

Society for General Microbiology

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Abstracts

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Full chapters of the following presentations will be published in a Symposium - *Community structure and co-operation in biofilms* - published for the Society General for Microbiology by Cambridge University Press.

TUESDAY 12 SEPTEMBER 2000

- 0910 J. WIMPENNY (University of Wales, Cardiff)
Overview of cooperation and community interactions in biofilms
- 0955 H.J. BUSSCHER (University of Groningen, The Netherlands)
Initial adhesion events
- 1115 D. DAVIES (Montana State University, USA)
Physiological events in early stages of biofilm formation
- 1200 P. STOODLEY (Montana State University, USA)
Factors influencing biofilm structure
- 1400 P. KOLENBRANDER (National Institutes of Health, Bethesda, USA)
Coadhesion in biofilms
- 1445 H.-C. FLEMMING (IWW Centre for Water Research, Mulheim, Germany)
Cohesiveness in biofilm matrix polymers
- 1600 H. LAPPIN-SCOTT (University of Exeter)
Detachment
- 1645 C. PICIOREANU (Delft University of Technology, The Netherlands)
Modelling and predicting biofilm structure

WEDNESDAY 13 SEPTEMBER 2000

- 0900 P. MARSH (CAMR, Porton Down)
Community interactions in biofilms
- 0945 S. MOLIN (Technical University of Denmark)
Probing complex biofilms
- 1100 L. EHLERS (National Research Council, Washington, USA)
Gene transfer in biofilms
- 1145 P. GILBERT (University of Manchester)
Population dynamics
- 1400 G. WOLFAARDT (University of Stellenbosch, South Africa)
Biodegradation by biofilm communities
- 1445 R. BAYSTON (University of Nottingham)
Biofilms and prosthetic devices
- 1600 D. ALLISON (University of Manchester)
Problems of control
- 1645 J. COSTERTON (Montana State University, USA)
Current status/future prospects

THURSDAY 14 SEPTEMBER 2000

Biofilms in implant-associated infections

0900 Implant infections - the orthopaedic perspective

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Derby Royal Infirmary

Use of predominantly metallic implants is the basis for the bulk of both trauma surgery and elective orthopaedics. Much work has been done emphasizing the importance of preventative measures such as laminar flow theatres, antibiotic prophylaxis and meticulous surgical technique. Despite this, an infection rate of between 1% and 3% is widely quoted for primary total hip arthroplasty, and in fracture surgery the infection rate can be as high as 15%.

Staphylococcus epidermidis, *Staphylococcus aureus* and coliforms are the main offenders, with their ability to adhere and develop biofilms on implants and surrounding cement. *In-vitro* studies demonstrate the importance of biomaterial design and surface preparation in decreasing the chance of this successful biofilm formation.

In fracture surgery, patients are often suboptimal, either being elderly patients with other comorbidities that increase the infection risk or patients with high energy open fractures that are technically infected before the implant is used. In elective surgery, factors such as diabetes or rheumatoid arthritis can significantly increase the chance of infection due to poor local immune systems and wound healing. Costs of implant infection are high, both for the patients and the economy. Revising a hip replacement for infection can cost six to seven times the original operation and there is a very real increase in severe medical complications in many of those who have to undergo major revision surgery. Current treatment principles rely on washout and debridement, high dose antibiotics and, more often than not, implant removal or replacement. Much reliance is also placed on gentamicin impregnated beads and antibiotic impregnated cement to optimise treatment of implant infection, but success is often limited.

There is little scope for improving preventative measures against implant infection. However, it is well recognised that the earlier a diagnosis is made, and the sooner treatment is commenced, the better the eventual outcome. The present challenge is to find a more effective way of reliably diagnosing infection, especially in the case of infected joint prostheses. The implications of removing a hip replacement unnecessarily are almost as inexcusable as not diagnosing an infection. The other challenge is to optimise current antibiotic treatment such that implants can be retained.

0935 Intravascular-catheter-related infections

I. RAAD

University of Texas, Houston, USA)

Abstract not submitted

1010 Preventing the complications associated with urinary tract devices

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Urinary tract devices such as ureteral stents and urethral catheters are used to facilitate urine drainage in the upper and lower urinary tracts, respectively. Unfortunately, bacteria readily colonise the device biomaterial forming a resistant microbial biofilm that can induce recurrent episodes of

bacteriuria. Colonisation of the biomaterial surface with urea-splitting bacteria such as *Proteus mirabilis*, *Proteus vulgaris* and *Pseudomonas* spp. causes alkalisation of the urine, thus lowering the solubility of struvite (magnesium ammonium phosphate, $\text{NH}_4\text{MgPO}_4\cdot 2\text{H}_2\text{O}$) and hydroxyapatite (calcium phosphate, $\text{Ca}_{10}(\text{PO}_4)_6\cdot \text{H}_2\text{O}$) which subsequently become deposited as encrusting material. The use of scanning electron microscopy has shown microbial biofilms in close association with encrusting materials on both urethral catheters and ureteral stents. Encrustation may lead to obstruction or blockage of urine flow with the associated urine retention causing pain and distress to the patient. Urinary tract devices are fabricated from a wide range of polymers including silicone, polyurethane, composite biomaterials and a range of hydrogel-coated biomaterials but biofilm formation and encrustation remain major concerns. Novel approaches to these problems from within the Medical Devices Group will be described, including developments in bioactive polymers, drug-polymer conjugates, biodegradable polymers, biomