investigates in more detail the project of the past. At the moment work has been carried on an older strain as well as the industrial strain at Pfizer (results subject to secrecy agreement). Analytical techniques such as HPLC, GC, image analysis are currently being used for determination of the antibiotic yield, residual oil, and microbial dimensions, respectively.

Image analysis has provided additional information on the morphology of the microorganism when utilizing oil of different droplet size. The results from the analytical techniques in combination with the ones from image analysis have been used to confirm the theory suggesting that utilisation of oil is highly dependent on the oil droplet size.

FB 06 Scale- up of high–cell–density *E. coli* fed–batch fermentation using multiparameter flow cytometry: effect of a changing microenvironment with respect to glucose and pH concentration

H. ONYEAKA, A.W. NIENOW & C.J. HEWITT
Centre for formulation Engineering, Dept of Chemical Engineering, The University of Birmingham, B15 2TT- Telephone: +44121-414-5371, fax: +44-121-414-5324, e-mail Hxo078@bham.ac.uk

Genetic engineering has allowed the manufacture of high value therapeutic and other heterologous proteins in large quantities, using suitable hosts. Fed-batch fermentation is commonly employed in industrial processes, but on scale up additional problems arises such as concentration gradients. These gradients are due to non–ideal mixing and as such mass transfer limitations will cause the micro-organism to be exposed to a rapidly changing microenvironment. Scale down simulation studies, have been successfully used by our group to examine the effect of glucose gradients on the physiology of *E. coli cell* during fed batch fermentation. This study has extended this work by assessing the effect of a changing microenvironment with respect to pH variation, oxygen limitation and glucose gradients on the physiology, and biomass yield of *E. coli* cells. Flow cytometric analysis of samples taken during the course of fermentation showed a progressive change in the physiological state of *E. coli* cells. A reduction of biomass yield was further observed in all the simulation studies when the cells spend longer time in the heterogeneous part of the fermenter (60 - 120 sec). The overall reduction in productivity can be explained as a switch of resources from product synthesis to cell maintenance.

Tuesday 17 September 2002

0900 Epidemiology of enterovirulent *E. coli* infections in the UK

H.R. SMITH
Laboratory of Enteric Pathogens, Central Public Health Laboratory, 61 Colindale Avenue, London NW9 5HT

The recognition of *Escherichia coli* as an important cause of diarrhoeal disease has led to identification of several categories. The first four were enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli*, enteroinvasive *E. coli* and Vero cytotoxin-producing *E. coli* (VTEC). Further groups of enterovirulent *E. coli* (EVEC) such as enteroaggregative *E. coli* (EAggEC) and diffusely adherent *E. coli* have been linked epidemiologically with diarrhoea in some studies. Recent hospital and community based studies have provided valuable data on these EVEC. In relation to the food chain it is still unclear which of the EVEC have an animal reservoir. "Classical" EPEC were originally defined as *E. coli* belonging to certain serogroups that caused epidemic gastroenteritis in young infants. These strains cause attaching and effacing (AE) lesions in the intestine but this AE property has also been identified in a wider range of *E. coli* from man and animals. VTEC are the most important *E. coli* group in Britain at present because of the emergence of VTEC of serogroup O157. Over two thirds of the VTEC O157 outbreaks result from animal contact or consumption of contaminated food or water. VTEC belonging to serogroups other than O157 are prevalent in animals but appear to cause little severe human disease in the UK. Recent studies on EAggEC have shown them to be a frequent cause of diarrhoeal disease and some foodborne outbreaks have been identified. There is a need for better tests to identify EVEC in both clinical specimens and foods and elucidate their role as foodborne pathogens.

0945 Evolution of virulence in pathogenic *E. coli*

T. WHITTAM
Michigan State University, USA

Abstract not received

1200 Mechanisms of acid tolerance in enteric bacteria

I. BOOTH
University of Aberdeen

The major mechanisms contributing to acid tolerance in enteric bacteria with emphasis on commensal and pathogenic *E. coli* will be discussed. The requirements for survival of acid stress will be delineated and our current understanding of how these requirements are met will be described. Our recent work on the habituation response will be reviewed.

1245 The effect of overexpression of Fur and other molecular chaperones on the survival of *E. coli* at low pH

J.A. WHYTE, C.P. O'BYRNE & I.R. BOOTH
Dept of Molecular and Cell Biology, University of Aberdeen

E. coli has the ability to adapt to mildly acidic conditions thus enhancing its survival at extreme acid pH. It is thought that proteins induced at the habituation pH may protect the cell when transferred to even lower pH. It is known that bacteria respond to stressful environmental conditions by increasing the production of specific proteins which alleviate or reduce damage incurred by the cell. The Ferric uptake regulator, Fur has been shown to be involved in acid stress by regulation of several acid shock proteins. Mutations in *fur* have been shown to render the cell acid-sensitive. Survival of acid pH may require that proteins either be retained in the folded state or can be re-folded. Molecular chaperones are involved in protein folding and the expression and activity of some of these are also stress-related. They prevent aggregation, assist re-folding and mediate degradation of misfolded proteins. This project is seeking to establish the relationship between Fur regulation and pH survival in commensal *E. coli* and to determine the importance of chaperones in acid tolerance.

The pBAD TOPO expression system allows the cloning of PCR amplified genes into a 4.1 kb linearised vector and regulation of expression via the araBAD promotor. The *fur* gene, along with those for several molecular chaperones, have been cloned into this system and transformed into an *E. coli* laboratory strain Frag 1. Overexpression of these genes is carried out using 0.4% arabinose and SDS analysis used to confirm overexpression. The viability of the strains after induction of expression of Fur or the chaperones at pH 7 is being investigated.

1400 *Animals, food and* Escherichia coli *O:157*

W.J. REILLY
Scottish Centre for Infection & Environmental Health, Glasgow

Abstract not received

1445 Use of *Escherichia coli* to model pathogen epidemiology in the pork abattoir

T.G. ALDSWORTH, K. WARRINER & C.E.R. DODD
Division of Food Sciences, School of Biosciences, The University of Nottingham, Sutton Bonington Campus, Leics LE12 5RD

The epidemiology of pathogenic microorganisms within the pork abattoir is still not clearly understood. In this study, the faecal indicator organism *Escherichia coli* was used to model the epidemiology of enteric pathogens during the processing of pork in the abattoir. Samples were collected from the skin surface of selected carcasses throughout the day, after each of the major operations involved with processing. Both the total aerobic and *E. coli* counts decreased after heating steps such as scalding and singeing, but increased subsequent to these steps when the carcasses were handled during scraping and polishing. Singeing, when applied to carcasses routinely, could reduce contamination significantly but the process was not always effective on every carcass. Subsequent operations then spread this residual contamination. Little change was seen in either the total aerobic or *E. coli* counts subsequent to polishing. However, using ERIC-PCR as a DNA-fingerprinting technique for *E. coli*, it was clear that there were often major contamination events during evisceration as the fingerprint patterns changed, even though there was no corresponding change in counts. To conclude, it is clear that, although the microbial population structure of the carcasses often changed substantially during processing, the viable counts did not demonstrate this.

1500 Construction of environmental biofilm consortia with defined internal architecture

J.S. ANDREWS, G. STEPHENS & G.H. MARKX
UMIST, Sackville Street, Manchester

Recent developments in the use of reporter genes such as the green fluorescent protein (GFP) and the use of dielectrophoresis to produce artificial structured microbial consortia, (ASMC) have allowed the study of interactions between bacteria within natural soil aggregates. Mixed cultures of the naturally occurring soil bacteria *Pseudomonas putida* and *Acinetobacter* sp. have been shown to interact in the degradation of the aromatic hydrocarbon benzyl alcohol. Moller *et al*. (Appl. Env. Microb. Vol. 64, 1998) demonstrated this interaction in a flow chamber by observing interactions between *P. putida* with a TOL plasmid *Pm*::*gfp* cassette inserted into its chromosome. When a consortium of *Acinetobacter* sp. and *P. putida Pm*::*gfp* is grown on benzyl alcohol, the TOL plasmid *Pm* is induced by benzoic acid. Fluorescence indicates an interaction is occurring between the two species. We have developed novel techniques for rapidly constructing biofilms with defined internal architecture based on

electrokinetics. Bacterial cells are guided to specific areas of a microelectrode array by dielectrophoresis, the movement of particles in non-uniform AC electric fields, and immobilised on the electrodes. We have constructed biofilms of *Acinetobacter sp*. and *P. putida::gfp* using AC electrokinetics. Fluorescence was observed, demonstrating an interaction between the bacteria in the biofilm. This novel technique provides a new tool for the study of interactions between environmentally and industrially significant bacterial species.

1600 The intimate secrets of enteropathogenic and Verocytotoxin-producing *E. coli*

G. FRANKEL
Imperial College London

Enteropathogenic and enterohaemorrhagic Escherichia coli (EPEC and EHEC respectively) subvert intestinal cell functions for the benefit of the bacterium. EPEC is an established human pathogen causing infantile diarrhoeal diseases while EHEC is an emerging zoonotic pathogen that can cause sever systemic complications due to elaboration of a potent Shiga toxin; cattle being considered its main reservoir.

Following initial attachment, EPEC and EHEC assemble a molecular syringe to inject effector proteins via a type III secretion system. Following injection, one of the effector proteins, the Translocated intimin receptor (Tir), becomes an integral plasma membrane protein and a receptor for the outer membrane bacterial adhesion molecule intimin. Intimin-Tir interaction triggers reorganisation of the host cell cytoskeleton and intimate bacterial attachment. In recent years, several antigenic variants of intimin have been described. This lead to development of a classification system for strains according to the type of intimin they expressed. Using human intestinal biopsies in vitro, small animal models and epidemiological studies, intimin types were then found to contribute to both host and tissue specificity. This presentation will cover several aspects of EPEC/EHEC biology and life style.

1645 Genomic indexing of pathogenic *E. coli* reveals new black holes

J.C.D. HINTON
Institute of Food Research, Norwich

Abstract not received

POSTERS:

FdBev 01 Interaction of *Escherichia coli* and *Listeria monocytogenes* with sprouting mung beans

KEITH WARRINER[1], FAOZIA IBRAHIM[1], MATTHEW DICKINSON[2], CHARLES WRIGHT[3] & WILLIAM M. WAITES[1]
[1]Division of Food Sciences, [2]Division of Plant Science, [3]Division of Agriculture and Horticulture, School of Biosciences, University of Nottingham, Sutton Bonington Campus, Loughborough, Leicestershire LE12 5RD

Sprouted seeds have been implicated in numerous outbreaks of food borne illness and represent a significant health risk. The study used a combination of plate counts, bioluminescent-labelled pathogens, nucleic acid viability stains and *in-situ* β-glucuronidase (GUS) staining to determine the interaction of model pathogens (*Escherichia coli* and *Listeria monocytogenes*) with sprouting mung beans. *E. coli*, initially introduced on beans became established as the dominant microflora on the surface and within the vascular system of growing sprouts. From bioluminescent profiles captured during sprouting, growth of *E. coli* occurred around the roots of sprouts. Numerous biofilms were visualised on the surface of hypocotyls but were composed of low/non-viable bacterial cells. In contrast, viable bacteria were recovered from the apoplastic fluid and sprouts treated with 10% v/v sodium hypochlorite (surface sterilised). The growth of *L. monocytogenes* within sprouting beans was also found to be high during sprouting but internal populations of the bacterium progressively decreased to below the level of detection (log_{10} 1 cfu/g) although a number did persist on the surface of hypocotyls. The internalisation of pathogens within sprouts would limit the efficacy of post-harvest biocidal washing and methods to prevent/remove contamination at the primary production level need to be developed.

FdBev 02 Optimisation of low level pathogen detection

CHARLOTTE ARCHARD, GAVIN NIXON, DANIEL HOPKINS & JACQUIE KEER
Bio-Analytical Innovation Team, Laboratory of the Government Chemist Ltd, Teddington, Middx TW11 0LY - Email: charlotte.archard@lgc.co.uk

Low level detection of pathogenic organisms as applied to diagnosis of infectious diseases or detection of low levels of pathogenic contaminants often challenges the limits of analytical sensitivity, especially in interrogation of challenging sample matrices such as food and environmental samples. Methods for low level detection have been investigated to determine critical points in analysis in order to facilitate optimisation of performance.

Two real–time amplification platforms and conventional nested PCR have been compared for the detection of *E. coli* O157, using *rfb*E and *fim*A as assay targets. All three amplification methods could reproducibly detect ten to a hundred copies of target genomic DNA, with sampling issues introducing variation at lower target levels. The sensitivity of all three methods was comparable, however considerations such as ease of use and ability to generate statistically meaningful data indicate that the ABI Prism® 7700 Sequence Detection system may be the platform of choice for routine low level detection.

Investigation of the performance of several methods for low level pathogen detection has allowed development of generic guidelines for assay design and optimisation of method sensitivity, which have the potential to improve trace detection strategies.

FdBev 03 Synergistic effect of UV, laser, microwave radiation and heat on *E. coli* in saline suspension

SIAVASH MAKTABI[1], ROGER PARTON[1] & IAN WATSON[2]
[1]Dept of Infection and Immunity, Joseph Black Building, IBLS, University of Glasgow, Glasgow G12 8QQ, [2]Dept of Mechanical Engineering, Laser and Optical Sytems Engineering Centre, University of Glasgow, Glasgow G12 8QQ

E. coli is an indicator organism in the food industry. Although laser sterilisation has well been studied in dentistry and medicine, there have been few studies within the food industry. UV radiation has been used for sterilisation of surfaces and water. The killing effect of microwave radiation has been investigated on many bacteria and food and there has been much controversy over its killing mechanism. In this study, the killing effect of laser, microwave and UV was studied on *E. coli* in saline suspension with applications pertinent for the food processing sector. The bacterial suspension was exposed to the treatment processes and viable cell counts were made before and after the treatment. A difference in the viable counts was apparent when the combined treatments was compared with the sum of the individual treatments alone. Similar results were obtained when conventional heating was used in place of microwave radiation. It was found that the order of the treatment processes may be important.

FdBev 04 Identification of *Lactobacillus plantarum* strains isolated from beer

ALTANZAYA YANSANJAV[1], MIROSLAV NEMEC[1] & IDA HOLLEROVA[2]
[1]Dept of Microbiology, Faculty of Science, Masaryk University, Tvrdeho 14, Brno 60200, Czech Republic, [2]Research Institute of Brewing and Malting, Lipova 15, 12044 Praha 2, Czech Republic

The Czech Republic is one of the major beer producers in Europe. It is important to have a state of the art quality control system to eliminate microorganisms that can spoil beer.

Microorganisms, which contaminate food products, can also contaminate beer despite of the fact that beer itself is not an optimal substrate for growth of microoganisms (low pH, relatively high content of ethanol and CO_2, presence of bitter compounds produced by hops and low concentration of nutrition for microorganisms). All species of yeast and Gram positive bacteria, especially lactic acid bacteria, can grow in beer. Therefore, microbiological controls are generally focused on these organisms.

Our work was focused on isolation and identification of *Lactobacillus plantarum,* a strain belonging to lactic acid bacteria group that can spoil beer. Two methods of identification were compared, namely, the classical biochemical test set API 50 CHL (BioMérieux) and analysis of cell proteins by SDS-PAGE. The results have shown that both methods were effective for identification of *Lactobacillus plantarum* . However, API 50 CHL results have had certain deviations in biochemical characteristics from typical reactions. On the other hand, the protein profile of studied strains was similar among the different strains (GELCOMPARE II).

The results presented above can be also confirmed by using a molecular-diagnostic method called ribotyping.

FdBev 05 The role of *fur* in the acid stress response of *Escherichia coli*

R.M. BIRCH, J.A. WHYTE, C.P. O'BYRNE & I.R.BOOTH
Dept of Molecular and Cell Biology, Institute of Medical Sciences, University of Aberdeen, Foresterhill, Aberdeen AB25 2ZD

Food-borne pathogens possess mechanisms that allow them to survive the acidic conditions of the stomach and subsequently colonise the intestine. *Escherichia coli,* as with many other food-borne pathogens, has the ability to adapt to mildly acidic conditions. This habituation, or adaptive acid tolerance response, enhances its survival at extreme pH (pH 2.0 - 3.0). The molecular basis of acid habituation remains poorly characterised. We have targeted genes thought to have some involvement in the acid tolerance response in other organisms, and created knock-outs of these genes in *E.coli* to assess the survival of mutants under conditions of acid stress. Currently, we are investigating the role of the *fur* gene in the acid stress responses of *E. coli*. We have created a *fur* knock-out in *E. coli* K-12. The mutant displays a weakened tolerance of acidic conditions that partially overrides the habituation response. Clear differences are seen between the survival of acid conditions in broth and in defined medium. The data will be discussed in the context of the stress experienced at acid pH in the different media.

Wednesday 18 September 2002

1400 Methods of study of multigenic diseases in humans

RICHARD C. TREMBATH
University of Leicester

Medical genetics has made significant impact on clinical practice, in part through the elucidation of the molecular basis of an ever-increasing number of inherited disorders. The breadth of such studies has increased recently through progress in the human genome project, together with access to deep and informative marker maps for genetic analysis.

Disease susceptibility implies a combination of exposure and a facilitative genetic background. Much effort is now being placed upon the definition of human genetic variation to common disease susceptibility including infections. This presentation will reflect upon the potential for such approaches to advance understanding of the molecular basis of susceptibility to infectious disease.

1440 Sibling familial risk ratio of meningococcal disease in UK caucasians

ELENE HARALAMBOUS[1], HELEN A. WEISS[2], ANDRZEJ RADALOWICZ[2], MARTIN L. HIBBERD[1], ROBERT BOOY[3], & MICHAEL LEVIN[1]
[1]Dept of Paediatrics, Imperial College Faculty of Medicine, St. Mary's Hospital, Paddington, London W2 1PG, [2]MRC Tropical Epidemiology Group, Infectious Diseases Epidemiology Unit, London School of Hygiene and Tropical Medicine, London WC1E 7HT, [3]Dept of Child Health, Queen Mary's School of Medicine & Dentistry, Barts and the London, Whitechapel, London E1 1BB

Background Despite evidence indicating a host genetic component to meningococcal disease (MD) susceptibility, to date this has not been quantified.

Methods and results To do this, the sibling risk ratio (λ_s) was calculated as the ratio of observed MD cases among 845 siblings of 443 UK Caucasian MD cases to the number expected, calculated from age-calendar year specific rates for England and Wales. 27 siblings contracted MD in comparison to an expected 0.89, generating a λ_s of 30.3 (95% CI: 20-44). Overestimation of λ_s due to *Neisseria meningitidis* exposure was taken into account by using a number of time cut-offs between index case and sibling MD onset. Excluding siblings with MD onset within one month of index case onset generated a λ_s of 11, decreasing slightly to 8.2 for risk of MD more than 12 months after index case onset. Social-class distribution did not differ between MD cases with affected siblings and the general population of England and Wales.

Interpretation This study is the first to calculate λ_s for MD (λ_s=30.3). Excluding siblings occurring within one month of index case MD onset indicates that host genetic factors contribute to at least one third of the sibling risk ratio.

1500 The UK mutagenesis programme for investigation of complex disease

STEVE D.M. BROWN
MRC Mammalian Genetics Unit and UK Mouse Genome Centre, Harwell

Systematic approaches to mouse mutagenesis are vital for future studies of gene function, including the identification of loci that impinge upon genetic susceptibility to infection. We have undertaken a major ENU mutagenesis programme incorporating a large genome-wide screen for dominant mutations (Nolan et al. *Nature Genetics* 25: 440-443). Nearly 30,000 mice have been produced and the majority screened employing a systematic and semi-quantitative screening protocol - SHIRPA (Rogers et al. *Mammalian Genome* 8: 711-713). SHIRPA is a hierarchical screening protocol employing a rapid and efficient primary screen for deficits in muscle and lower motor neuron function, spinocerebellar function, sensory function, neuropsychiatric function and autonomic function. Moreover, in the primary screen blood is collected from mice and subjected to a comprehensive clinical chemistry analysis. Subsequently, secondary and tertiary screens of increasing complexity can be employed on animals demonstrating deficits in the primary screen. Progeny testing of mice carrying abnormal phenotypes indicates that 2% of mice from the screen carry a new heritable dominant phenotype. Over 150 mutants have been confirmed as heritable and added to the mouse mutant catalogue and, overall, we can extrapolate that we have recovered around 500 mutants from the screening programme. For further information on the project and details of data derived from the screening see: *http://www.mgu.har.mrc.ac.uk.* We are currently using frozen sperm and IVF for the rapid generation of small backcrosses in order to map many of the newly catalogued mutations to the mouse genome. We have mapped over 70 mutants to date and confirmed that many of the novel phenotypes represent mutations at previously unidentified loci in the mouse genome.

The generation of a new mutant map of the mouse will be a powerful resource available to the mouse and human genetics communities at large for future gene function studies. The use of ENU mutagenesis for the discovery of loci involved in infection raises particular challenges. But any tractable and systematic analysis of the genes involved in susceptibility to infection requires a genome-wide approach using both phenotype-driven and gene-driven mutagenesis methods.

1610 *In vivo* and *in vitro* studies of genetic resistance to systemic salmonellosis in the chicken encoded by the *SAL1* locus

PAUL WIGLEY, NAT BUMSTEAD & PAUL BARROW
Institute for Animal Health, Compton Laboratory, Compton, Newbury, Berkshire RG20 7NN

Genetic resistance to systemic salmonellosis in the chicken is mediated through a single locus on chromosome 5 termed *SAL1*. Inbred chicken lines with the *SAL1* locus display increased resistance to systemic salmonellosis caused by *Salmonella enterica* serovar Typhimurium and serovar Enteritidis in young chicks and to fowl typhoid caused by *Salmonella enterica* serovar Gallinarum in older birds. Resistance is not linked to sex, MHC or to *NRAMP1,* which contributes only a minor role to resistance to systemic salmonellosis in the chicken. In experimental infections resistant inbred lines (lines Wl, 6_1 & N) have a greatly increased LD_{50}, and at *post mortem* show greatly reduced pathological changes when compared with susceptible inbred lines (7_2, C & 15I). Primary macrophages from adult inbred line chickens (Wl) rapidly cleared *S. gallinarum in vitro*, whereas *S. gallinarum* persisted for 48 h in susceptible (7_2) line cells. No difference in nitric oxide production was found between lines, however, resistant line macrophages produced a strong oxidative burst not found in susceptible line cells when challenged with *S. gallinarum*. Primary macrophages from resistant line chicks also showed increased oxidative burst to *S. typhimurium* and *S. enteritidis* challenge. These findings suggest increased macrophage killing has an important role in resistance.

1630 Identifying genetic susceptibility factors for malaria and tuberculosis: candidate gene studies and genome-wide screens

A. HILL
University of Oxford

Abstract not received

0900 NRAMP1 and susceptibility to infection in mice and humans

JENEFER M. BLACKWELL
Cambridge Institute for Medical Research, University of Cambridge School of Clinical Medicine, Wellcome Trust/MRC Building, Addenbrooke's Hospital, Hills Road, Cambridge CB2 2XY

Genetic variation in response to infection provides a powerful tool for analysis of infectious disease. In our laboratory we have used both population-based and family-based genetic allelic association and linkage analyses to study the role of candidate genes in disease susceptibility, and in determining response to vaccination. This has demonstrated roles for NRAMP1, the IL4-IL-9 gene cluster, a cluster of chemokine genes on chromosome 17, and genes within the class II and class III regions of the MHC in determining susceptibility to leprosy, tuberculosis and leishmaniasis, and in regulating immune response to mycobacterial and leishmanial antigens. Genetic and functional characterization of murine and human *Nramp1/NRAMP1* encoding the natural resistance associated macrophage protein has been a major focus of our research. The gene encodes a polytopic integral membrane protein with 10-12 membrane spanning domains. Confocal and EM-gold studies demonstrate that Nramp1 localizes to late endosomes/lysosomes of macrophages. We used the *Xenopus* oocyte expression system to demonstrate that Nramp1 is a $H^+/Fe^{2+},Zn^{2+},Mn^{2+}$ antiporter. Its action as a divalent cation transporter influences antigen processing for presentation via class II, endosomal fusion, resistance to infection, response to vaccination, and susceptibility to rheumatoid and juvenile rheumatoid arthritis. *Work supported by The Wellcome Trust.*

0940 Unravelling the genetics of the host response to meningococcal disease

M.L. HIBBERD, E. HARALAMBOUS, S.O. DOLLY, J. GOULDING, S. KONDAVEETI, E. ORAGUI & M. LEVIN
Dept of Paediatrics, Imperial College Faculty of Medicine, St. Mary's Hospital, Paddington, London W2 1PG

Introduction In the majority of people, *Neisseria meningitidis* becomes a commensal of the nasopharyngeal epithelium for at least some part in their life. Despite this, meningococcal disease is a rare occurrence, suggesting a complex relationship between colonisation, infection and disease. Susceptibility is likely to be influenced both by the environment (in particular bacterial colonisation) and the host response. A number of genes are likely to contribute to the host response, and variations in them may well form the basis of an individual's susceptibility. We postulated that variation within both the complement regulator, factor H, (fH) and a mediator of intracellular bacterial killing, SLC11A1, (formerly NRAMP1) genes might predict disease.
Subjects Patients from the paediatric intensive care unit at StMary's Hospital and 2 UK wide studies (including families and controls), donated samples and filled in questionnaires.
Methods and results For fH, a polymorphism at position 496 (C to T) was investigated by PCR and restriction enzyme digestion. The proportion of C/C homozygotes was increased in 161 St Mary's patients (59%) compared to 92 controls (33%) ($p = 0.00006$) as was the C allele frequency (0.75 vs 0.57, $p = 0.00001$). An additional 118 nuclear families showed increased transmission of the C allele to patients (61%) compared to the T allele (39%) ($p = 0.04$).

SLC11A1 promoter region microsatellite alleles (1 to 4) were categorised by size. The St Mary's patients (215) showed an increased frequency of the 2-2 genotype (15%) compared to 8% in 92 controls ($p = 0.002$). Investigation of disease outcome showed that no St Mary's patients with the 2-2 genotype died ($p = 0.04$). A second UK study ($n = 122$) confirmed this ($p = 0.008$).
Conclusions The fH gene C/C genotype is both strongly associated and linked with disease, suggesting that this polymorphism down regulates complement activation during the host response.

Allele 2 of the SLC11A1 promoter region microsatellite is known to drive lower levels of expression, predicting lower levels of intracellular killing and macrophage activation, possibly explaining its association with disease. Interestingly, the 2-2 genotype also seems to protect from severe disease.
It seems increasingly clear that small, genetically determined, differences in the host have large effects on the success of the infectious response and these can be used to identify key pathways of protection.

1100 Metalloproteinase-7 contributes to joint destruction in *Staphylococcus aureus* induced arthritis

I.GJERTSSON[1], L. MATRISIAN[2] & A. TARKOWSKI[1],
[1]Dept of Rheumatology and Inflammation Research, Göteborg University, Sweden, [2]Dept of Cancer Biology, Vanderbilt University School of Medicine, USA

Septic arthritis induced by *Staphylococcus aureus* causes a rapid destruction of joint cartilage and periarticular bone. The mechanisms behind this phenomenon are not fully understood. Earlier studies have shown that cytokines and metalloproteinases are of importance in bone metabolism. The aim of this study has been to investigate the significance of metalloproteinase-7 expression in *S. aureus* induced septic arthritis.

MMP-7 deficient mice and congeneic controls were intravenously inoculated with an arthritogenic dose of *S. aureus* LS-1. MMP-7 deficient mice developed significantly less severe arthritis both clinically and histopatologically with regard both to synovitis and destruction of bone and cartilage. Despite this finding, bacterial growth in the deficient animals was significantly increased. In vitro responses to staphylococcal antigens/superantigens did not differ between MMP-$7^{+/+}$ and MMP-$7^{-/-}$ mice with respect to cytokine and chemokine production.

In conclusion expression of MMP-7 contributes to staphylococcal elimination but simultaneously aggravates arthritis.

1120 ENU mutagenesis and the production of new innate immune phenotypes

BRUCE BEUTLER, KASPER HOEBE, XIN DU, KOICHI TABETA & ZUPING ZHOU
The Scripps Research Institute, La Jolla, CA, USA

The nature of the innate immune sensing apparatus in mammals was deciphered using forward genetic methods: methods that proceed from phenotype to gene. An endotoxin-resistant mouse strain, C3H/HeJ, was used to map the general LPS response gene, which was then identified as TLR4. This discovery provided the first critical insight into how the innate immune system detects infection. It is now known that in humans, ten TLRs act in concert to detect infectious pathogens. Phenotypes like the LPS resistance of C3H/HeJ mice are in short supply. Hence, forward genetic analysis of complex biological phenomena, including the innate immune response, now depends largely upon random germline mutagenesis, using ethylnitrosourea (ENU). Specific aspects of the innate immune response are kept under surveillance, and phenotypic variants are produced. In our laboratory, we have now identified a new mutation (*Lps2*) that abolishes responses to endotoxin. This mutation is believed to involve a component of the LPS receptor that has not previously been detected. In the long run, all of the genes that subserve innate immune responses may be identified through mutagenesis, provided that sufficiently clever and specific screens can be devised.

1200 Mapping QTL controlling susceptibility to infection by *Streptococcus pneumoniae* in mice

PAUL DENNY[1], ELAINE HOPES[1], NEILL GINGLES[2], KARL W. BROMAN[3], WILLIAM MCPHEAT[5], JOHN MORTEN[4], JANET ALEXANDER[2], PETER W. ANDREWS[2] & STEVE D.M. BROWN[1]
[1]MRC UK Mouse Genome Centre & Mammalian Genetics Unit, Harwell, Oxon OX11 0RD, [2]Dept of Microbiology and Immunology, University of Leicester, Leicester, [3]Dept of Biostatistics, Johns Hopkins University, Baltimore, Maryland, USA, [4]Research and Development Genetics, AstraZeneca, Alderley Park, Macclesfield, Cheshire, [5]Cell

Biology and Biochemistry, AstraZeneca R&D Mölndal, Pepparedsleden 1, 431 83 Mölndal, Sweden
The pneumococcus, *Streptococcus pneumoniae* is responsible for the majority of cases of community-acquired pneumonia and is also a significant cause of meningitis, bacteremia and otitis media in children. Antibiotic drugs are the standard therapy, but antibiotic resistance is increasingly common in pneumococcal strains and has epidemic potential. Vaccination offers protection against a spectrum of pneumococcal serotypes, but horizontal gene transfer may ultimately negate the effectiveness of both vaccines and antibiotics. It is clear that a better understanding of the host response to this bacterium is essential for improved preventative and therapeutic treatments. There is a significant genetic contribution to host susceptibility to pneumococcal infection, but the limited numbers of case-confirmed pedigrees or sibling pairs preclude linkage analysis. We have therefore developed a genetic model using inbred mouse strains that exhibit differences in susceptibility to pneumococcal infection. Our previous work showed that BALB/c and CBA/Ca are resistant and susceptible to infection by *S. pneumoniae,* respectively[1]. We therefore established crosses between these strains to allow us to follow the inheritance of susceptibility. We followed two traits, survival time and the development of invasive pneumococcal disease, as measured by levels of circulating bacteria.

A complex phenotypic distribution was seen in the (BALB/c x CBA/Ca)F2 (CCBAF2) generation, with many animals surviving the full length of the experiment, and others succumbing to infection early on, in a way similar to the CBA/Ca parent. Many CCBAF2 animals also failed to develop significant bacteremia, but there was not an absolute correlation between bacteremia level and survival. We will present details of the phenotypes and the results of a genome scan performed on the CCBAF2 generation using a set of polymorphic markers.

1. Gingles, N.A. et al. Role of genetic resistance in invasive pneumococcal infection: identification and study of susceptibility and resistance in inbred mouse strains. *Infection and Immunity* **69**, 426-34 (2001).

POSTERS:

Clinical Microbiology Group

CM 01 Detection of staphylococcal enterotoxin genes in informal milk samples from Piracicaba, SP, Brazil

LEA CHAPAVAL, SIU MUI TSAI, JOSÉ ELIAS GOMES & DAVID H. MOON
Cellular and Molecular Biology Laboratory,Centro de Energia Nuclear na Agricultura, University of São Paulo, Piracicaba, São Paulo, Brazil - (leca@cena.usp.br)
Staphylococcus aureus is a major pathogen involved in sub-clinical mastitis in dairy cattle and in many countries it has been identified as one of the major causes of food poisoning outbreaks involving dairy based products. For PCR many preparation methods are unsuitable for PCR because they do not provide a satisfactory quantity/quality of DNA template. This study describes a rapid procedure for isolation of genomic DNA from *Staphylococcus aureus* isolates without the need for Lysostaphin and found that our method is capable of producing staphylococcal DNA in sufficient quantity and quality for the amplification of staphylococcal enterotoxin genes (SEA, B, C, D and E) and the toxin TSST-1 from environmental isolates. In our survey of informal milk (64) we found that 98% were positive for *Staph. aureus* by culture, but we were unable to detect the presence of staphylococcal enterotoxins (SEA-E) by the commercially available kit VIDAS-SET, bioMérieux, France. The PCR results show that 73% of the isolates were positive for one or more genes, 64% for SEA, 4.7% for SEB, 1.5% for SEC and 32.8% for TSST-1. It was interesting to note that 29.7 % of the isolates were positive for SEA and TSST-1.

CM 02 Evaluation of different molecular typing methods of *Salmonella typhi* strains isolated from Suez Canal area

KHALIFA IBRAHIM KHALIFA & SAID HAMID ABBADI
Faculty of Medicine, Suez Canal University, Ismailia, Egypt
Forty one strains of *Salmonella typhi* were isolated from 203 patients who attended fever hospitals in Suez Canal area (Suez, Ismailia and Port Said cities) complaining of fever and their clinical pictures were suggestive of typhoid fever. All the isolates were identified by the conventional biochemical reactions and further charecterized by three molecular typing methods, plasmid profile, pulsed field gel electrophoresis (PEGE) and ribotyping. Plasmid profile revealed that only 13 out of 41 isolates (31.7 %) harbored plasmids (5 isolates has one plasmid of the same size and 8 isolates had the same plasmid profile). All the isolates were typeable by PFGE using *Xba*I and *Spe*Ienzymes for restriction of DNA. Considerable dgree of genetic diversity was observed among the isolates as evidenced by 16 different *Xba*I patterens and 11 *Spe*I patterens and 14 (34.2 %) isolates had identical *Xba*I and *Spe*I patteren (XISI). *Xba*I enzyme was found to be the more appropriate enzyme for PFGE studies of *S. typhi* isolates. All the isolates were typeable by ribotyping using *Pst*I for restriction of DNA. Eleven ribogroups out of fourty-one isolates were detected and *Pst*I proved to be the most useful enzyme.

CM 03 Virulence characteristics of VISA isolated in different parts of the world

LUCY M. EVERETT & CURTIS G. GEMMELL
Dept of Bacteriology, University of Glasgow, Castle Street, Glasgow Royal Infirmary, Glasgow G4 0SF
Vancomycin-intermediate *Staphylococcus aureus* (VISA) strains may differ phenotypically from strains of methicillin-resistant *S.aureus*. We compared six VISA strains from America, Japan and the United Kingdom, a methicillin sensitive *S.aureus* (MSSA) and EMRSA16.

Cell wall thickness was examined using electron microscopy and selected strains were assayed for the production of protein A and toxic shock syndrome toxin (TSST-1) by ELISA, and alpha-haemolysin through haemolysis of rabbit erythrocytes. All strains were tested for susceptibility to phagocytosis by human polymorphonuclear cells and their ability to induce respiratory burst.

All VISA cell walls were thicker than EMRSA16 and MSSA. Cell bound protein A was detected on only one VISA strain. Three VISA produced 32-64 haemolytic units (HU) / 50µl of culture and three produced 4-8HU. EMRSA16 produced three-fold more TSST-1 than any of the VISA strains.

The presence of a thickened cell wall made no significant difference to the susceptibility of five VISA strains to phagocytic uptake. The absence of surface protein may have been compensatory. Induction of respiratory burst by four VISA strains was significantly lower than EMRSA16. We speculate that increased cell wall thickness whilst not affecting the susceptibility of VISA to phagocytic ingestion, it does affect their ability to generate respiratory burst. Higher protein A expression in MSSA may be responsible for reduced respiratory burst compared to EMRSA16.

CM 04 Evaluation of PCR in the diagnosis of endocarditis

S. LANG[1,3], R. WATKIN[2], P.A. LAMBERT[3], W.A. LITTLER[2] & T.S.J. ELLIOTT[1]
[1]Clinical Microbiology and [2]Cardiology, Queen Elizabeth Hospital, University Hospital NHS Trust Birmingham, Birmingham B15 2TH, [3]Life & Health Sciences, Aston University, Aston Triangle, Birmingham B4 7ET
The diagnosis of infective endocarditis (IE) is based on the Duke criteria, which can be inconclusive when blood cultures are negative. Without a definitive aetiological diagnosis directed antimicrobial therapy cannot be instigated which may result in increased morbidity and mortality.

A broad-range PCR technique was used to directly amplify prokaryotic DNA present within heart valve tissue from patients undergoing valve replacements, of whom 21 were confirmed

"definite" for IE by the Duke criteria, 10 were considered as "possible" IE patients and 35 had no known microbial infection. DNA sequencing of the amplicon and database analysis allowed identification of the infecting microorganism.

The application of PCR analysis confirmed 9/17 (53%) positive blood culture results and microorganisms were identified in 2/4 (50%) of blood culture-negative patients with definite IE. In the possible IE group, evidence was provided for the conversion of 2/9 (22%) to the definite category and for 4/9 (44%) to be re-categorised as IE negative.

PCR using broad-range prokaryotic primers confirmed previous blood cultures (including *Staphylococcus*, *Streptococcus* and *Bacillus* species), identified the infecting microorganism for patients with persistently negative blood cultures, and provided supporting evidence for conversion of possible IE patients to either the definite or rejected category of infection.

CM 05 Lipid changes in *Staphylococcus epidermidis* adapted to chlorhexidine resistance by serial passage

MANIJEH GHODS[1], PETER A. LAMBERT[1] & TOM S. JELLIOT[2]
[1]Microbiology Research Group, Pharmaceutical Sciences and Research Institute, Aston University, Birmingham B4 7 ET, [2]Clinical Microbiology, University Hospital NHS Trust, Birmingham B15 2TH

The susceptibility of three strains of *Staphylococcus epidermidis* to chlorhexidine (CHX) was decreased 16-fold by serial passage in the presence of increasing concentrations of CHX. One of the strains showed cross-resistance to triclosan (TLN) and benzalkonium chloride (BKC), one strain was cross resistant to povidone-iodine (PI) alone while the third strain showed cross-resistance to benzalkonium chloride alone. By contrast, sensitivity tests with antibiotics including gentamicin, fusidic acid, vancomycin, erythromycin, penicillin G and ciprofloxacin carried out for the above strains showed no increased resistance.

Mechanisms of resistance to chlorhexidine (CHX) in *Staphylococcus epidermidis* were investigated. All of the CHX-adapted strains showed an increase in the proportions of whole cell fatty acids and phospholipids in the cytoplasmic membrane compared with their parent, sensitive strains.

The results suggest that continuous exposure to CHX can induce resistance, which appears to be related to changes in lipid content, but that no cross-resistance to commonly used antibiotics occurs.

CM 06 Plasmid typing: a tool for further sub-division of epidemic MRSA in the UK

Z. Al-DOORI[1], D. MORRISON[1], A. BROWN[1], G. EDWARDS[1] & C. GEMMELL[1,2]
[1]Scottish MRSA Reference Laboratory, [2]Glasgow University, Dept of Bacteriology, Glasgow

PFGE, the present gold standard for typing MRSA, can subtype the dominant UK epidemic clones EMRSA-15 and EMRSA-16. However, several PFGE subtypes are prevalent. The aim in this study was to evaluate the usefulness of plasmid typing for further differentiation of EMRSA-15 and EMRSA-16. The study included: comparison of conventional and Qiaprep-Spin plasmid extraction; screening 87 MRSA from 15 Scottish hospitals for typability and discrimination; outbreak investigation. Qiaprep-Spin compared favourably with conventional extraction. PFGE identified 14 subtypes amongst 53 EMRSA-15. 8% were non-typable by plasmid typing and eight types were identified. The commonest EMRSA-15 PFGE subtype was differentiated into four plasmid types. PFGE identified 12 subtypes amongst 34 EMRSA-16. 53% were non-typable by plasmid typing and eight types were identified. One of the commonest EMRSA-16 PFGE subtype was differentiated into four plasmid types. Sixteen "outbreak" EMRSA-15 included 10 which were indistinguishable by PFGE typing, however, plasmid typing further sub-divided these 10 isolates into three groups. In conclusion, Plasmid typing, with the less toxic Qiaprep-Spin method, provided useful differentiation amongst EMRSA-15 and EMRSA-16, however, the high incidence of non-typability in EMRSA-16 indicates it will be less useful for this clone.

CM 07 Suppression of Triton-X100-induced autolysis of a methicillin-resistant *Staphylococcus aureus* strain by epicatechin gallate

P.D. STAPLETON[1], S. SHAH[1], J.M.T. HAMILTON-MILLER[2] & P.W. TAYLOR[1]
[1]Dept of Pharmaceutics (Microbiology), School of Pharmacy, 29-39 Brunswick Square, London WC1N 1AX, [2]Dept of Medical Microbiology, Royal Free & University College Medical School, London NW3 2QG

Epicatechin gallate (ECG), a constituent of green tea, can modulate methicillin resistance in *Staphylococcus aureus* (MRSA), rendering previously resistant strains susceptible to β-lactam antibiotics. The process by which this is achieved has not been elucidated. Elevated rates of autolysis have been associated with high-level methicillin resistance and mutations in auxiliary genes that lower the levels of methicillin resistance are also known to reduce the rates of autolysis and cell wall turnover (DE Jonge *et al*., 1991, J. Bacteriol. 173:1105-1110). To investigate whether ECG affected autolysis a homogeneous methicillin-resistant *S. aureus* strain BB568 was grown in the absence and presence of ECG (25, 50 and 100 mg/L) and the rates of triton-X100-induced autolysis assessed. ECG was found to significantly reduce the rate of autolysis (78% lysis vs 30-35% over a 2.5 hour time period for untreated and treated cells respectively). In addition, assessment of lytic enzyme activity, both spectrophotometrically and by bacteriolytic enzyme profiling, revealed that ECG (≥ 50 mg/L) suppressed the secretion of lytic enzymes into the growth medium. In conclusion, ECG reduces both rates of autolysis and lytic enzyme secretion, which may be a contributory factor in the ability of ECG to reduce methicillin resistance in *S. aureus*.

CM 08 The use of intact cell MALDI for the sub-typing of MRSA

KATHRYN A. JACKSON[1], VALERIE EDWARDS-JONES[2] & ANDREW J. FOX[3]
[1]Kratos Analytical, Wharfside, Trafford Wharf Road, Manchester, [2]Dept. Biological Sciences, Manchester Metropolitan University, Manchester, [3]Manchester Public Health Laboratory, Withington Hospital, Manchester

Rapid identification and sub-typing of Methicillin resistant *Staphylococcus aureus* is essential for outbreak recognition and infection control.

The use of MALDI-TOF mass spectrometry on intact cell micro-organisms; Intact Cell MALDI (ICM), has been shown by numerous workers to be effective for discrimination to species level identification. In order to achieve sub-typing of microorganisms it has been essential to develop standardised methods. For MRSA, such a method was developed, incorporating standardised conditions for both the culture of the isolates and the MALDI analysis.

This method was used for the ICM analysis of a collection of isolates, which had been characterized previously using conventional and molecular subtyping methods, namely antibiograms, phagetyping and PFGE. These isolates included diverse sporadic and epidemiologically related isolates. Examination of the data, using software developed "in-house", permitted cluster analysis and additionally illustrated that key areas of the spectra allow identification and subtyping of MRSA. Particular strain marker peaks have been identified.

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

Microbial Infection Group

MI 01 Lethal photosensitisation of *Staphylococcus aureus*

MICHELLE L. EMBLETON[1,2], SEAN P. NAIR[1], BARRY COOKSON[3] & MICHAEL WILSON[2]
[1]Cellular Microbiology Research Group and [2] Dept of Microbiology, Eastman Dental Institute for Oral Health Care Sciences, University College London, [3]Laboratory of Hospital Infection, Central Public Health Laboratory, Colindale, London

The increasing resistance of *Staphylococcus aureus* to conventional antimicrobials and the prevalence of methicillin-resistant *S. aureus* (MRSA) in hospital-acquired infections, necessitates the development of alternative strategies for their control and treatment. One possibility is lethal photosensitisation, in which target cells are treated with a photosensitive chemical (photosensitiser). Upon irradiation with light of a defined wavelength, the photosensitiser produces high-energy singlet oxygen and free radicals that damage plasma membranes leading to cell death. The effectiveness of lethal photosensitisation in killing *S. aureus* was evaluated using various strains, including MRSA, and three different photosensitisers, toluidine blue O, azure A and tin (IV) chlorin$_{e6}$ in combination with HeNe laser light. The susceptibility of the bacteria in lag, exponential and stationary phases was also investigated. All three photosensitisers were able to kill *S. aureus* with TBO being the most effective, giving a reduction in the viable count of up to 5 log units. The degree of killing obtained was dependent on both the strain and photosensitiser used, and varied with the growth phase from which the bacteria were harvested. In conclusion, lethal photosensitisation is a highly effective way of killing *S. aureus*/MRSA, and may be a viable option for the treatment of localised infections and decolonisation regimens.

MI 02 Toll-like receptor 2 and host susceptibility to severe *Staphylococcus aureus* disease

C.E. MOORE[1], S. SEGAL[1], A.B. BERENDT[2], A.V.S. HILL[1] & N.P.J. DAY[1]
[1]Nuffield Dept of Clinical Medicine, John Radcliffe Hospital, Oxford, [2]Bone Infection Unit, Nuffield Orthopaedic Centre, Oxford

The toll receptors are ancient, conserved proteins essential for the innate immune response to a variety of stimuli including bacteria, viruses and fungi. Toll-like receptor 2 (TLR2), part of the human innate immune system, binds directly to peptidoglycan on the surface of *Staphylococcus aureus*, triggering an inflammatory response which results in the production of a number of cytokines. Previous work has suggested a link between staphylococcal disease and a coding mutation in the *tlr2* gene, though this association was statistically weak and the number of patients studied small.

We examined whether this polymorphism was associated with staphylococcal disease in a large case-control study of severe *S. aureus* infection, recruited in a Caucasian population based in Oxfordshire. DNA from 428 severe disease patients and 696 neonatal cord blood controls was typed using a method involving PCR followed by RFLP analysis. We found no association between the previously implicated polymorphism and severe *S. aureus* disease, suggesting that this polymorphism is not an important susceptibility factor. Another recently discovered polymorphism in the *tlr*2 gene has been associated with *Streptococcus pneumoniae* infection and is currently under investigation.

MI 03 A new pathogenicity island of *Staphylococcus aureus*

WILLEM VAN WAMEL, MAARTJE RUYKEN, KOK VAN KESSEL & JOS VAN STRIJP
Eijkman-Winkler Institute, UMC Utrecht, The Netherlands

Recently our lab described CHIPS a Chemotaxis Inhibitory Protein of *Staphylococcus aureus*. CHIPS interacts specifically with C5a receptor (C5aR) as well as the formylated peptide receptor (FPR) of human neutrophils resulting in the specific and total down regulation of the response to both receptors.
In strain Newman the gene for CHIPS (*chp*) was found to be located on a new pathogenicity island (SaPI4) encoding beside for *chp*, the genes for staphylokinase (*sak*) and enterotoxin A (*sea*). SaPI4 is located at the 5' end of a prophage that incorporates in the gene for β-toxin (*hlb*).

Eighty-five random selected clinical strains and five classical lab strains were tested for SaPI4 using PCR and southern blotting. PCR reactions were preformed with primers pairs specific for *hlb*, *chp*, *sak*, *sea* and enterotoxin P (*sep*). In addition southern blot analyses were performed on EcoR1 or XhoI digested chromosomal DNA of every strain, using dig labeled *chp*, *sak*, *sea* or *sep* probes. SaPI4 was found in 90% of the *S. aureus* strains. Seven variants of SaPI4 were discovered, type A: *chp-sak-sea* was found in 12.2% of the strains, type B: *chp-sak* in 24.4%, type C: *chp* in 14.4%, type D: *sak-sea* in 16.6%, type E: *sak* in 14.4%, type F: *chp-sak-sep* in 4.4%, type G: *sak-sep* in 3.3% and in 10% of the strains no variant of SaPI4 was detected.

Do SaPI4 variants exist in different *S. aureus* families? To address this question, we preformed pulse field gel electrophoreses (PFGE) of all strains. Combining the PFGE-types and the SaPI4-types it became clear that SaPI4a was found in 3 PFGE-types, SaPI4b in 4 PFGE-types, SaPI4c in 1 PFGE type, SaPI4d in 4 PFGE-type, SaPI4e in 5 PFGE types, SaPI4f in 1 PFGE-types, and SaPI4g in 1 PFGE-type.

Of all variants bacteriophage production was induced by mitomycin C, bacteriophages were collected and used for lysogenization of *S. aureus* R5 (broad phage host). Lysogenic R5 derivatives were found for all variants except for SaPIc. The different lysogens were analyzed by southern blotting using dig labeled *chp*, *sak* and *sea* probes (depending on the SaPI4 type) and *hlb* probes. In all cases the SaPI4 containing bacteriophages were incorporated into *hlb.* Furthermore in all lysogens expect for one, one copy of the prophage was found. Finally if a lysogen carried *chp*, CHIPS was detected by ELISA

By mitomycine C treatment of the lysogens, large quantities of the different bacteriophages were obtained. Bacteriophage DNA was isolated and digested with EcoR1. Nine different phages carried SaPI4.

These data show SaPI4, carrying *chp*, *sak*, *sea* or *sep*, to be a wide spread, highly variable mobile DNA fragment.

MI 04 Plant estrogen genistein as anti-staphylococcal agent

MARGARETA. VERDRENGH & ANDREJ TARKOWSKI
Dept of Rheumatology, Göteborg University, Guldhedsgatan 10A, S-413 46 Göteborg, Sweden

Background The isoflavone genistein is found in high levels in soy beans. It has been shown to exert anti-tumoral properties, while its impact on bacteria is less well documented. The appearance of staphylococci resistant to antibiotics contributes to increasing number of fatal infections. Our aim was to examine the effect of genistein on the growth of staphylococci.
Methods Bacteria were cultured in broth containing genistein at different concentrations. At different time points bacterial growth was monitored by viable counts. Following bacterial strains were analyzed: *S. aureus* LS-1, Newman, 67-0 (MRSA), 1061, PLs(MRSA), *Streptococcus bovis*, *Bacillus cereus*, and *Escherichia coli.*
Results Genistein at 100μM exhibited inhibitory effect on all staphylococcal strains used. The inhibiton varied between 5 fold in case of bacteria grown in log-phase and 12 to 60-fold when thawed bacteria were used. Also the growth of other gram-positive bacteria was clearly inhibited by genistein. In contrast, growth of *E. coli* was not suppressed by genistein.
Conclusion Isoflavone genistein exerts a suppressive effect on the growth of gram-positive bacteria, including *Staphylococcus aureus*.

MI 05 Frequencies of novel and putative exotoxin genes in strains of *Staphylococcus aureus* from bovine mastitis

DAVIDA SMYTH[1], PATRICK J. HARTIGAN[2], WILLIAM J. MEANEY[3] & CYRIL J. SMYTH[1]
[1]Dept of Microbiology, Moyne Institute of Preventive Medicine, [2]Dept of Physiology, Trinity College, Dublin 2, [3]Teagasc, Dairy Production Research Centre, Moorepark, Fermoy, Republic of Ireland

In the last five years a rapid expansion in the discovery of new staphylococcal extracellular toxins has occurred, in particular superantigens or potential superantigens, including nine new enterotoxins, fifteen so-called exotoxins (SETs) and three other putative enterotoxins. This aim of this study was to determine the frequencies of novel enterotoxin genes in Irish strains of *Staphylococcus aureus* associated with bovine mastitis. Twenty-one bovine *S. aureus* strains representative of the major Irish clonal types along with a number of human clinical control strains were subjected to PCR analysis and Southern blots. A multiplex PCR was used (Monday, S.R. & Bohach, G.A. (1999). *J Clin Microbiol* **37,** 3411–3414) to screen for enterotoxin genes *sea–sej.* A multiplex PCR was also designed to facilitate the screening for the more recently described genes *sek–seo.* Of the twenty-one bovine strains tested only 9 (43%) possessed genes for novel toxins. Interestingly the nine enterotoxin-gene positive strains belong to one Irish clonal type. The genes encoding the SET proteins appear to be more widespread. All of the bovine strains tested by PCR analysis and by Southern blotting with probes specific for *set* genes appear to have a *set* locus which varies in size and composition.

MI 06 Genetic resistance to intestinal colonisation and faecal shedding of *Salmonella* in inbred chicken lines

PAUL WIGLEY, NAT BUMSTEAD, KERRIE MARSTON, MARGARET LOVELL & PAUL BARROW
Institute for Animal Health, Compton Laboratory, Compton, Newbury, Berkshire RG20 7NN

Salmonella food poisoning in man, through consumption of contaminated poultry, remains a major problem. Numbers of human cases remain high despite improved hygiene and increased vaccination leading to a decrease in infected flocks. This probably reflects cross-contamination of meat during slaughter and processing. Currently available control methods: vaccination, competitive gut flora preparations and antibiotics have variable efficacy or economic or public health implications. Therefore control of gastrointestinal tract colonisation and faecal shedding through genetic resistance is an attractive option. The ability of *S. typhimurium* to colonise the gut and be shed in faeces was determined in six-week old inbred lines of chickens. Differences were found between lines in the numbers of birds shedding and the numbers of *Salmonella* colonising the caeca. Lines with low excretion (6_1 & W1) were termed resistant, those with high excretion (7_2 & N) termed susceptible. Similar, though less pronounced, results were found with *S. enteritidis* and *S. infantis*. No difference in serum anti-*Salmonella* IgG or IgA titre or the inhibition of *Salmonella* growth by caecal contents was found between lines. Resistant lines showed a slight increase in circulating heterophils (PMNs) and in intestinal flow rate indicated by production of faecal droppings. In an F1 generation resistance was found to be dominant, not linked to sex, MHC or *SAL1*, the gene implicated in resistance to systemic salmonellosis in chicken.

MI 07 Structure of the fibrinogen-binding domain from a *Staphylococus epidermidis* adhesin

M.G. BOWDEN[1], S.L. DAVIS[1], S. GURUSIDDAPPA[1], P. KARTHE[2], S.V.L. NARAYANA[2] & M. HÖÖK[1]
[1]Center for Extracellular Matrix Biology, Institute of Biosciences and Technology, Texas A&M University Health Science Center, Houston, TX, USA, [2]University of Alabama at Birmingham, AL, USA

Staphylococcus epidermidis is an important opportunistic pathogen and is a major cause of foreign body infections. We have characterized the ligand-binding region of SdrG, a fibrinogen-binding microbial surface component recognizing adhesive matrix molecules from *S. epidermidis.* The recombinant minimum-binding region of SdrG (rSdrG(273-597)) was shown to bind specifically to the N-terminal 25 residues of the Fg ß-chain. A peptide alanine-amino acid replacement strategy was used to define the interactions between rSdrG and peptide ß6-20 in solid-phase assays. The Fg ß6-20 peptide residues found to be necessary for rSdrG binding were confirmed by the rSdrG(273-597)-ß6-20 co-crystal structure recently solved. Recombinant SdrG(273-597) contains two similarly folded domains. The fold observed is a new variant of the immunoglobulin motif that we have called DE-variant or the Dev-IgG fold. Mutagenic substitution of rSdrG residues S83 and D84, predicted to make contact with the ß6-20 peptide, resulted in proteins with no or markedly reduced affinity for human fibrinogen. The characterization of the structure of rSdrG with its ligand provides a wealth of information that can be used in the rational design of small inhibitors of SdrG binding to fibrinogen.

MI 08 Molecular analysis of coagulase-negative *Staphylococcus* mutants deficient in response to catecholamine inotropes

R.D. HAIGH[1], M. JOHNSON[1], J.A. MORRISSEY[1], P.H. WILLIAMS[1], M. LYTE[2] & P.P.E. FREESTONE[1]
[1]Dept of Microbiology and Immunology, University of Leicester, University Road, Leicester, LE1 9HN, [2]Dept of Surgery, Minneapolis Medical Research Foundation, Hennepin County Medical Centre, Minneapolis, MN 55404, USA

Opportunistic infection is a major problem in intensive care units. Of particular concern is sepsis as a result of colonisation of indwelling catheter lines by coagulase-negative staphylococci (C-NS), in particular the skin commensal *Staphylococcus epidermidis*. The environmental factors which influence growth and biofilm production by the C-NS are currently poorly understood; however, we have shown that the catecholamine inotropes, a class of drugs regularly administered to patients through intravenous lines, were able to massively increase the growth and the formation of biofilms by these organisms. In order to analyse the genes involved in the response of C-NS to catecholamine inotropes we have constructed an *S. epidermidis* Tn917-LTV1 transposon insertion library. The library has been arrayed and the mutants then assessed for noradrenaline responsiveness *via* growth in a noradrenaline-supplemented serum-based medium. Growth deficient isolates will be analysed by Southern blot analysis to confirm single chromosomal insertions. The flanking chromosomal DNA will be rescued as an *E. coli* shuttle vector, and disrupted genes will be identified by DNA sequencing from transposon specific primers. Ultimately, this research aims to establish the need for, and to inform the design of, inotropic treatment regimens that would reduce the risks of catheter colonisation and sepsis by C-NS.

MI 09 Determination of the active residues in binding function of AlbA protein by alanine-scanning mutagenesis

LIXING WENG, QI LI, JINLING XU & LIANHUI ZHANG
Laboratory of Biosignals and Bioengineering, Institute of Molecular Agrobiology, 1 Research Link, The National University of Singapore, Singapore 117604

A new Alba gene isolated from Klebsiella oxytoca (ATCC 13182) encodes a 26 kda protein which binds albicidin without further catalytic digestion activity. For deeper insight into the position of binding residues, an extensive mutational analysis was carried out by using site-directed mutagenesis and alanine scanning. This new protein has four histidine residues, site-directed mutagenesis of H78G, H141G and H189G did not affect enzyme activity suggesting that these residues are not essential for binding activity. However, H125G dropped 30% of the binding ability, indicating a role of this residue in function. H125E, H125A, H125L kept part of enzyme activity and deletion of His125 caused losing binding ability completely. We singly

mutated amino acid residues from E81 to Q178 in AlbA protein with alanine, expressed the mutants in E. coli DH5a cells and studied the effects of the mutation on the binding activity of albicidin with each mutant. Several mutants such as K106A, W110A, Y113A, L114A, Y126A and W162A decreased about 30% of the binding ability when they were expressed at the same protein level and Y126A showed a significantly reduced (60%) binding activity relative to the native enzyme. We propose that Lycine-106, Tryptophan-110, Tyrosine-113, Tryptophan-114, Histidin-125, Tyrosine-126 and Tryptophan-162 together form the binding domain of AlbA.

MI 10 Characterisation of a novel siderophore transport operon in *Staphylococcus aureus*

JANE HAMMACOTT[1], ALAN COCKAYNE[1], JULIE MORRISSEY[2] & PAUL WILLIAMS[1]
[1]Institute of Infections and Immunity, Queen's Medical Centre, University of Nottingham, NG7 2UH, [2]Dept of Microbiology and Immunology, University of Leicester, University Road, Leicester LE1 9HN

Iron is an essential growth requirement for most organisms and iron acquisition is likely to be an important virulence determinant. We are studying iron acquisition in *S. aureus* with a view to understanding virulence mechanisms and identifying novel drugs targets.

Analysis of the *S. aureus* genome has identified a novel ABC transporter operon, similar to a *Bacillus subtilis* operon, *yfmCDE*. These operons show similarity to known ferrichrome transporters including *fhuCBG* from *S. aureus*. However, previous studies have shown that disruption of *fhuG* results in the inability of *S. aureus* to grow on ferrichrome, making it unlikely that the *yfmCDE* homologue encodes a ferrichrome transporter. We are currently investigating the possible role of this operon in iron acquisition.

The *yfmC* homologue, encoding the lipoprotein component of the transporter, has been over-expressed, purified and antibodies generated. Preliminary studies of the expression profile of this protein indicate that it is not strongly regulated in response to iron levels. We are currently comparing the expression profile of *yfmC* under different growth conditions, since we have previously found that expression of some iron-regulated proteins is regulated differently in different culture media. Interestingly, the anti-YfmC antibody cross-reacts with a protein of similar size present in membrane extracts of *S. epidermidis* suggesting that the presence of a similar operon in this organism.

MI 11 Molecular characterisation of the interactions between *Staphylococcus aureus* and elastin

ROBERT DOWNER, FIONA ROCHE & TIMOTHY J. FOSTER
Moyne Institute of Preventive Medicine, Trinity College Dublin, Ireland

The elastin-binding protein of *Staphylococcus aureus* (EbpS) was reported as the surface protein responsible for the binding of soluble tropoelastin to bacterial cells. Fractionation of staphylococcal cells revealed that the protein is not a cell wall protein but is associated with the cytoplasmic membrane. Analysis of EbpS-PhoA and EbpS-LacZ fusions expressed in *Escherichia coli* revealed that EbpS is an integral membrane protein with both the N terminus and C terminal terminus located on the extracellular face of the cytoplasmic membrane. Studies to test binding of *S. aureus* to immobilised elastin peptides showed that EbpS was not responsible for bacterial adherence, casting doubt on the classification of this protein as an MSCRAMM. An *ebpS* deficient strain of *S.aureus* grew slower than the isogenic wild-type strain during exponential growth suggesting that EbpS may have a role other than soluble elastin binding. Experiments with bacterial binding to immobilised elastin revealed that the fibronectin-binding proteins, FnBPA and FnBPB, are responsible for the adherence of staphylococcal cells to immobilised elastin peptides. This is the first report of *S. aureus* binding to immobilised elastin and this interaction may be important for bacterial colonisation of elastin rich tissue such as lung, skin and heart valves.

MI 12 Location of the binding site for clumping factor B (ClfB) of *Staphylococcus aureus* in the A -chain of human fibrinogen

E.J. WALSH[1], S. PERKINS[2], M. HÖÖK[2] & T.J. FOSTER[1]
[1]Dept of Microbiology, Moyne Institute of Preventive Medicine, Trinity College, Dublin 2, Ireland. [2]Institute of Biosciences and Technology, Texas Medical Center, Houston, Texas USA

The fibrinogen-binding activity of ClfB, a microbial surface component recognising adhesive matrix molecules of *S. aureus* has been characterised. Western ligand blot analysis showed that a recombinant form of the N-terminal A-region of ClfB (rClfB44-542) bound to the native Aα-chain of human fibrinogen (Fg). Recombinant ClfB44-542 and *S. aureus* expressing ClfB adhered to microtitre plates coated with a recombinant form of the Aα-chain expressed in *E. coli*. ClfB mediated adherence to the Aα-chain of Fg could be inhibited by rClfB44-542. Recombinant truncates of the Fg Aα-chain were constructed and the binding site for ClfB was localised to the COOH-terminal portion of the Aα-chain (α-c domain, residues 220-610). The α-c domain of Fg is important in the regulation of fibrin polymerisation and it has been reported that this region contains novel high affinity plasminogen and plasminogen activator binding sites, which play an important role in fibrin polymerisation. The binding site for ClfB was located between residues 334 and 437 of the □-c domain. To further define the ClfB binding site a series of synthetic peptides representing this region were synthesised and their ability to inhibit the binding of rClfB44-542 to Fg is being tested. The binding of ClfB to the α-c domain and interfering with fibrinolysis is also being investigated.

MI 13 Molecular analysis of the Fbl protein of *Staphylococcus lugdunensis*

JENNIFER MITCHELL & TIM FOSTER
Microbiology Dept, Moyne Institute of Preventative Medicine, Trinity College Dublin, Ireland

The Fbl protein of *Staphylococcus lugdunensis* is closely related to the fibrinogen-binding clumping factor, ClfA of *Staphylococcus aureus*. This study showed that Fbl promotes binding of *S. lugdunensis* to fibrinogen. The *fbl* gene was present in all strains of *S. lugdunensis* tested. The ligand binding A regions of Fbl and ClfA are 48% identical. However the sequence of the N-terminal 174 residues of region A were completely different whereas residues 175-493 of Fbl are 60% identical. Expression of Fbl on the surface of the heterologous expression host *Lactococcus lactis* caused these cells to clump in soluble fibrinogen and to adhere in a dose-dependent manner to immobilised fibrinogen. Fbl and ClfA bound to the same region of the γ-chain of fibrinogen. The fibrinogen binding activity of Fbl in *S. lugdunensis* and *L. lactis* was shown to be inhibited by anti-ClfA-region A antibodies. Recombinant ClfA region A inhibited the fibrinogen binding activity of Fbl when expressed on the surface of *L. lactis*. Therefore Fbl is a clumping factor of *S. lugdunensis* that promotes binding of *S. lugdunensis* to the γ-chain of fibrinogen.

MI 14 Characterisation of staphylococcal ferritin homologues

J.A. MORRISSEY[1], A. COCKAYNE[2], K.J. BRUMMELL[3] & P. WILLIAMS[2,4]
[1]Dept of Microbiology and Immunology, University of Leicester, [2]Institute of Infections and Immunity, University of Nottingham, [3]Dept of Molecular Biology and Biotechnology, University of Sheffield, [4]School of Pharmaceutical Sciences, University of Nottingham

Ferritins are important iron storage proteins in many bacteria and may also play a role in resistance to oxidative stress. We have begun to investigate the physiological function of ferritin homologues in *S. aureus* and *S. epuidermidis*.

Analysis of genome sequences identified a single ferritin homologue in *S. aureus* which was amplified by PCR and cloned into the expression vector pET30a. Over-expressed protein was used to generate monospecific antiserum, and immunoblotting studies identified the ferritin protein in cell lysates of both *S. aureus* and *S. epidermidis*. In common with many other bacteria, ferritin expression in *S. aureus* was maximal in stationary phase and was positively regulated by iron. In contrast, ferritin expression in *S. epidermidis* was constitutive. As previously reported, ferritin expression was found to be constitutive in an *S. aureus* PerR mutant - a manganese responsive regulator - but interestingly, addition of manganese and iron to wild type *S. aureus* still resulted in enhanced ferritin expression suggesting that iron rather than manganese may play the key role in regulating ferritin expression in this bacterium.

MI 15 Functional characterisation of a putative ABC-type metal ion transporter in the Staphylococci

K.A. RIDLEY[1], A. COCKAYNE[1], S. STOLNICK[2] & P. WILLIAMS[1,2]
[1]Institute of Infections and Immunity, University of Nottingham, [2]School of Pharmaceutical Sciences, University of Nottingham

Metal ion uptake systems are poorly understood in the Staphylococci. Previously, a putative metal ion transporter termed *sit*ABC has been discovered in *S. epidermidis* and a homologue, termed *mnt*ABC, has been identified in *S. aureus*.

Expression of the substrate-binding protein SitC has been shown to be upregulated in response to iron and manganese starvation though the true substrate specificity of this protein remains to be determined. Studies have been conducted to identify the substrate specificity of SitC to further characterise the role of the *sit* transport system in metal ion homeostasis in *S. epidermidis*.

SitC was over-expressed in *E. coli* and based on the predicted metal-binding function of SitC, metal chelate chromatography was employed for purification, which additionally indicated a low affinity Ni^{2+}-binding capacity for this protein.

Biophysical analysis of SitC substrate-binding specificity has been performed using isothermal titration microcalorimetry which confirmed the low affinity interaction with Ni^{2+} observed during purification. The relative affinity of SitC for other metal ions is currently being investigated and comparative studies with purified MntC will indicate if observed sequence differences between the two proteins correlate with differences in the ligand binding profiles.

MI 16 Imaging *Staphylococcus aureus* invasion in real time

S.N.A. QAZI[1], T. SELF[4], P. WILLIAMS[2] & P.J. HILL[1,3]
[1]Institute of Infections and Immunity, Queen's Medical Centre, University of Nottingham, Nottingham, NG7 2UH, [2]School of Pharmaceutical Sciences, University of Nottingham, Nottingham NG7 2RD, [3]School of Biosciences, University of Nottingham, Loughborough, Leicestershire LE12 5RD, [4]Institute of Cell Signalling, Queen's Medical Centre, University of Nottingham, Nottingham NG7 2UH

It is now widely recognised that *Staphylococcus aureus* can become internalised in a wide variety of mammalian in cells, and that the intracellular location of the bacteria may contribute to persistent staphylococcal infections. We have developed methodologies, using a combination of fluorescent proteins with readily available dyes and confocal microscopy, to study the invasion of cell lines by *S. aureus* and their subsequent replication over a specific time courses. The technology allows us to readily assess which part of the infection cycle is affected in *S. aureus* mutants.

MI 17 Photoactivated Phenothiazinium dyes as antimicrobial agents

ZIA SAYED, FRED HARRIS & DAVID PHOENIX
Centre for Forensic Science, University of Central Lancashire, Preston PR1 2HE

The illumination of cytotoxic photosensitising molecules leads to the generation of highly reactive free radical species able to damage membranes, DNA and other cellular structures. Phenothiazinium dyes and close analogues are highly effective photosensitising agents and here, have been tested for antibacterial activity against *Staphylococcus aureus*. Dyes were found to possess biostatic and biocidal activity with MIC's and MLC's in the micromolar range but which were reduced up to tenfold when illuminated with a light dose of 6.3 J cm^{-2}. The planar molecules of Phenothiazinium dyes are known to intercalate with DNA, suggesting a possible killing target. Dye ability to damage *S. aureus* DNA was investigated using the 'Comet' assay. Cells of the organism were trapped in low melting Agar and after incubation with illuminated dyes, were lysed under electrophoretic conditions. DNA released was visualised and showed the 'trail' pattern characteristic of fragmented DNA, implying that the dyes tested were able to damage DNA. This ability was confirmed by HPLC analysis, which showed dye attack on DNA to produce 8-hydroxy-2'-deoxy-guanosine with levels enhanced by illumination.

MI 18 Use of hydrophobic moment analysis to investigate structure / function relationships of α-helical antimicrobial peptides

SARAH DENNISON, FRED HARRIS & DAVID PHOENIX
Centre for Forensic Science, University of Central Lancashire, Preston PR1 2HE

Many eukaryotic organisms ubiquitously produce α-helical peptides with antimicrobial action. This action involves membrane penetration but by varied mechanisms. Here, the membrane interactive potentials of a number of such peptides, active against Gram-negative, Gram-positive, and both classes of bacteria, were characterised using hydrophobic moment analysis. Regression analysis of the results revealed a negative correlation between mean hydrophobic moment and mean hydrophobicity of peptides active against Gram-negative bacteria, and, Gram-negative and Gram-positive bacteria. This implied that a characteristic balance between peptide amphiphilicity and hydrophobicity was necessary for membrane interaction of. Graphical analysis showed these α-helical peptides to possess wide hydrophobic faces and narrow hydrophilic faces, characteristic of α-helical peptides, which use a pore mechanism of membrane interaction. Similar analysis of peptides active against Gram-positive bacteria showed their mean hydrophobic moments to cluster around 0.5 and show little correlation with their mean hydrophobicities. These results suggest that possession of a threshold amphiphilicity alone is important for membrane interaction of these peptides and suggests a carpet mechanism for membrane penetration.

Wednesday 18 September 2002

0905 Entry and exit of proteins to and from the ER in *S. cerevisiae*

C. STIRLING
University of Manchester
Abstract not received

0940 Early steps in protein secretion by the yeast *Yarrowia lipolytica*

J.M. BECKERICH
Laboratoire de Microbiologie et Génétique Moléculaire, INA-INRA CNRS, Institut National Agronomique, 78850 Thiverval-Grignon, France
Yarrowia lipolytica is an industrial yeast which has the outstanding property to secrete large quantities of proteins, especially the alkaline extracellular protease (AEP). We have shown that AEP is transported by co-translational translocation in the endoplasmic reticulum (ER) and that the majority of the translocation sites on the ER membrane are devoted to this type of translocation. To study this pathway, we have taken advantage of a thermosensitive mutant of the 7S RNA, the nucleotidic moiety of the Signal Recognition Particle. Suppressors and co-lethal mutations were selected. Several of them were studied. The product of *SLS1* identified by a co-lethal mutataion was shown to be in the ER lumen acting as a partner of Kar2p, the main hsp70 of the ER. Tsr1p identified by a suppressor mutation, was located in the ER membrane. Sls2p was mapped far downstream from the ER. Tsr3p was shown to be a cytoplasmic protein related to the cytoskeleton. Altogether, these results highlight the tight coupling of the events along the secretory pathway and the effectiveness of the *Yarrowia* model to explore the protein secretion pathway.

1015 Role of the VFT complex in endosome to Golgi membrane traffic

SYMEON SINIOSSOGLOU & HUGH R.B. PELHAM
MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH
Membrane traffic requires vesicles to fuse with a specific target, and SNARE proteins and Rab/Ypt GTPases contribute to this specificity. In the yeast *Saccharomyces cerevisae,* the Rab/Ypt GTPase Ypt6p is required for fusion of endosome-derived vesicles with the late Golgi. We have previously shown that activation of Ypt6p depends on its exchange factor, Ric1p-Rgp1p, a peripheral membrane protein complex restricted to the Golgi. In order to understand how activated Ypt6 mediates membrane fusion, we looked for proteins that specifically recognize Ypt6-GTP. We found that a conserved trimeric protein complex, VFT (Vps52-53-54), that was shown before to be required for efficient recycling of late Golgi proteins, binds directly to the GTP form of Ypt6.

Localization of VFT to the Golgi requires Ypt6p, but is unaffected in mutants, in which late Golgi integral membrane proteins, including SNAREs, are mislocalised. The VFT complex also binds directly to the N terminal domain of the SNARE Tlg1p, both *in vitro* and *in vivo*, in a Ypt6p independent manner. We suggest that the VFT complex links vesicles containing Tlg1p to their target, which is defined by the local activation of Ypt6p. We are currently investigating (a) the interactions of the different VFT subunits with Ypt6p and Tlg1p (b) whether the VFT complex plays a role in the assembly of SNARE complexes and membrane fusion.

1120 Vesicle trafficking in filamentous fungi

N. READ
University of Edinburgh
Abstract not received

1155 Secretion genes and their regulation in *Aspergillus*

C. VAN DEN HONDEL
TNO Nutrition and Food Research, Zeist, The Netherlands
Abstract not received

1400 Protein degradation at the Endoplasmic Reticulum

THOMAS SOMMER
Max-Delbrück-Center for Molecular Medicine, Germany
The secretory pathway of eukaryotic cells harbors an elaborate protein quality control system, which prevents the deployment to the secretory pathway of misfolded or unassembled proteins. This system is localized in the Endoplasmic Reticulum (ER). ER associated protein degradation (ERAD) is an important component of this quality assurance system and directs misfolded proteins for destruction by the cytoplasmic ubiquitin-proteasome pathway.

ERAD can be divided mechanistically into five steps: First, misfolded proteins are detected in the ER-lumen. Second, the proteolytic substrates are targeted to and inserted into an aqueous transport channel that includes the multispanning membrane protein Sec61p. Third, the substrates are transported back into the cytosol (dislocation). Fourth, dislocated substrates are marked with the polypeptide ubiquitin by membrane-bound components of the ubiquitin system. These include the ubiquitin-conjugating enzymes Ubc1p, Ubc6p and Cue1p assembled Ubc7 and the ubiquitin ligase Hrd1p. Fifth, the ubiquitin-conjugated and dislocated molecules, which are still attached to the cytosolic surface of the ER-membrane are mobilized by the Cdc48p/Ufd1p/Npl4p ATPase complex. Finally, the cytosolic 26S-proteasome complex digests the misfolded proteins.

1435 Signalling during secretion in filamentous fungi

MERJA PENTTILÄ, MARKKU SALOHEIMO, TIINA PAKULA & MARI VALKONEN
VTT Biotechnology, FIN-02044 VTT, Finland
The filamentous fungus *Trichoderma reesei* is an industrial fungus that produces in particular cellulose and hemicellulose hydrolysing enzymes used in various industrial applications. Production of these enzymes is repressed by glucose but in inducing conditions the fungus can secrete several tens of grams of cellulases into the culture medium. The strong cellobiohydrolase (CBHI) promoter has also been used for heterologous protein production in this fungus and the CBHI coding region as a fusion partner.

We have studied the signalling pathways occurring in response to overloading the secretory pathway or production of heterologous proteins in *T.reesei.* Several *sec* genes and folding factor genes have been isolated, as well as the UPR (unfolded protein response) pathway regulator *hac1* and the kinase *ire1*. As in *Saccharomyces cerevisiae,* upon UPR induction active HAC protein is generated through unconventional splicing but in addition it seems that also mRNA start site selection and translational control are involved. We have shown that overexpression of the active form of HAC leads to increased protein secretion in both yeast and filamentous fungi. An other novel signalling pathway that appears to function in *T.reesei* is controlling transcription of the endogenous secreted proteins. Under secretion stress conditions, provoked by drugs or expression of foreign proteins, transcription of secreted proteins is down-regulated. Subtraction libaries and proteomics methods are used to analyse further cellular responses to secretion stress.

1550 Hyphal extension and branching in relation to protein secretion

ODED YARDEN
Dept of Plant Pathology and Microbiology, Faculty of Agricultural, Food and Environmental Quality Sciences, The Hebrew University of Jerusalem, Rehovot 76100, Israel

Most protein secretion occurs around the region of the advancing tip in filamentous fungi. However, the relevance of the number of hyphal tips on protein secretion level in cultures of filamentous fungi is still under debate. Our limited understanding of the possible link between hyphal branching and secretion may be due to the fact that most of the evidence provided is based utilizing strains obtained by screens for hyperbranching mutants, with limited information concerning the genetic basis for hyperbranching and the possible mechanism linking hyperbranching with secretion. The availability of multiple *Neurospora crassa* morphological mutants and the amenability of this organism to classical and molecular genetic analyses and manipulations make this organism an ideal model for studying hyphal elongation and branching. By genetic dissection of some of these mutants it has become evident that hyperbranching can be a consequence of a wide variety of genetic alterations that include both "house-keeping" as well as regulatory components of the fungal cell. These include elements of the translational and glycosylation machinery as well as kinases and phosphatases involved in controlling intracellular processes. The use of pharmacological agents to mimic the genetic defects and potentially influence protein secretion will be discussed.

1625 The secretion of glycosylated proteins in *Candida albicans* and their interaction with the human host

N.A.R. GOW
Dept of Molecular and Cell Biology, Institute of Medical Sciences, University of Aberdeen, Aberdeen AB25 2ZD

The outer layer of the cell wall of *Candida albicans* is heavily enriched in glycosylated proteins. Protein glycosylation is important to ensure correct folding of the proteins and to protect the secreted protein from environmental hydrolases. In addition, we are investigating the hypothesis that specific carbohydrate epitopes have specific biological roles during infection, for example by acting as adhesive ligands, antigens or immunoregulatory molecules. We are exploring the role of the *O*- and *N*-linked mannans in the host-fungus interaction via the analysis of strains in which a range of genes encoding mannosyl transferases (*MNT1-5, OCH1*) or regulators of mannosyl transferases (*PMR1*, *MNN4*) have been disrupted. This generates genetically stable strains with specific truncations in cell wall mannans. Many *mnt*Δ mutants were attenuated in virulence in animal infection models. *O*-mannan is implicated in adhesion to both epithelia and endothelia. Deletion of several *MNTs* suggests that glycosylation of certain surface proteins may be required for yeast-hypha morphogenesis under certain conditions. The acid-labile phosphomannan, a component of N-mannan, has been implicated as a receptor for recognition of macrophages, however phosphomannan-depleted *mnn4* mutants were found to be ingested normally by these phagocytes. Analysis of glycosylation mutants to date therefore demonstrates that carbohydrate epitopes of mannoproteins do indeed play key roles in several aspects of *C. albicans* pathogenesis including adherence, cell wall permeability, drug sensitivity, virulence, and yeast-hypha morphogenesis.

Thursday 19 September 2002

0910 Structural adaptations of proteins and membranes in cold-adapted microorganisms

NICHOLAS J. RUSSELL
Dept of Agricultural Sciences, Imperial College, Wye, Ashford, Kent TN25 5AH

Psychrophilic microorganisms that live at or even below 0°C possess specially adapted proteins and membranes, compared with mesophiles and thermophiles, in order to counter the thermodynamic loss of structural flexibility that occurs as temperature is lowered to such cold values. Their proteins have three-dimensional structures with modifications to the intramolecular bonds, compactness of the core, surface interactions with solvent water, and inter-subunit interactions, which all serve to lower the apparent optimum temperature for enzyme activity, and increase the specific activity (k_{cat}) or physiological efficiency (k_{cat}/K_m) at low temperatures (typically 0-30°C). Such changes are at the expense of increased thermolability at moderate temperatures. The same is true of cytoplasmic and membrane proteins, but in membranes there are additional modifications to lipid composition. This ensures that both the membrane fluidity and phase behaviour are regulated so that membrane proteins can function and correct permeability characteristics are maintained. This talk will consider the adaptations in enzymes and membranes of cold-adapted microorganisms, which enable them to grow at low temperatures at rates comparable with those of mesophiles and thermophiles at higher temperatures.

0950 Expression of the cold-shock genes in Gram-negative bacteria

M.P. GALLAGHER, N. SIENKIEWICZ, N.J. HOLDEN, J. FAGE-BUTLER, I.W. HUTCHISON & F.B. WARD
Institute of Cell & Molecular Biology, School of Biology, University of Edinburgh, Mayfield Road, Edinburgh EH9 3JR

Bacteria are generally capable of growing over a temperature range of 30-40 degrees Celsius, although this range is often narrower for highly host-adapted pathogens. A variety of Gram negative mesophilic bacteria have been found to exhibit an adaptive stress response upon incubation at low temperature, which is referred to as the cold shock response. Studies initially in *E. coli*, revealed that several members of a set of small highly conserved proteins, referred to as the CspA family, are highly expressed during the adaptive process. Other members of this family, in contrast, appear not to be cold induced. Using a proteomics approach, we have examined the response of exponential and stationary phase cultures of the food-borne pathogen Salmonella, to incubation at temperatures above and below the minimum required for cell division. In all cases, the cells appear to attempt adaptation. However the degree and kinetics of the process vary significantly. Transposon mutagenesis and reporter gene studies have been used as a complementary approach to identify cold regulated genes. These have uncovered a curious mixture of targets which will be discussed during the presentation.

1100 Low-temperature adaptation in lactic acid bacteria

JEROEN A. WOUTERS[1,2]
[1]NIZO Food Research, P.O. Box 20, 6710 BA Ede, The Netherlands, Phone: ++31-318-659576, Fax: ++31-318-650400, E-mail: jwouters@nizo.nl, [2]Laboratory of Food Microbiology, Wageningen University, Wageningen, The Netherlands

Lactic acid bacteria (LAB) are frequently used to start industrial food fermentations. During production of these starter cultures and during manufacture and storage of the fermented products, LAB experience a variety of temperature changes. For these reasons, the cold-adaptive response of *Lactococcus lactis*, a mesophilic LAB that is widely used in the manufacturing of cheese, attracts interest. Because of the implications of cold-shock proteins (CSPs) in freeze protection and their presumed central role in cold adaptation, the role of these proteins of *L. lactis* is investigated. For *L. lactis* MG1363 a family of five *csp* genes is identified. The *L. lactis* chromosome was found to contain two sets of two tandemly located and cold-inducible *csp* genes (*cspA/cspB* and *cspC/cspD*) and a single, constitutively expressed *cspE* gene. By using *L. lactis* strains specifically overproducing the respective CSPs, it was found that these proteins protect against freezing and might be involved in the regulation of (non-7-kDa) cold-induced proteins (CIPs). In addition, we characterized the effects of multiple *csp* gene disruptions on adaptation to cold and gene regulation of *L. lactis*. Deletion of *csp* genes affects freeze survival of *L. lactis*, the production of the remaining counterparts of the lactococcal CSP family as well as the production of several CIPs.

1140 The role of RNA helicases in low temperature adaptation

RICARDO CAVICCHIOLI
School of Biotechnology and Biomolecular Sciences, The University of New South Wales, Sydney, NSW, 2052, Australia

An important factor that constrains microbial growth in cold environments or following cold shock, is the formation of deleterious secondary structures in RNA. In particular, cold-stabilised structures in mRNA may prevent translation initiation or elongation, and mRNA degradation. RNA helicases unwind duplex RNA thereby alleviating some of these problems. Low temperature regulated RNA helicases have been identified in members of the *Bacteria* and *Archaea*, and shown to have biochemical and genetic properties that indicate an important role in thermal adaptation. Regulation of RNA helicase gene expression in *Escherichia coli, Anabaena* (cyanobacteria) and *Methanococcoides burtonii* (methanogen) appears to occur through a common mechanism involving a long 5'-untranslated region (5'-UTR). This presentation discusses the role of RNA helicases in low temperature adaptation. Important advances in the field are highlighted while providing a focus on the RNA helicase from the Antarctic archaeon, *M. burtonii.*

1400 Low temperature spoilage yeasts

I.N. ROBERTS[2], H. STEELS[1], C.J. BOND[2], S.A. JAMES[2] & M. STRATFORD[1]
[1]Food Microbiology, Unilever R & D, Colworth House, Sharnbrook, Bedford MK44 1LQ, [2]National Collection of Yeast Cultures, Food Safety Science Division, Institute of Food Research, Colney Lane, Norwich NR4 7UA

At ambient temperature, the most notorious spoilage yeasts are found in the genera *Saccharomyces*, *Zygosaccharomyces* and *Dekkera*, all being highly-fermentative, preservative-resistant ascomycetes. In contrast, spoilage yeasts at low temperature are predominantly basidiomycetes, and the great majority are non-fermenting, causing spoilage by surface growth and film-formation. Foods spoiled include frozen chicken, fish and peas, and chilled vegetables and salads. Low temperature spoilage yeasts include *Rhodotorula glutinis*, *Trichosporon beigelii*, *Debaryomyces hansenii* and *Yarrowia lipolytica*.

A new spoilage yeast species, *Zygosaccharomyces lentus*, was recently discovered amongst strains previously identified as *Z. bailii*. *Z. lentus* was established as a new taxon by 26S D1/D2 rDNA sequencing, heat and oxidative stress sensitivity, and lack of growth in aerobic shaken culture at temperatures above 25ºC. This new species showed an unusual ability to grow and cause spoilage at 4ºC. *Zygosaccharomyces kombuchaensis* was

recently discovered in the 'tea fungus' used to make the oriental fermented tea, kombucha, together with *Acetobacter* spp.. *Z. kombuchaensis* was shown by ribosomal DNA sequencing to be a novel species, and the closest relative of *Z. lentus*. Subsequent analysis of *Z. kombuchaensis* revealed that this species shared the unusual low temperature growth characteristics, confirming its close genealogical relationship to *Z. lentus*.

1440 The cold shock response in *saccharomyces* species

KATHERINE A. SMART & JESSICA LECLAIRE
School of Biological and Molecular Sciences, Oxford Brookes University, Oxford OX3 0BP

At the end of a brewing fermentation, the yeast sediments to the bottom of the vessel and is recovered, through a process known as cropping, before transfer to a holding tank where it is stored until required for use in a subsequent fermentation. Cold shock has been postulated to occur during yeast sedimentation, cropping and storage.

The Cwp2 mannoprotein is one of the most abundant proteins in the cell wall and is believed to play a role in wall stabilisation along with another homologous constituent, Cwp1. During cold shock it has been suggested that wall proteins encoded by *TIP1, TIR1, TIR2* and *TIR4* are expressed whereas *CWP1* is down-regulated. Whilst this has been demonstrated to occur for haploid strains of *Saccharomyces cerevisiae*, no similar study has been conducted using industrial strains. In this paper we demonstrate that brewing yeast storage at 4°C elicits a cold shock specific response resulting in modifications in cell surface physical characteristics and wall functionality. These observations confirm the differential expression of cell wall mannoproteins and specific cold shock wall serine rich peptides for industrial strains of *Saccharomyces cerevisiae* and *Saccharomyces pastiaranus* following exposure to cold environments.

1550 Identification by genome-wide expression analysis of aquaporins and other, novel proteins as determinants of freeze tolerance in yeast

JOHAN M. THEVELEIN[1], AN TANGHE[1], BARBARA LEYMAN[2], KATLEEN LEMAIRE[2], FRANÇOISE DUMORTIER[1], SAM VAN DE VELDE[1] & PATRICK VAN DIJCK[2]
[1]Laboratorium voor Moleculaire Celbiologie and [2]Flemish Interuniversity Institute for Biotechnology-VIB, Institute of Botany and Microbiology, Katholieke Universiteit Leuven, Kasteelpark Arenberg 31, B-3001 Leuven-Heverlee, Flanders, Belgium. E-mail: johan.thevelein@bio.kuleuven.ac.be

The initiation of fermentation in the yeast *Saccharomyces cerevisiae* triggers a rapid loss of general stress resistance. This is inconvenient for industrial applications in which actively fermenting cells are subject to specific stress conditions, such as in the preparation of freeze doughs. We have isolated mutants deficient in fermentation-induced loss of stress resistance (*'fil'* mutants). The *fil1* mutant contained a specific mutation in adenylate cyclase confirming the role of glucose activation of the cAMP-PKA pathway for the loss of stress resistance. The *fil2* mutant contained a mutation in Gpr1, a G-protein coupled receptor which mediates glucose as well as sucrose activation of the cAMP pathway. Genome-wide gene expression analysis of the *fil1* mutant and subsequent investigation of several upregulated genes led to the identification of six novel genes important for the high resistance of the mutant. They all encode small proteins with unknown function that appear to be membrane bound. We have also isolated *fil* mutants directly in a commercial bakers' yeast strain. This was done by UV-mutagenesis of the tetraploid/aneuploid strain S47, preparation of small doughs with the mutagenised culture and subjecting the doughs to multiple rounds (up to 200) of freeze/thaw treatments. In this way we isolated strain AT25, which showed a better freeze tolerance than the parent strain S47 and behaved in a very similar way as S47 for most other properties although it turned out to be a diploid strain. Genome-wide gene expression analysis of several strains derived from AT25 has identified the aquaporin (water channel) genes *AQY1* and *AQY2* as determinants of freeze tolerance. Deletion of the aquaporin genes in a laboratory strain reduced freeze tolerance while overexpression enhanced freeze tolerance, also in the AT25 strain. Also expression of the human aquaporin gene, *hAQP1,* in yeast improved freeze resistance. A correlation between freeze tolerance and aquaporin expression was also observed in *Schizosaccharomyces pombe* and *Candida albicans*. These findings support a role for aquaporin-mediated plasma membrane water transport activity in determination of freeze tolerance. This appears to be the first clear physiological function identified for microbial aquaporins. We suggest that rapid osmotically driven efflux of water during the freezing process reduces intracellular ice crystal formation and resulting cell damage.

1630 Physiological and genetic adaptations in antarctic lichen-forming fungi

PAUL S. DYER, GARETH J. MURTAGH & PETER D. CRITTENDEN
School of Life and Environmental Sciences, University of Nottingham, University Park, Nottingham NG7 2RD

Lichens are the dominant components of the terrestrial "vegetation" in dry (ice-free) exposed terrain in both maritime and continental Antarctica. At least 350 species have been recorded, including many cosmopolitan lichens but also some species with a distribution limited to the Antarctic region. Studies are underway to investigate possible physiological and genetic adaptations present in the fungal 'mycobiont' partner that may allow survival in the hostile antarctic environment. Thalli of the bipolar lichen *Xanthoria elegans* were collected from polar and temperate locations worldwide and ascospore discharge used to establish axenic cultures. These were used in physiological experiments which revealed that isolates from different geographic locations exhibited significant growth differences. Temperature had a significant effect on relative growth rate (RGR) such that *X. elegans* originating from sites with lower mean temperatures had significantly higher RGRs at all test temperatures between 2^{o} and 18 ^{o}C. A larger 'growth engine' with enhanced metabolic activity might be an adaptation to growth in colder climates. Differences between isolates were also present in a molecular phylogenetic analysis based on rDNA sequence and RAPD tree data. Research is now ongoing to analyse the breeding systems of antarctic lichen-forming fungi, to determine whether they exhibit 'homothallic' (selfing) or 'heterothallic' (obligate outcrossing) breeding systems. Homothallism has been suggested to be an adaptation for growth in extreme environments.

POSTERS:

PBMG 01 Hyphal growth in fission yeast

EVELYN AMOAH-BUAHIN & JOHN ARMSTRONG
School of Biological Sciences, University of Sussex, Brighton, Sussex BN1 9QG

Many yeasts can switch from unicellular to mycelial growth as a means of colonizing a substrate. This growth pattern is often associated with infection. We have studied *Schizosaccharomyces. pombe* under varying environmental conditions and have found that Leupold's strains of *S. pombe* are unable to form the hyphal growth pattern, however, the *S. pombe* strain NCYC132 has been observed in earlier reports to occasionally form abnormal growth patterns. Our studies on this strain have showed that it can undergo a transition to pseudo-hyphal growth in response to environmental stresses. We have identified nutritional cues governing the growth switch, but the genes and signaling pathways involved are completely unknown.

S. pombe is thought to use both its MAPK and cAMP signaling pathways for mating. The question in mind now, is which pathway *S. pombe* uses for hyphal formation. Determining the genes involved in this process would be a step closer to answering this question. Information and techniques from *S.*

pombe, can be used or adapted to investigate the molecular basis of hyphal formation in *S. japonicus* var. *japonicus,* a dimorphic fission yeast which exhibits yeast to mycelium dimorphism. This study should help expand our understanding of how the model organism, *S. pombe* survives under environmental stress.

PBMG 02 Growth of *Staphylococcus aureus* on mucin and mucin-derived sugars

JOANNA C. WILKINS, KAREN A. HOMER & DAVID BEIGHTON
Dept of Oral Microbiology, GKT Dental Institute, King's College London, Caldecot Road, London SE5 9RW

Staphylococcus aureus, a commensal of the nasopharynx, is implicated in a range of infections including respiratory infections in cystic fibrosis patients. Mucins and mucin-derived sugars, (sialic acid, galactose, N-acetylgalactosamine, N-acetylglucosamine and fucose) are likely to be carbohydrates available in the nasal cavity of healthy individuals and the lungs of cystic fibrosis patients. As part of a study to examine the catabolism of mucin-derived sugars by *S. aureus* the sequenced strain (EMRSA-16; strain 252) was grown in a defined medium supplemented with glucose, galactose N-acetylneuraminic acid, N-acetylgalactosamine, N-acetylglucosamine or porcine gastric mucin. Whole cellular proteins from cells grown under different culture conditions were extracted and separated by two-dimensional PAGE. Differences in the protein profiles were identified and we have undertaken to assign putative functions to those proteins by peptide mass fingerprinting. Through the examination of existing sequence databases, proteomic methods and functional assays we have identified proteins implicated in the pleotrophic effects associated with the utilisation of amino-sugars including the intracellular catabolic enzymes neuraminate pyruvate lyase and N-acetylglucosamine-6-phosphate deacetylase. The identification of proteins, and their functions, used by *S. aureus* to replicate and persist *in vivo* are important steps towards understanding its pathogenicity.

PBMG 03 Isolation and characterisation of a cryptic plasmid from *Propionibacterium granulosum*

MARK D. FARRAR & KEITH T. HOLLAND
Skin Research Centre, Division of Microbiology, School of Biochemistry & Molecular Biology, University of Leeds, Leeds LS2 9JT

The cutaneous propionibacteria are the most prevalent residents of sebum-rich areas of human skin. Studies of these organisms have been limited by a lack of genetic manipulation methods. Our aim is to develop a genetic transfer system for the cutaneous propionibacteria.

Strains of *P. acnes* and *P. granulosum* were screened for the presence of endogenous plasmids. None of the *P. acnes* strains carried plasmids but two strains of *P. granulosum* carried a single plasmid of approximately 3.5 kb. One plasmid, pMF283, was sequenced fully. This plasmid was 3.54 kb in size and sequence analysis revealed three open reading frames. One gene, designated *rep*, encoded a protein with homology to the Rep family of plasmid replication proteins. This protein possessed motifs that indicated the plasmid replicates by the rolling circle mechanism. A second ORF encoded a protein that shared weak homology with Tra proteins, involved in transfer of DNA by conjugation. A third ORF encoded a protein of unknown function.

This is the first description of a plasmid from a member of the cutaneous propionibacteria and provides some evidence of DNA transfer in these organisms via conjugation. We are currently developing a vector system for these organisms.

PBMG 04 Induction of *Listeria monocytogenes* flagellin gene at temperatures below 20°C

FAYE M. BARNARD, AISHA NAZLI, TANIA M. PEREHINEC & CATHERINE E.D. REES
University of Nottingham, School of Biosciences, Sutton Bonington Campus, Loughborough, Leics LE12 5RD

Listeria monocytogenes is classically described at motile between 20-25°C, showing a characteristic tumbling form of motility. Analysis of proteins induced when *Listeria monocytogenes* cells were grown at 10°C revealed that a protein of approximately 30KDa is induced. N-terminal aminoacid sequence analysis of the induced protein indicated that this was the *Listeria* flagellin protein, FlaA. Use of a Pr_{flaA}::*luxAB* chromosomal reporter gene showed that the *flaA* promoter was induced at these low temperatures, and cultures grown in motility media demonstrate that cells can actively swim. Hence although the tumbling motility phenotype is abolished, cells produce flagellae and are actively motile. This suggests that the tumbling observed in rich broth between 20-25°C may be some interference with the chemotactic signalling mechanism rather than a reflection of the normal pattern of swimming by this organism.

PBMG 05 Cold shock proteins in *Listeria*: evidence for role of DNA binding proteins in low temperature induction

CATHERINE E.D. REES & FAYE M. BARNARD
University of Nottingham, School of Biosciences, Sutton Bonington Campus, Loughborough, Leics LE12 5RD

One of the most notable features of *Listeria monocytogenes* as a human pathogen is its psychrotrophic nature and its ability to proliferate at low temperature contributes to its ability to cause food poisoning. We have identified 3 major cold-shock gene homologues (*cspA* and *cspB*); a third gene homologue (*cspC*) has been identified from genome sequences. Analysis of proteins induced following cold shock show high level induction of one potential Csp protein and low level induction of a further two. *cspA* mRNA transcripts accumulate within 10 min of temperature downshift and, unlike in *E.coli* and other mesophiles, this mRNA remained detectable as long as the cells remained at low temperature. Promoter sequences showed strong homology to those of *Bacillus cspB* and alignment of gene sequences identified a conserved "Downstream Box" motif common to Gram-positive bacteria but distinct from that identified in *E. coli*. EMSA ('gelshift') analysis provided evidence for protein binding to an IHF consensus sequence found in the promoter region of *cspA*. However no IHF homologues have yet been identified in Gram-positive organisms but the *cspA* promoter showed lower levels of activity in an *ihf*$^-$ *E. coli* strain compared to the *ihf*$^+$ parent.

PBMG 06 Methylotrophs from Antarctica

SYEDA AZRA MOOSVI & ANN P. WOOD
King's College London, Microbiology Group, Life Sciences, Franklin Wilkins Building, 150 Stamford Street, London SE1 9NN

Dimethylsulfide (DMS) arises primarily from marine biogenic activity and is oxidised in the atmosphere to methanesulfonic acid (MSA), dimethylsulfone ($DMSO_2$) and other sulfur compounds which are deposited in rain and snow and hence become part of the biogeochemical sulfur cycle. They are known to have inputs to the Antarctic environment. Increasing levels of MSA have been measured over the years in Antarctic ice cores reflecting greater marine biogenic activity of global significance. Recently research has focused upon the isolation and characterisation of specialised bacteria that are capable of using such sulfur compounds, thereby degrading them and reducing the pollutant effect of these in nature.

Pure cultures of bacteria have been isolated from lake, soil and sediment samples of Signy Island, South Orkney, Antarctica. All cultures are Gram-negative, facultative methylotrophs, capable of growth on a range of heterotrophic and methylotrophic growth substrates. All the strains isolated to date are mesophilic, growing between 15 and 37 ^oC. Detailed studies

were carried out on one lake isolate (25S) and one soil isolate ($25E_1$) enriched on $DMSO_2$ and MSA respectively. Methylotrophic enzyme assays indicate that these strains use the serine pathway together with previously documented enzyme pathways for breakdown of C_1 sulfur substrates. Strain 25S appears to use a reductase pathway for metabolism of $DMSO_2$ involving $DMSO_2$- and DMSO-reductase enzymes while strain $25E_1$ appears to use an MSA monooxygenase enzyme complex for metabolism of MSA.

These results in addition to physiological studies, scanning electron microscopy and 16S rRNA sequencing of selected isolates suggest that strain 25S is most closely related to *Hyphomicrobium sulfonivorans* although enzyme profiles suggest strain differences. Strains $25E_1$ appears to be *Afipia felis* which has not previously been reported to be methylotrophic.

PBMG 07 What factors confer fitness to the pathogen *Staphylococcus aureus*?

RUTH C. MASSEY[1], CATRIN MOORE[2], NICHOLAS DAY[2] & SHARON J. PEACOCK[1]
[1]Nuffield Dept of Clinical Laboratory Sciences, [2]Nuffield Dept of Medicine, University of Oxford, John Radcliffe Hospital, Oxford OX3 9DU

The survival of any organism requires optimal fitness within the environment in which it exists. The pathogen *Staphylococcus aureus* survives in many diverse environmental niches: as a carriage isolate in the noses of 30% of the human population; as an invasive isolate in an otherwise healthy host and as an invasive isolate in an immuno-compromised hospitalised host. Many studies have shown that the acquisition of antibiotic resistance determinants is costly to the fitness of an organism upon removal of the antibiotic from the environment. Others have suggested that the expression of virulence factors, and the invasiveness this infers is unnecessary expenditure of energy, and must be costly to the overall fitness of the organism. In this study we have measured the relative fitness of each organism in a strain collection consisting of carriage isolates, community-acquired invasive isolates and hospital-acquired invasive isolates. This collection of isolates has been well defined in relation to clonality, the number of virulence factors present in each strain, and the antibiotics each strain is resistant to. We present data to suggest that there are strong associations between the relative fitness of an organism and whether it was community or hospital acquired, and found that the presence of the Mec element and specific virulence factors are a significant cost to the fitness of this organism.

PBMG 08 Small colony variants: phenotypic variants of *S. aureus* that confer population wide antibiotic-resistance

RUTH C. MASSEY[1], ANGUS BUCKLING[2] & SHARON J. PEACOCK[1]
[1]Nuffield Dept of Clinical Laboratory Sciences, University of Oxford, John Radcliffe Hospital, Oxford OX3 9DU, [2]Dept of Biology and Biochemistry, University of Bath, Bath BA2 7AY

Bacterial antibiotic resistance is often associated with a fitness cost in the absence of the antibiotic. As a means of circumventing this fitness cost the bacterium *S. aureus* has evolved a switching mechanism that results in the rapid emergence of antibiotic-resistant small colony variants (SCVs) upon exposure to antibiotics. Surprisingly, the emergence and growth of SCVs in antibiotic containing media also facilitate the growth of the remaining antibiotic sensitive population. Preoteomic analysis indicates that the switch from wild type to SCV is associated with a change in metabolic respiration from aerobic to anerobic. There is a concurrent lowering of environmental pH, which allows the survival and growth of wild type bacteria in the presence of an otherwise lethal dose of the antibiotic. These observations suggest that co-operative behaviour may play a role in the pathogenesis of this organism, and perhaps explains why persistence and relapse is such a common feature of infection.

PBMG 09 A sequence derived from a clinical isolate of *Staphylococcus aureus* shares homology with both a pathogenicity island and bacteriophage DNA

S.G. SHAWCROSS[1], M.M. DAWSON[1], H.A. FOSTER[2] & V. EDWARDS-JONES[1]
[1]Dept of Biological Sciences, Manchester Metropolitan University, Manchester M1 5GD, [2]Dept of Biological Sciences, University of Salford, Lancashire M5 4WT

When some strains of *Staphylococcus aureus* are grown in the presence of the anti-microbial compound silver sulphadiazine (AgSD) there is a shift in the *tst*-1-specific restriction digestion banding pattern. AgSD interacts with DNA, which may induce Pathogenicity Island (SaPI) excision and result in changes in the relative positions of *tst*-1 & surrounding restriction enzyme sites. Strain RN4282 (a SaPI-1 carrier) was used to investigate the effects of AgSD on the excision/circularisation of the SaPI-1. Using PCR primers, which amplify across the circularisation junction of SaPI-1, we have found preliminary evidence for an increase in SaPI-1 excision/circularisation. Clinical and laboratory isolates (n=34) were screened for evidence of circularised SaPI-1. Amplicons were obtained from four strains: RN4282 (control), T1 (NMTSS) and 2 wound isolates. When the T1-derived amplicon (521 bp) was sequenced it showed limited internal sequence homology with the RN4282-derived amplicon (567 bp). Southern hybridisation & restriction digestions of the wound isolate amplicons showed that they resemble the T1-derived amplicon. Database (BLAST) searching with the T1-derived sequence revealed some homology with phi PVL proviral DNA, the *S. aureus* phage L54 attP site and *S. aureus* temperate phage phiSLT. This highlights the likely common origin of SaPIs and bacteriophages.

PBMG 10 Microbial ecology and freeze adaptive mechanisms for antarctic lake bacteria

JACK GILBERT, JOHANNA LAYBOURN-PARRY, PHIL HILL & CHRISTINE DODD
Food Microbiology / Life and Environmental Sciences, Nottingham University, Sutton Bonington Campus, Leics LE12 5RD

38 lakes from the Vestfold Hills in Eastern Antarctica were sampled to assess bacterial community structure and water chemistry. 866 isolates were cultured and amplified ribosomal DNA restriction analysis was used to remove replicates and determine the phenetic relationships between bacteria. Community molecular fingerprinting techniques, e.g. DGGE, were also applied to sample filters collected from the lakes to assess culturing bias levels in the culture collection. Changes in water chemistry (dissolved organic carbon, phosphates, nitrates, nitrites, ammonia and chlorophyll *a*) were analysed throughout one year on five of the chosen lakes. These environmental parameters along with temperature and salinity were used to determine reasons for successional and spatial variability in the bacterial community within individual lakes, and to determine reasons for possible changes in community structure between lakes.

Bacteria were also screened, using a novel assay, for thermal hysteresis activity to determine methods of cold adaptation. Ice active bacteria were characterised by 16S rDNA sequencing and assessed using DGGE as to their dominance in the community to determine what, if any, advantage such adaptations impart on the bacteria.

PBMG 11 Comparative genomic analysis of hospital and community acquired *Staphylococcus aureus* strains

MATTHEW T.G. HOLDEN & THE PATHOGEN SEQUENCING UNIT
The Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge CB1 10SA

Whilst *Staphylococcus aureus* has gained notoriety as a serious nosocomial pathogen that exhibits increasing levels of resistance to all available antibiotics, it can also cause serious invasive disease in the community. We have sequenced the 2.8 Mb

genomes of two strains of *Staphylococcus aureus* that cause disease in these distinct clinical settings. The two strains chosen for sequencing are a recent U.K. hospital-acquired strain, representative of the epidemic methicillin resistant *S. aureus* (MRSA) strain EMRSA-16 (Strain 252), and a hyper-virulent community acquired methicillin sensitive *S. aureus* (MSSA) strain (Strain 476). The two complete genomes of these clinically distinct strains provide a unique opportunity for improving understanding of the mechanisms of epidemic spread, colonization of the host, persistence and virulence.

Comparative genomic analysis has revealed that, as with other *S. aureus* genomes, the two genomes are co-linear with discrete regions of difference. These vary in size from few nucleotides to tens of kb, and contain examples of horizontally transferred genetic elements, including: IS elements, transposons, bacteriophage and plasmids. Amongst the coding sequences found in these regions there are many unique or *S. aureus*–specific genes that have similarity to known virulence or drug resistance proteins; some of which may provide clues to the observed differences in virulence of the two strains. In addition, specific mobile regions of the *S. aureus* chromosome have been previously characterized that are associated with methicillin resistance (SCCmec) or pathogenicity (SaPI). Analysis of these regions in the MRSA and the MSSA strains, and in comparison to other sequenced strains, reveals the presence of difference sets of genes, thus highlighting the importance of these regions in generating and maintaining genetic diversity in *S. aureus* populations.

PBMG 12 The first glimpse of psychrophilic genomes

RICARDO CAVICCHIOLI[1], NEIL SAUNDERS[1], LAURA GIAQUINTO[1], SOPHIE MAZARD[1], AMBER GOODCHILD[1] & PAUL CURMI[2]
[1]School of Biotechnology and Biomolecular Sciences, Sydney, [2]School of Physics, The University of New South Wales, Sydney, NSW, 2052, Australia

The field of extremophiles is expanding at an extrordinary rate and in the last decade research on cold ("psychro") adapted organisms and their cellular products has greatly accelerated. Complete genome sequences now exist for a range of extremophiles (halophiles, acidophiles, radio-tolerant, hyper/thermophiles) from the *Bacteria* and *Archaea* domains. Despite these advances no genomes have been completed for psychrophilic/tolerant organisms. Of the 16 complete archaeal genomes (June 2002), 5 are for methanogens (and at least 4 others are in progress): *Methanopyrus kandleri* (98°C), *Methanococcus jannaschii* (85°C*), Methanothermobacter thermautotrophicus* (65°C*), Methanosarcina acetivorans* (37°C), *Methanosarcina mazei* (37°C*)*. Recently, we initiated genome sequencing of the psychrophilic methanogen, *Methanogenium frigidum* (15°C), and the Joint Genome Institute commenced the psychrotolerant methanogen, *Methanococcoides burtonii* (23°C). The completion of these genomes will expand our understanding of the organismal and metabolic biocomplexity that has evolved in cold-adapted *Archaea*, and provide the reference for comparing mechanisms of archaeal cold-adaptation with those in *Bacteria* and *Eukarya*. This presentation will describe the findings which are emerging from the partial psychro-methanogen genome sequences. Trends which may be gleaned about thermal adaptation from the genomic comparisons will be described; including analyses of data sets for methanogens, *Archaea*, prokaryotes and all genomes. In addition to bioinformatic studies, tools being developed for phenome analysis will be described.

PBMG 13 Isolation and characterization of K5 type yeast killer protein

FATİH İZGÜ, DEMET ALTINBAY & EDA BENER
Dept of Biological Sciences, Middle East Technical University, 06531, Ankara,Turkey

Yeast strains with killer phenotype (K^+) secrete into the medium polypeptide toxins that are classified into eleven different types (K1 – K11). Our previous studies revealed that some of these toxins were inhibitory not only to other yeast species but also gram (+) pathogenic bacteria. As yeast killer proteins are promising antimicrobial agents, we have aimed to purify and characterize the K5 type yeast killer toxin by using *Hansenula anomala* NCYC 434 as the source of this protein. The optimum production of the K5 type killer protein by *H.anomala* at pH 4.0 – 4.5 and at 22 – 24°C in a killer zone assay test . The killer protein in the culture supernatant was concentrated bu ultrafiltration and applied to ion exchange perfusion chromatography (BioCAD 700 E protein workstation). The active fraction was further purified by size exclusion chromatography. The protein migrated as a single band on discontinous gradient SDS PAGE and had a MW of 48, 200.The pI value of K5 type protein was determined at pH 3.8 by high voltage vertical gel electrofocusing. The glyco- nature and amino acid sequence determinations are under investigation in our laboratory. These results will be of help in large scale purification of this protein for industrail purposes.

Monday 16 September 2002

0910 Oral bacteria: diversity and ecology

FLOYD E. DEWHIRST & BRUCE J. PASTER
The Forsyth Institute, Boston, USA

Oral microbial diversity by 16S rRNA gene sequencing

Studies using molecular techniques have shown that cultural studies severely underestimate the microbial diversity in most environments. We have therefore used 16S rRNA methods to obtain sequence information for both culturable and un-cultivated organisms present in the oral cavity.

Samples were obtained from many different oral sites, including periodontal pockets, teeth, cheek, tongue, tonsils, palette and throat, and from subjects that were healthy or had gingivitis, periodontitis, ANUG, NUP, Noma, and halitosis. Following extraction of DNA, bacterial 16S rDNA genes were amplified with universal, treponemal, or bacteroides specific primers and cloned into *Escherichia coli.* Over 6,000 clones have been sequenced and indicate that there are over 600 human oral species or phylotypes present. The sequences of approximately half of the clones match the sequences for named species or unnamed oral isolates. The other half of the sequences differ from sequences of cultivated organisms and match only other clone sequences. The species or phylotypes fall into 11 bacterial phyla, including two with no cultivable members. This research is part of ongoing efforts to identify essentially all of the organisms found in the human oral cavity, and based on 16S rRNA sequence information, to develop microarrays for simultaneously monitoring the entire oral microbial composition of sites in clinical studies of oral health and diseases.

0950 Systematics of oral non-sporing, gram-positive anaerobes

WILLIAM WADE & JULIA DOWNES
Oral Molecular Microbial Ecology Research Programme, King's College London, London SE1 9RT

Gram-positive non-sporing anaerobes are found in two phyla within the domain *Bacteria*: *Actinobacteria* (high G+C) and *Firmicutes* (low G+C). The principal members of this group within the phylum *Actinobacteria* are found in the family *Coriobacteriaceae*. This family includes the genus *Atopobium* and the recently-described genera *Cryptobacterium*, *Olsenella* and *Slackia* which are frequently isolated from oral infections. Many species belonging to this group within the *Firmicutes* have hitherto been included in the genus *Eubacterium*. *Eubacterium* is defined by default and has traditionally been a dumping ground for Gram-positive non-sporing anaerobes not included in other, better-established, genera. Following the restriction of *Eubacterium sensu stricto* to the type species *E. limosum* and *E. barkeri* and *E. callenderi*, polyphasic studies have been performed to clarify the taxonomy of this group. Phylogenetic studies show that taxa formerly classified as *Eubacterium* are widely distributed among the phylum in families such as *Lachnospiraceae*, *Erysipelotrichaceae*, *Synotrophomondadaceae* in addition to *Eubacteraceae*. Members of one branch of the latter family, consisting mainly of asaccharolytic organisms, are strongly associated with oral infections, including periodontitis and dento-alveolar abscesses. Despite a number of recent revisions to the taxonomy of this group, much remains to be done.

1100 Population genetics of oral bacteria

MOGENS KILIAN
Dept of Medical Microbiology and Immunology, Bartholin Building, University of Aarhus, DK-8000, Aarhus C, Denmark

Molecular analyses have demonstrated that bacterial species must be viewed as populations of clones that share basic house-keeping functions, but otherwise may have very different properties. Analysis of the genetic structure of bacterial populations can elucidate the genetic mechanisms that cause this diversity. More importantly, population genetic analysis may identify particularly virulent variants within a species, thus providing a better background for the identification of important virulence factors; yield a more detailed understanding of host-parasite relationships; and explain why temporal variations may occur in the prevalence of bacterial infections. The presentation will demonstrate how application of these concepts to oral microbiology has made it possible to address many important questions about (1) patterns of acquisition, transmission, and dynamics of the oral microflora, (2) the existence of particularly virulent forms of oral bacteria, (3) and the molecular mechanisms responsible for geographic and temporal variations in oral disease frequency and severity.

1140 Primer bias in the molecular analysis of the subgingival microflora

A. DE LILLO[1], M.A. MUNSON[1], L. KYRIACOU[1], F.P. ASHLEY[1], R.M. PALMER[1], A.J. WEIGHTMAN[2] & W.G. WADE[1]
[1]King's College London, [2]Cardiff University

Molecular ecological analysis is well established for the characterisation of bacterial communities. In this study, molecular and cultural analyses of subgingival plaque in periodontitis and the specificity of three PCR primer pairs were compared. Subgingival plaque was collected from two subjects with adult periodontitis. DNA was extracted from the samples and 16S rDNA amplified with primer pairs 27F, 1492R (A); 27F, 1525R (B) and 530F, 1525R (C). Amplified genes were cloned, sequenced and identified by comparison with sequences held in reference databases. 126 taxa were identified among 430 clones sequenced. Predominant taxa in the libraries combined were *Filifactor alocis* (8.2 %), *Streptococcus parasanguis* (5.1 %) and *Selenomonas infelix* (5.1%). There were gross differences between libraries in terms of the taxa detected. For example, members of the phylum *Bacteroidetes* made up 33.1% of the clones in library B but only 10.9% and 5% of libraries A and C respectively. Taxa belonging to the *Actinobacteria* and *TM7* phyla were only detected in library B. Library C failed to detect any representatives of *Spirochaetes* and *Fusobacteria*. Total species richness in the three libraries was estimated to be 245 taxa (Chao1 statistic). Observed and estimated numbers of taxa shared between the libraries were 33 and 137 (A and B), 28 and 70(A and C) and 24 and 107 (B and C).

In conclusion, this study has shown that different "universal" primer sets preferentially amplify different taxa present in the bacterial communities under study. Multiple primer sets are recommended for use in molecular ecology studies to minimise this source of bias.

This study was supported by a grant from the Wellcome Trust (ref: 061118)

1200 Surface-associated proteins of Streptococci and Enterococci

KAREN HOMER
Dept of Oral Microbiology, GKT Dental Institute, King's College London

Surface-associated proteins of Gram-positive bacteria have numerous physiological functions including roles in adhesion and virulence. Many of these proteins are immunogenic and may provide targets for vaccine development. The publication of genomic sequences for a number of bacteria has allowed us to use proteomic techniques of two-dimensional gel electrophoresis and mass spectrometry (MALDI-ToF-MS and LC-MS/MS) to conduct comprehensive identification of surface-localised proteins. We have analysed surface-associated proteins of enterococci and streptococci, including members of the viridans group and *Streptococcus pyogenes*. Several "anchorless" proteins, including enolase and glyceraldehyde-3-phosphate dehydrogenase, were expressed by the majority of species. Some proteins, including protein translation elongation factors and trigger factor, have been localised to the cell surface for the first time in these organisms. The function of these proteins on the surface of streptococci and enterococci has yet to be determined. We also provide evidence for the expression of numerous forms of ABC-type transporters and components of sugar transport systems, the expression of which is dependent on the species under consideration and the response of bacteria to different environmental conditions including pH and nutrient source.

1330 Antigenic diversity of oral pathogens

RUDOLF GMÜR
University of Zurich, Switzerland

The microbiota of the human oral cavity and the host response to them have coevolved over a long time. Little is known about how this process has actually shaped the "partners", but there is a consensus that the interactions between oral bacteria (present in form of complex biofilms) and the host are best considered as a continuum between symbiosis, commensalisms and amphbiosis (opportunistic pathogenicity). Highly adapted hostile bacteria have to evade or counter the host's defence but at the same time need the host response they provoke to satisfy their nutritional requirements. Investigating the antigenic diversity at the bacterial cell surface may provide important information to improve the understanding of this delicate host-pathogen relationship, is considered by many as an essential basis for vaccine development, and, in another context, will promote seroclassification of oral microorganisms, in particular at the sub-species level. An overview over recent progress in assessing the antigenic diversity of selected oral bacteria (*Actinobacillus actinomycetemcomitans*, *Bacteroides forsythus, Fusobacterium nucelatum*, *Porphyromonas gingivalis)* in relation to different experimental approaches will be presented. The species differ remarkably with respect to both the extent of surface antigen heterogeneity expressed by different isolates and the type of antigens responsible for the diversity.

1410 Ecological control of oral microbial communities

P.D. MARSH[1,2] & D.J. BRADSHAW[3]
[1]CAMR, Salisbury SP4 0JG[2], Leeds Dental Institute, Leeds LS2 9LU, [3]Quest International, Ashford TN24 0LT

Dental plaque is an example of a biofilm and microbial community. Patterns of gene expression alter when bacteria colonise the tooth, while plaque microbial communities display emergent properties, i.e. the community is more than the sum of its component species. The community life-style results in an extended habitat range, increased metabolic efficiency, greater protection from environmental stresses, and enhanced pathogenicity. Although plaque is found naturally on teeth, and contributes to the host defences by excluding exogenous species, it is also associated with common diseases affecting humans: caries and periodontal diseases. The predominant microflora in disease differs from that in health, although pathogens can be detected at healthy sites using molecular approaches. Disease, therefore, is due to an imbalance in the proportions or metabolism of the resident microflora. Modelling studies have demonstrated that these perturbations are driven by changes in local environmental conditions. For example, low pH following the metabolism of dietary carbohydrates selects for mutans streptococci and predisposes sites to caries, while an increased flow of gingival crevicular fluid enriches plaque for the anaerobic and proteolytic bacteria implicated in periodontal disease. This knowledge can be exploited to prevent pathogen overgrowth by interfering with these selection forces (ecological control).

1450 Proteomic analysis of oral bacteria

D. BEIGHTON
GKT Dental Institute, London

To define a proteome it is usually necessary for the genomic sequence of an organism to be available. The proteome of organism is the expressed proteins and is subject to variation. Many species of oral bacteria including, *Streptococcus mutans*, *Streptococcus gordonii*, *Streptococcus mitis*, *Treponema denticola*, *Prevotella intermedia*, *Porphyromonas gingivalis*, *Fusobacterium nucleatum* (subspecies *polymorphum*), *Lactobacillus acidophilus*, *Actinobacillus actinomycetemcomitans* and the yeast, *Candida albicans* have been, or are being sequenced. Although these sequences represent the major recognised oral pathogens there is little reported analysis of the proteomes of these organisms growing under different conditions.

Recent advanced in electrophoresis, chromatography and mass spectroscopy (MS) enable the identification of proteins produced by these bacteria and novel search programmes (MS-BLAST and FASTS), used in conjunction with *de novo* sequencing, enable functions to be assigned to proteins from unsequenced bacteria. While ICAT and related techniques enable the quantitation of the effect of environment on protein expression. These MS techniques are used to identify post translation modifications and to explore the composition of protein complexes. These proteomic methods will considerably increase the understanding of microbial growth, microbial interactions and their role in the oral diseases.

1600 Environmental control of oral bacterial gene expression

BILL BONASS
Division of Oral Biology, University of Leeds, LS2 9LU

Oral bacteria have to respond to an ever-changing environment in order to remain in the mouth. In addition to the dietary changes that occur each day there are changes associated with disease e.g. temperature, pH and oxidative stress. A range of techniques has been used to identify environmentally regulated genes and proteins in *Porphyromonas gingivalis and Streptococcus mutans* including 2-dimensional electrophoresis, SELDI, RT-PCR and differential display of RNA. Using these approaches a number of genes and proteins have been identified including *ragAB,* that appears to be down-regulated by shifts of temperature from 37^{o}C to 41^{o}C suggesting that *P. gingivalis* may down-regulate virulence determinants in an attempt to avoid a potentially damaging host-cell response. Studies on *S. mutans* have identified a number of genes that are regulated by a reduction in pH including a *dnaJ* homologue, which is upregulated, by a drop in pH from 7.0 to 5.0 indicating an increased requirement for molecular chaperones at reduced pH. The identification of these environmentally regulated genes suggests novel mechanisms that may operate in oral bacteria to enable them to rapidly adapt to change.

1640 Genetic exchange in the oral microbiota

PETER MULLANY, ADAM P. ROBERTS, IAN DAVIS, AURÉLIE VILLEDIEU, MARTHA DIAZ TORRES, DERREN READY, RACHEL PIKE & MICHAEL WILSON
Dept of Microbiology, Eastman Dental Institute, University College, 256 Gray's Inn Road London WC1X 8LD

Antibiotic and heavy metal resistance is widespread among members of the oral microbiota. Work in our laboratory has

concentrated on understanding the genetic basis of this resistance. These investigations, and those of others, have revealed that the same antibiotic resistance genes can be found in many different bacterial genera in the oral cavity and in gut bacteria, providing strong evidence of either gene transfer between these organisms or transfer via an intermediate organism. Further investigation of these resistance genes showed that some of them were contained within conjugative transposons and plasmids. Our work has demonstrated that antibiotic resistance-encoding conjugative transposons and mercury resistance-encoding plasmids can transfer by conjugation. Further experiments have used the constant depth film fermentor (CDFF) to create microcosm dental plaques. When donor organisms containing conjugative transposons were introduced into this system, genetic exchange between members of the microcosm was demonstrated, again providing strong evidence that gene transfer could occur in the oral cavity. In conclusion, the oral microflora contains many different antibiotic resistance genes, a large proportion of which are located on mobile genetic elements. It is highly probable that the transfer of these different mobile elements occurs readily in the oral cavity.

POSTERS:

SE 01 Physiological and biochemical characterisation of methylotrophs isolated from the human body

VASILIKI ANESTI & ANN P. WOOD
King's College London, Microbiology Group, Life Sciences, 150 Stamford Street, Franklin-Wilkins Building, London SE1 9NN

The microbial flora of human feet and mouth is of both cosmetic and medical relevance. The primary causes of oral malodour or halitosis and the distinctive odour of the feet are the volatile sulfur compounds (VSC): hydrogen sulfide (H2S), dimethylsulfide (CH3SCH3) and methyl mercaptan (CH3SH). In the human mouth they are produced by putrefactive activities of microorganisms in saliva, the gingival crevice and on the tongue. However in the feet the most important volatile sulfur compound is methyl mercaptan which has a typical cheesy odour and is produced by microorganisms able to metabolize methionine. The odour of feet is very often referred to as "cheesy".

Pure cultures of bacteria have been isolated from the mouth of healthy people and from samples from people suffering from periodontal disease which have been provided by the Guy's Dental Institute. Selected strains already isolated from human feet have been provided for further physiological and biochemical investigation. All cultures were methylotrophs, capable of growth on heterotrophic and methylotrophic growth substrates. All strains grow best at 37°C. Methylotrophic enzyme assays and SDS-PAGE showed that they use the serine pathway for breakdown of C-1 substrates. Only one tested strain uses the ribulose monophosphate pathway.

The above results in addition to 16S rRNA sequencing of selected isolates confirm for the first time the presence in the mouth of three groups of microorganisms isolated in this study and able to grow on volatile sulfur compounds: the *Methylobacterium*, the *Hyphomicrobium* and the *Brevibacterium* genus. Moreover in the human feet a new methylotrophic *Methylobacterium* species has been isolated, and strains identified as *Micrococcus luteus* and *Pseudomonas* species have been also found.

SE 02 A novel marine bacterium, *Pseudoalteromonas phenolica* sp. nov. produces anti-methicillin-resistant *Staphylococcus aureus* (MRSA) substance, MC21-A

ALIM ISNANSETYO & YUTO KAMEI
Marine and Highland Bioscience Center, Saga University, Saga 847-0021, Japan

A marine bacterium, strain O-BC30^T which produces an anti-metichillin-resistant *Staphylococcus aureus* (MRSA) substance was classified to a new species of the genus *Pseudoalteromonas* based on the phenotypic characterization, sequence analysis of the 16S rDNA and DNA-DNA hybridization. The G+C content of DNA was 40.0±0.6 mol%. The isolate failed to grow in the NaCl-free medium or in the medium supplemented with 1-8% (w/v) NaCl. Based on the 16S rDNA similarities, this new *Pseudoalteromonas* species was closely related to *P. luteoviolacea* and *P. piscicida*. Analysis of DNA-DNA relatedness exhibited low level of DNA hybridization (19.6% with *P. lutioviolacea* and 22.4% with *P. piscicida*). Because O-BC30^T produces phenolic anti-MRSA substances, *Pseudoalteromonas phenolica* sp. nov. is the proposed name for this new species. The phenolic anti-MRSA substance, MC21-A was purified from the MeOH extract of O-BC30^T cells by several column chromatographic techniques. MC21-A was determined to be [1,1`-biphenyl]-2,2`-diol,3,3`,5,5`-tetrabromo by spectrometric analyses. Its anti-MRSA activity against ten clinical MRSA isolates was comparable to that of vancomycin (MC21A MICs, 1 - 2 μg/ml; vancomycin MICs, <0.25 to 2 μg/ml). Time killing study also demonstrated that MC21-A was bactericidal and its killing rate was much higher than that of vancomycin

SE 03 Deletions and insertions in the *Streptococcus mutans* chromosome

W.G. ROBINSON, L.A. OLD, D.S. SHAH & R.R.B. RUSSELL
Dental School, University of Newcastle, Newcastle upon Tyne NE2 4BW

Many isolates of *Streptococcus mutans* lack the ability to ferment melibiose and other sugars such as β-glucosides. We have previously reported that the melibiose-negative phenotype is most commonly due to a chromosomal deletion that includes the muliple sugar metabolism (*msm*) operon. In the present study, sequence information from the *S. mutans* genome project was used to design PCR primers to explore the extent of the deletion. In all melibiose-negative strains examined, an 18 kb stretch of chromosome including *msm* and *gal* operons was deleted and replaced by a novel insertion element, IS*Smu*5. Strains that were unable to utilise β-glucosides were found to have a separate 4 kb deletion in the *bgl* regulon that is proposed to be due to homologous recombination between two short 12 bp stretches of identical sequence. Analysis of the IS*Smu*5 sequence of independent melibiose-negative isolates from different parts of the world suggests that the deletions may be an ancient event. It also appears that loss of the affected genes is selectively neutral.

Tuesday 17 September 2002

The Promega prize scheme aims to encourage both communication skills and technical excellence in young scientists. The two best presentations in this session will win £200 each and go forward to compete in the Young Life Scientist of the Year final.

1405 The human cytomegalovirus 72 kilo-Dalton major immediate early protein interacts physically and functionally with a constituent of ND10 bodies, hDaxx

D.L. WOODHALL[1], L.A. BRYANT[1], G.W.G. WILKINSON[2], S. EFSTATHIOU[3] & J.H. SINCLAIR[1]
[1]Dept of Medicine, University of Cambridge, Cambridge, [2]Dept of Medicine, University of Wales College of Medicine, Cardiff, [3]Dept of Pathology, University of Cambridge, Cambridge

The major immediate early proteins of human cytomegalovirus (HCMV) play a pivotal role in the control of viral and cellular gene expression during productive, lytic infection. One of the functions of the 72-kDa major immediate early protein (IE72) is to redistribute promyelocytic leukaemia protein (PML), disrupting ND10 nuclear domains.

We have identified the cellular protein hDaxx, a component of ND10 nuclear domains, to be an in vivo and in vitro interaction-partner of IE72, and that the modification of IE72 by the Small Ubiquitin-like MOdifier (SUMO-1) may also be important for this interaction. We have demonstrated by immunohistochemistry and FACS analysis that cells expressing high levels of hDaxx are refactory to infection at low multiplicity of infection (MOI), by green-fluorescent-protein-tagged (GFP) HCMV and other GFP herpesviruses. This inhibition can be overcome by infection at high MOI, indeed, increased GFP expression can be detected in cells subsequently super-infected with a non-GFP-tagged virus. Moreover, we have shown that titration of hDaxx into IE-expressing cells leads to a partial recovery of PML in ND10 bodies.

These data suggest that hDaxx plays a role in maintaining the integrity of ND10 upon infection, and is an important target for IE72 during productive HCMV infection.

1420 Characterisation of a Δ*hasA* allelic replacement mutant of *Streptococcus equi* subspecies *equi*

D.A. WEST, J.P. MAY, J.D. SLATER & D.J. MASKELL
University of Cambridge, Centre for Veterinary Science, Madingley Road, Cambridge CB3 0ES

Streptococcus equi subspecies *equi* (*S. equi*) is a highly contagious respiratory pathogen of Equidae and is the causative agent of strangles. The genome sequence of *S. equi* (www.sanger.ac.uk) has enabled the identification of genes with similarities to virulence genes in other streptococci, for example *hasA* (hyaluronan synthase) which is required for capsular polysaccharide biosynthesis. This presentation describes the generation and phenotypic characterisation of an allelic replacement *hasA* mutant (Δ*hasA*).

The *hasA* gene was PCR amplified and cloned using the TOPO vector system (Invitrogen). A central 439 bp region of the gene was deleted and an erythromycin antibiotic resistance cassette inserted. The construct was electroporated into *S. equi* and erythromycin resistant colonies screened by Southern hybridisation. A single mutant was selected for further investigation.

To assess the impact of disrupting *hasA* on capsule, the hyaluronic acid content of the Δ*hasA* mutant was quantified using a colorimetric assay. In wild type *S. equi* capsule production was growth-phase dependent; being maximal during log phase and reduced during stationary phase. In contrast no hyaluronic acid capsule was detected for Δ*hasA* at any stage of growth.

The importance of *S. equi* capsule in resistance to phagocytosis was investigated. Briefly, polymorphonuclear leukocytes from horse blood were prepared by gradient centrifugation and incubated with pre-opsonised log phase Δ*hasA* or wild type *S. equi.* Colony counts were determined before and after incubation to determine neutrophil killing of bacteria. Neutrophils killed the Δ*hasA* mutant much more efficiently than they were able to kill wild type *S.equi*.

In conclusion an allelic replacement Δ*hasA* mutant of *S. equi* was generated that lacked a hyaluronic acid capsule. This was used in experiments that showed that the presence of capsule is essential for resistance of *S. equi* to equine neutrophil killing.

1435 AspA - a conserved, autotransported, subtilisin-like serine protease in *Neisseria meningitidis*

D.P.J. TURNER, K.G. WOOLDRIDGE, D.A.A. ALA'ALDEEN
Division of Microbiology and Infectious Diseases, University Hospital, Nottingham

Autotransporter proteins are important virulence factors in many Gram-negative bacteria. In an attempt to identify novel meningococcal virulence determinants, the meningococcal genomic sequence was screened *in silico* for previously unrecognised autotransporter proteins. This led to the identification of Autotransported serine protease A (AspA: NMA0478; NMB1969), a novel *c*. 112 kDa subtilisin-like serine protease (pyrolysin family). AspA is conserved in serogroup A, B and C meningococci and exhibits significant homology to SphB1, which is essential for the maturation and secretion of Filamentous haemagglutinin in *Bordetella pertussis*. *aspA* was cloned and expressed. Antibodies against recombinant AspA (rAspA) were detected in patients' convalescent sera. Rabbit antiserum (RαAspA) was raised against rAspA and used to screen a panel of meningococcal strains. AspA was expressed in 8/10 hypervirulent lineages examined, but not AspA mutant strains. Fractionation experiments confirmed that the precursor protein was present in the outer membrane, whilst the secreted forms of AspA were detected in culture supernatants. Surface-exposure was confirmed by immunogold-staining and electron microscopy. RαAspA exhibited bactericidal activity against the homologous strain (MC58). Site-directed mutagenesis of S^{426} in the serine protease site, abolished AspA secretion in *E. coli*, confirming auto-cleavage. In conclusion, AspA is a novel, conserved, immunogenic, cross-reactive and surface-exposed meningococcal autotransporter protein. Anti-AspA antibodies are bactericidal. Further investigation of AspA as a putative meningococcal virulence determinant and vaccine candidate is warranted.

1450 Antibiotic production by *Erwinia carotovora* subsp. *carotovora*: what's driving the Car?

N.J.L. SIMPSON, G.E. BROWSE, K.S. LILLEY & G.P.C. SALMOND
Dept of Biochemistry, University of Cambridge, Building O, 80 Tennis Court Road, Cambridge CB2 1QW

Erwinia carotovora subsp. *carotovora* produces the simple β-lactam antibiotic 1-carbapen-2-em-3-carboxylic acid (Car), which is under strict quorum sensing control. The signal molecule *N*-(3-oxohexanoyl)-**L**-homoserine lactone (OHHL) is synthesised by CarI and upon interaction with CarR leads to transcription of the *car* cluster. Production of plant-macerating exoenzymes is also regulated by OHHL and hence many regulators have been discovered which interact with both systems. Three aspects of regulation of Car production have been investigated in this project. Sequence analysis of spontaneous streptomycin resistant Car-negative mutants has revealed single amino acid substitutions at Lys-43 in RpsL (encodes the S12 subunit of the 30S ribosome). A substitution to

Thr, Asn or Leu results in a Car-negative phenotype but a mutation to Arg appears to have no effect on Car. The effect of RpsL on Car production was shown to be at the level of transcription of the *car* biosynthetic locus, via a combination of *lacZ* and primer extension studies. Previously, overexpression of RsmA has been shown to result in reduced levels of exoenzyme and *carI* mRNA. RsmA may function by binding to and degrading specific mRNA transcripts. This work has shown that RsmA also negatively regulates Car production and that it is not acting solely through *carI* message stability. Additionally, Erwinia possesses at least two other CarR homologues, EccR and CarK, the precise roles of which are unclear. A proteomic approach has been taken in order to identify proteins under the control of these two regulators.

1505 The Hepatitis C virus NS5A protein inhibits Activating Protein – 1 (AP1) function by perturbing MAPK signalling pathways

ANDREW MACDONALD, ANDREW A. STREET, CHRISTOPHER McCORMICK, KATHERINE J. CROWDER & MARK HARRIS
School of Biochemistry & Molecular Biology, University of Leeds

The Hepatitis C virus (HCV) non-structural 5A protein (NS5A) is a multifunctional phosphoprotein that has been shown to associate with a variety of cellular signalling proteins. Of particular interest is the observation that a highly conserved C-terminal Class II polyproline motif in NS5A mediated association with the SH3 domains of members of the Src family of tyrosine kinases (Macdonald *et al*., submitted 2002). In this study we wished to further analyse the down-stream consequences of NS5A expression. Utilising a transient luciferase reporter system within NS5A expressing Hepatoma cell lines we investigated the molecular mechanisms for NS5A-dependent inhibition of the mitogenic and stress stimulated transcription factor, Activating protein – 1 (AP1). A combination of dominant active signalling proteins, pharmacological inhibitors, PMA and epidermal growth factor stimulation demonstrated that AP1 down-regulation was due to MAPK signalling pathway perturbation. Further, using immunoprecipitation kinase assays we revealed that in the presence of NS5A expression both ERK1/2 phosphorylation and kinase activity are reduced. By a combination of western and northern analysis it was observed that levels of cFos transcription factors but not cJun are reduced in NS5A expressing cells. Using site directed mutagenesis we demonstrated that the AP1 inhibition is critically dependent on the previously described C-terminal polyproline motif within the NS5A protein. Expression of an NS5A protein with proline to alanine amino acid changes within this motif failed to inhibit AP1 reporter activity. Most importantly, using a recently developed novel delivery system for expression of the whole HCV genome (McCormick *et al*., 2002), we were able to demonstrate AP1 perturbation in Hepatoma cell lines. Thus, our data provide new insight into the signalling pathways modulated by the NS5A protein that may account for the oncogenic and immuno-modulatory effects observed with HCV infection.

1550 Cellulose synthesis in biofilm formation by the plant colonising bacterium *Pseudomonas fluorescens* SBW25

S. GEHRIG
University of Oxford

Pseudomonas fluorescens SBW25 is capable of colonising liquid-solid (L-S) interfaces by forming a classical 'mushroom-channel' type biofilm. Uniquely, however, a mutant strain of SBW25 is also able to colonise the air-liquid (A-L) interface by forming a substantial mat attached at the edges to the vial walls. This mutant, known as the Wrinkly Spreader (WS), produces rapidly-spreading colonies with a characteristic wrinkled morphology. The primary cause of A-L biofilm formation and the rapidly-spreading phenotype on agar plates appears to be the over-production of acetylated cellulose (AC), triggered by a single point mutation in the *wsp* regulatory pathway controlling AC production and bacterial attachment. AC is expressed by enzymes encoded by the *wss* operon (*wssA-J*). *wssBCDEF* and *wssGHI* encode the cellulose synthase and acetylation subunits respectively. The proteins encoded by *wssA* and *wssJ* show significant similarity to the cell-cycle protein MinD, and may be involved in the polar localisation of the AC synthase complex. This suggestion has been supported by fluorescent microscopy in which AC was found to be specifically located at the cell poles in WS, but not localised at all in a cell-shape mutant (*mreB*) of WS which produces spherical rather than rod-shaped cells. The *wss* operon is known to be specifically expressed by SBW25 in the rhizosphere, and a *wss*$^-$ strain is less capable of colonising sugar beet seedlings than the wild-type strain. This observation, coupled with the involvement of *wss* in A-L biofilm formation, suggests that localised-AC production is an important factor allowing the rapid planar expansion across plant surfaces during rhizosphere colonisation.

1605 Microarray analysis of *Campylobacter jejuni*

OLIVIA CHAMPION[1], NICK DORRELL[1], JASON HINDS[2], GEMMA MARSDEN[2], PHILIP D. BUTCHER[2] & BRENDAN W. WREN[1]
[1]Dept of Infectious & Tropical Diseases, LSHTM, London WC1E 7HT, [2]Bacterial Microarray Group, St George's Hospital Medical School, London SW17 0RE

The genome sequence of the human intestinal pathogen *Campylobacter jejuni* NCTC 11168 was published in February 2000. To exploit this information further we constructed a low-cost whole genome microarray using PCR products amplified with a single pair of vector primers from selected clones of an ordered plasmid library of the NCTC 11168 strain, which was a by-product of the genome sequencing project. Whole genome microarray comparisons of eleven *C. jejuni* strains of diverse origin identified 364 genes that were absent or highly divergent in one or more of these isolates. Many of these strain-specific genes are associated with the biosynthesis of surface structures including flagellar, lipo-oligosaccharide and capsule. The *C. jejuni* species-specific genes contribute mainly to metabolic, biosynthetic, cellular and regulatory processes, but many virulence determinants are also conserved. Further evidence that the capsule accounts for Penner serotype specificity was provided by comparison of the capsule biosynthesis locus which revealed conservation of all the genes in this region in strains with the same Penner serotype as the sequenced strain, but showed many genes absent or highly divergent in this region in strains of a different serotype. This study revealed extensive genetic diversity among *C. jejuni* strains and paves the way towards identifying correlates of pathogenicity and developing improved epidemiological tools. However such low-cost microarrays have limitations as not all genes are represented by a unique PCR product, resulting in problems with cross-hybridisation and signal specificity. Consequently a gene-specific microarray has been constructed, with PCR products designed to have minimal cross-hybridisation to the rest of the genome. This array also contains a number of unique genes from strains other than the sequenced NCTC11168 strain. These genes are from regions including the LOS biosynthesis locus, the capsule locus and a putative virulence plasmid. This composite microarray is therefore more representative of the species.

A comparison has been carried out between the clone array and the gene specific array. A collection of clinical and environmental isolates have also been investigated using the gene specific array and a brief overview of these results, progress and future direction will be presented.

1620 Molecular adaptation of rhizobia to the environment

MICHELLE BARR & PHILIP POOLE
School of Animal and Microbial Sciences, University of Reading, Whiteknights, PO Box 228, Reading RG6 6AJ

Bacteria of the family Rhizobiaceae can interact with leguminous plants, allowing a metabolic symbiosis to occur. The bacteria

reduce atmospheric nitrogen to ammonia in specialised root organs called nodules. The nitrogen is then exported to the plant in exchange for a carbon source. Much is known about the molecular basis for nitrogen fixation and the processes allowing nodulation and fixation to occur. However, little is known about genes specific for rhizobial survival in the rhizosphere. An *in vivo* selection strategy is being developed to investigate rhizosphere colonization and the early steps in symbiosis between *R. leguminosarum* and pea. *In vivo* expression technology (IVET) utilizes a mutant defective in survival in the environment that can be rescued for growth when complemented with a gene expressed downstream of a promoter probe library. *purN* deletion mutants of *Rhizobium leguminosarum* 3841 and *Sinorhizobium meliloti* 1021 are constructed and have been shown to cause auxotrophy in the environment. Vectors to contain the promoter probe library have been made containing the rescue gene and a marker gene to analyze gene expression. The *R. leguminosarum* auxotroph and promoter probe vectors will be used to isolate genes induced specifically in the environment, which may contribute to environmental adaptation.

1635 Strain typing of *Mycobacterium bovis*

LOUISE BAILEY[1,2,3], ORLA FLYNN[4], EAMON COSTELLO[4], DON O' GRADY[4], RORY O'BRIEN[1,2,3] & MARK ROGERS[1,2,3]
Conway Institute for Biomolecular Science[1], Dept of Zoology, [2]University College Dublin, Belfield, Dublin 4, Tuberculosis Investigation Unit, Faculty of Veterinary Medicine, University College Dublin, Ballsbridge, [3]Dublin 4, Central Veterinary Research Laboratory, Abbotstown, Dublin 15, Ireland

A novel DNA probe (pUCD) for the strain typing of *Mycobacterium bovis* (*M. bovis*) was characterized. Four tandem repeats (pUCD 1, pUCD 2, pUCD 3, pUCD 4) were derived from the full-length pUCD probe. Restriction Fragment Length Polymorphism (RFLP) analysis was carried out on 21 *M. bovis* isolates using pUCD 1, 2, 3 and 4 as oligonucleotide probes. A 36bp probe found in the Direct Repeat (DR) region of the *Mycobacteria* tuberculosis genome was combined with the oligonucleotides in a mixed hybridization. A Variable Number Tandem Repeat (VNTR) typing system was developed using the pUCD repeats as individual PCR targets. A comparison was made of twenty isolates typed by VNTR-PCR and by the pUCD oligonucleotide probes. It was found that the VNTR-PCR typing system compared favourably with pUCD in terms of its ability to discriminate between isolates. The VNTR-PCR typing system was found to be a more desirable typing system because typing could be completed in a day as opposed to three days with RFLP. This study was extended to ascertain whether the polymorphic properties of pUCD were due to mutations in *Alu I* restriction sites or changes in the number of tandem repeats. The initial study of 21 *M. bovis* isolates was extended to 100 *M. bovis* isolates chosen at random from various sources.

1650 How many species of *Streptomyces* are pathogenic?

ERIKA T. QUINTANA
Dept of Agricultural and Environmental Science, Microbial Resources Laboratory, University of Newcastle, Newcastle upon Tyne NE1 7RU

Actinomycetoma, a localised chronic, destructive and progressive infection of the skin, subcutaneus and eventually bone, is a serious health problem in many tropical and subtropical countries, notably India, Mexico, Senegal, Somalia and Sudan. The causative agents of actinomycetoma are species belonging to the genera *Actinomadura*, *Nocardia*, and *Streptomyces*, thus on the latter only *Streptomyces somaliensis* has been widely recognised as a causal agent of disease. In the present study, twenty-four strains were isolated from cases of actinomycetoma in the Sudan and putatively identified as *Streptomyces somaliensis* on the basis of culture and morphological properties. Complete sequences of the 16S rDNA, nucleic acid fingerprinting techniques, substractive hybridization and phenotypic properties of the isolates and representative streptomycetes were carried out. The isolates from the Sudan formed five well defined subclades in the 16S rDNA streptomycete tree and the integrity of each of the subclades is supported by their corresponding molecular fingerprinting patterns. Substractive hybridization of the type strain of *Streptomyces somaliensis* and one of the sudanese isolates shown the independent integrity of each strain as different agents of actinomycetoma. The sudanese isolates can be distinguished from all other streptomycetes on the basis of the molecular and phenotypic data. It is proposed that one of the subclades be recognised as a new pathogenic species of streptomycetes under the name *Streptomyces sudanensis*. The fact that some of the clinical significant isolates constitute novel species clearly has implications on the treatment and a reappraisal on the current picture of the agents of actinomycetoma.

Dissection of virulence attributes in the pathogen, *Candida albicans* - a Gordian knot

ALISTAIR J. P. BROWN
Aberdeen Fungal Group, Molecular and Cell Biology, Institute of Medical Sciences, University of Aberdeen, Foresterhill, Aberdeen AB25 2ZD

Candida albicans is a major fungal pathogen of humans. It causes recurrent superficial oral and vaginal infections, and life-threatening systemic disease in severely immuno-compromised patients. For many years, a major objective of many laboratories has been the characterization of factors that promote the virulence of *C. albicans*. These are thought to include yeast-hypha morphogenesis, adhesion, secreted aspartyl proteinases, and phenotypic switching. Initially, efforts to determine their contributions to *C. albicans* virulence were hampered by the diploid and apparent asexual nature of *C. albicans*, its alternate decoding of the CUG codon (which blocks expression of standard reporter genes), and the limited molecular toolbox for *C. albicans*. However, the last decade has seen rapid advances in *C. albicans* molecular biology. *Saccharomyces cerevisiae* has proved a vital model for the isolation of *C. albicans* genes. Specialized *C. albicans* reporters, stable plasmids and gene knock-out technologies have been developed. Mating has been demonstrated in this "asexual" fungus, raising the possibility of an exploitable sexual cycle in the not too distant future. A detailed physical map is nearing completion. Most significantly, a near complete genome sequence is now available, allowing the application of genomic tools to this fungus. These advances have led to a rapid acceleration in the molecular analysis of *C. albicans* virulence. However, a complex picture is emerging. Individual virulence attributes are polygenic traits. Some virulence attributes are encoded by complex gene families. Furthermore, specific subsets of virulence genes appear to be co-regulated. This complicates the analysis of *C. albicans* virulence. However, it provides the fungus with the flexibility to express appropriate virulence attributes as its microenvironment evolves during the establishment and progression of a *C. albicans* infection.

Main Symposium
Tuesday 17 September 2002

0940: *Staphylococcus epidermidis* virulence and pathogenesis

F. GOETZ[1], S. CRAMTON[1] & R. LANDMANN[2]
[1]Microbial Genetics, University Tuebingen, Germany, [2]Div. Infectious Diseases, University Hospital Basel, Switzerland

The genetic and molecular basis of biofilm formation in staphylococci is multifaceted. The ability to form a biofilm affords at least two properties: the adherence of cells to a surface and accumulation to form multi-layered cell clusters. A trademark is the production of the slime substance PIA, a polysaccharide composed of β-1,6-linked *N*-acetylglucosamines with partly de-acetylated residues, in which the cells are embedded and protected against the host's immune defense and antibiotic treatment. Mutations in the corresponding biosynthesis genes (*ica* operon) lead to a pleiotropic phenotype; the cells are biofilm- and hemagglutination-negative, and less adhesive on hydrophilic surfaces. We identified a novel glucosaminidase (Gad) that is responsible for the degradation and/or turnover of PIA. A *gad* deletion-replacement mutant is associated with more polysaccharide and forms a thicker biofilm than an isogenic wild type strain. A number of biofilm-negative mutants have been isolated in which PIA production appears to be unaffected. Two of the characterized mutants are affected in the major autolysin (*atlE*) and in D-alanine esterification of teichoic acids (*dltA*). In a recently established mouse tissue cage model the virulence of the wild type *S. aureus* SA113 was compared with that of its *ica*-negative mutant. Finally the presence of virulence genes of *S. aureus* N315 are compared with that of *S. epidermidis* RP62A.

Cells & Cell Surfaces Group
Posters:

CCS 21 Identification and characterisation of novel *Staphylococcus epidermidis* antigens

M.R. POURMAND & S.J. FOSTER
Dept of Molecular Biology and Biotechnology, University of Sheffield, Western Bank, Sheffield S10 2TN

Staphylococci are human pathogens of increasing importance due to the spread of antibiotic resistance. Antibody therapy and vaccination are treatment options to overcome the diseases related to these pathogenic bacteria. *S. epidermidis* is of particular concern in respect of nosocomial infections associated with indwelling medical devices. Novel potential targets for therapeutic antibodies are products of staphylococcal genes expressed during human infection. Using a direct screening technique we have identified over 57 *in vivo* antigens from *S. epidermidis*. Several antigens have been characterized and found to be novel putative surface proteins involved in binding host ligands of the extracellular matrix.

CCS 20 Identification and characterisation of *Staphylococcus aureus* components expressed during human infection

KIRSTEN J. BRUMMELL, SIMON R. CLARKE, PHILIP W. MCDOWELL & SIMON J. FOSTER
Dept of Molecular Biology and Biotechnology, University of Sheffield, Firth Court, Western Bank, Sheffield, S10 2TN

The effective treatment of *Staphylococcus aureus* infection is becoming increasingly difficult by conventional antibiotic therapy due to an increase in *S. aureus* strains exhibiting wide-spectrum resistance to antibiotics. Therefore, it is important that alternative treatment options, such as vaccination are investigated. An ideal vaccine candidate should be an exposed protein expressed during human infection. In order to identify *in vivo* expressed antigens, human sera was used to probe bacteriophage lambda libraries of *S. aureus*. 355 clones were isolated yielding 115 loci. Several potential vaccine components have been identified including a novel Autolysin and the surface proteins MRP 1 and MRP 2. These components have been overexpressed, with further studies currently being carried out to assess their role and regulation in pathogenesis. These studies include the cross-reactivity of antigens with sera from a range of infections, ligand binding studies, reporter gene fusions, and mutational analysis.

Environmental Microbiology Group
Posters:

EM 10 Proteomic studies of the extreme acidophile "*Ferroplasma acidarmanus*"

MARK DOPSON, CRAIG BAKER-AUSTIN & PHIL BOND
School of Biological Sciences, University of East Anglia, Norwich NR4 7TJ

The extremely acidophilic archaea "*Ferroplasma acidarmanus*" was isolated from a highly acidic acid mine drainage site in Iron Mountain, California where it constituted 85 ± 7% of the microorganism population by fluorescent *in situ* hybridization. "*Ferroplasma acidarmanus*" grows via the oxidation of ferrous iron or yeast extract at approximately pH 1 and in metal concentrations up to 10's g/l. The growth substrates for "*Ferroplasma acidarmanus*" have been investigated and although many organic and inorganic substrates stimulated a physiological response in the form of increased oxygen consumption, only mixotrophic growth on ferrous iron in conjunction with yeast extract, cas amino acids or a vitamin mix and heterotrphic growth on yeast extract alone supported balanced growth.

"*Ferroplasma acidarmanus*" will be challenged with various concentrations of metals, inhibitors, uncouplers, pH variations and growth conditions and the protein expression analysed by 2D gel electrophoresis. Differentially expressed proteins will be identified using mass spectrometric analysis and the genes cloned from the sequenced genome. This will provide insights into the physiological strategies employed by "*Ferroplasma acidarmanus*" and improve the understanding of acidophilic archaea.

EM11 Repair of UV damage in the extremely halophilic archaeon, *Halobacterium salinarium*

LUCIO MARCELLO, CATHY VERMONT & SHIRLEY MCCREADY
School of Biological and Molecular Sciences, Oxford Brookes University, Gipsy Lane, Oxford OX3 0BP

Halobacterium salinarium is an extremely halophilic archaeon that (unlike most other known archaea) tolerates high levels of sunlight in its natural environment. We have previously demonstrated the existence of a 'dark' excision repair mechanism in this organism and the recently-completed genome sequence shows that *Halobacterium* carries genes homologous to the prokaryotic genes, *uvrA*, *uvrB*, *uvrC* and *uvrD*. Probably, therefore, a 'prokaryotic' excision repair complex operates in this organism for excision of UV lesions, although there are also homologues of eukaryotic repair genes – notably *S. cerevisiae RAD3*, *RAD25* and *RAD27* (this latter is called *rad2* in *S. pombe* and *FEN1* in mammals). However, whilst excision repair presumably contributes to survival after UV damage, it is probable that photoreactivation is of greater biological significance for repair of DNA damage caused by sunlight.

Photoreactivation has been shown to be very efficient in *Halobacterium* and we have shown that the two principal UV-induced DNA lesions, cyclobutane pyrimidine dimers (CPDs) and 6-4 photoproducts (6-4 pps), are both repaired more efficiently in the presence of visible light than in the dark. However, 6-4 photolyases occur less commonly than CPD photolyases, and it was not clear whether the rapid repair in the light of 6-4 photoproducts was due to a 6-4 photolyase or simply due to 'freeing-up' of the excision repair enzymes when CPDs were photoreactivated. To establish whether there is a 6-4

photolyase in *Halobacterium*, we have created a *phr2* deletion mutant. Using an immunoassay for UV damage, we have shown that the deletion mutant fails to photoreactivate CPDs, confirming that *phr2* encodes a CPD photolyase. We have also shown that 6-4 pps are very efficiently photoreactivated in the mutant, suggesting that there is also a 6-4 photolyase. In addition to *phr2*, *Halobacterium* has a second member of the photolyase/cryptochrome gene family, *phr1*, and we are currently making a *phr1* deletion mutant to establish whether *phr1* codes for a 6-4 photolyase. Interestingly, the *phr2* mutant is not significantly more UV sensitive in the dark than in the light, despite rapid repair of 6-4 photoproducts, suggesting that 6-4 pps may not be significantly lethal lesions in *Halobacterium*.

Eukaryotic Microbiology Group
Thursday 19 September 2002

1020: Biochemical and cytological studies of a bacterial actin homologue

RUT CARBALLIDO-LÓPEZ, LAURA JONES, YING LI, DIRK-JAN SCHEFFERS, RICHARD DANIEL & JEFF ERRINGTON
Sir William Dunn School of Pathology, University of Oxford, Oxford OX1 3RE

Little is known about how bacterial cell shape is determined. Traditionally, the cell wall was thought to be critical for cell shape. Indeed, most mutations that affect cell shape lie in genes associated with cell envelope synthesis. We recently showed that two shape determining genes of *B. subtilis*, *mreB* and *mbl* (*mreB* like), form helical actin-like filaments, which run around the cell periphery, just under the cell membrane[1]. Van den Ent *et al*.[2] then showed that the crystal structure of an MreB-like protein from *Thermotoga* is congruent with that of actin.

Biochemical studies with Mbl protein, both in crude extracts of *B. subtilis* cells and with purified recombinant protein support the actin-like properties of the protein. The protein binds and hydrolyses ATP, and sedimentation and cross linking assays show that polymerization has similar salt requirements to that of eukaryotic actins. Fluorescence recovery after photobleaching (FRAP) experiments have revealed that the cytoskeletal filaments of Mbl have dynamic properties *in vivo*.

We are currently investigating the nature of the link between the MreB and Mbl proteins and the cell wall synthetic machinery. Preliminary experiments suggest that some penicillin-binding proteins and cell wall precursor synthetic proteins are targeted to particular sites in the cell envelope. This suggests that the MreB family of proteins may govern cell shape by directly controlling the insertion of new wall material.

References: 1. Jones, L.J.F., Carballido-Lopez, R., and Errington, J. (2001) Control of cell shape in bacteria: helical, actin-like filaments in *Bacillus subtilis*. *Cell* **104**: 913-922. / 2. van den Ent, F., Amos, L.A., and Löwe, J. (2001) Prokaryotic origin of the actin cytoskeleton. *Nature* **413**: 39-44.

Microbial Infection Group
Posters:

MI 19 Expression of *Staphylococcus aureus* virulence genes during invasion of bovine mammary epithelial cells

SANKALE SHOMPOLE, LINDA E. LIOU, GREGORY A. BOHACH & KENNETH W. BAYLES
Microbiology, Molecular Biology & Biochemistry, University of Idaho, Moscow, Idaho 83844 USA

In *Staphylococcus aureus*, expression of surface and secreted proteins during an infection is controlled in part, by the *agr* regulatory system. It has been proposed that this system also controls gene expression during invasion of host cells. This hypothesis was tested using QRT-PCR to monitor the transcription of RNAIII, protein A (*spa*), alpha- and beta-hemolysins (*hla* and *hlb*) respectively, during invasion of cultured bovine mammary epithelial cells (MAC-T cells). Total RNA from internalized *S. aureus* cells was harvested at 1 hr intervals starting at pre-invasion (T_0) up to 8 hr (T_8) post invasion. Relative quantity of gene transcripts was evaluated relative to T_0. Transcription of RNAIII ranged from 1-1500-fold at T_1-T_2 to 11,000-fold at T_4. The fold increase dropped to 1000-fold levels T_5-T_8 post-invasion. Expression of *spa* generally repressed by *agr* was 7-fold at T_1. Thereafter, transcription levels at T_2-T_8 remained low. Subsequent increased expression of RNAIII at T_4 impacted negatively on *spa* expression post-invasion. Expression of *hla* increased from 3-fold at T_1 to 340-fold at T_5 but dropped to 15-30-fold levels at T_6-T_8. Expression of *hlb* indicated a similar pattern although the magnitude was lower starting at 5-fold at T_2 and increasing to 68-fold at T_5. The increased transcription of *hla* and *hlb* after T_4 correlated with known positive regulatory effects of RNAIII on expression of exoprotein genes. In conclusion, these results suggest that the *S. aureus* quorum sensing mechanism in infected MAC-T cells is activated between T_4-T_5 post-invasion and results in activation of the *agr* regulatory system and increased RNAIII transcription. Activation of *agr* and the increased transcription of the effector molecule RNAIII, negatively down-regulated *spa* expression and positively up-regulated expression of exoproteins that are postulated to facilitate escape of *S. aureus* from the endosome and subsequent replication in the cytoplasm.

MI 20 Identification and characterisation of a permease required for infection in *Staphylococcus aureus*

M.J. HORSBURGH[1], M. WILTSHIRE[1], E. INGHAM[2] & S.J. FOSTER[1]
[1]Dept of Molecular Biology & Biotechnology, University of Sheffield, Firth Court, Western Bank, Sheffield, S10 2TN, [2]Dept of Microbiology, University of Leeds, Leeds

Understanding the components that contribute to the virulence of *Staphylococcus aureus* is important for rationally developing new preventative or therapeutic treatments. We have identified a permease of *S. aureus* that is required for successful infection in a murine abscess model of infection. Insertional inactivation of the permease gene produces a significant reduction in the number of bacteria recovered after seven days ($P<0.002$) when compared to its isogenic parent strain. The permease gene was shown to be transcribed monocistronically by probing RNA using Northern blot and the promoter region was mapped by primer extension. The expression of the permease gene is modulated by both Agr and Sar, the major regulators of putative virulence determinants in *S. aureus*. The permease has homology to a number of amino acid transporters of the LysP/Roc families.

The permease mutant exhibits reduced microaerophilic growth on sera plates compared to wild-type. This defect is completely rescued when an excess of phenylalanine is added to the sera plates suggesting the permease may transport one or more aromatic amino acids. The strain used is not auxotrophic for aromatic amino acids suggesting that scavenging sufficient aromatic amino acids from the host is an *in vivo* requirement during *S. aureus* infection.

Physiology, Biochemistry & Molecular Genetics Group
Wednesday 18 September 2002

1145: Vesicle trafficking in filamentous fungi

NICK.D. READ & PATRICK C. HICKEY
Fungal Cell Biology Group, Institute of Cell and Molecular Biology, University of Edinburgh EH9 3JH

Vesicle trafficking underpins hyphal tip growth and protein secretion in filamentous fungi. Although the significance of vesicle trafficking has long been appreciated, we know relatively little about it in fungal hyphae. Experimental methods for analysing vesicle trafficking in living hyphae at high spatial resolution with the confocal microscope will be described and results obtained with the membrane-selective dye FM4-64 shown. The primary mechanism by which the dye is internalised by fungal cells is by energy-dependent endocytosis. The dye then becomes distributed to different organelles, including secretory vesicles within the Spitzenkörper of growing hyphal tips, via the vesicle trafficking network in a time-dependent manner. Fluorescence recovery after photobleaching (FRAP) experiments have demonstrated the vectorial transport of

secretory vesicles to the Spitzenkörper. Internalised dye within secretory vesicles is recycled back to the plasma membrane and is not readily lost to the surrounding medium. A model of the vesicle trafficking network in fungal hyphae will be presented. A further model (the 'Exocytosis-Endocytosis Equilibrium' [or 'Triple E' model]) of how vesicle trafficking may regulate hyphal tip morphogenesis will also be proposed.

Poster:
PBMG 14 Oxidative stress resistance in *Staphylococcus aureus*. Role and regulation of superoxide dismutases

M.H. KARAVOLOS & S.J. FOSTER
Dept of Molecular Biology and Biotechnology, University of Sheffield, Western Bank, Sheffield S10 2TN

Oxidative stress resistance is important for the life of *Staphylococcus aureus*. Superoxide stress occurs internally as a by-product of metabolism and also externally during host cell attack. To combat this *S. aureus* has two superoxide dismutases (SODs), designated SodA and SodM, and also a complex system involving Mn^{2+} ions to resist killing. By construction of multiple mutant strains we have shown the importance of the enzymatic and elemental dismutation reactions. In a disk sensitivity assay, Mn^{2+} was able to completely rescue an isogenic *sodA sodM* mutant when challenged by paraquat, underlining the importance of Mn^{2+} as a SOD-independent superoxide scavenger. In the same system, a strain missing *sodA* and the two major Mn^{2+} uptake systems (*mntA mntH*) was not fully rescued by Mn^{2+} further demonstrating the importance of this element. Also, Mn^{2+} was able to improve the survival of a *sodA sodM* isogenic mutant when it was challenged by paraquat during growth in liquid culture medium. Transcriptional analysis has revealed complex regulatory circuits controlling SOD expression. Finally, the importance of SODs was evaluated using a mouse abscess model of infection in which, *sodA* and *sodM* as well as the double isogenic mutant (*sodA sodM*) were attenuated compared to the parental strain SH1000.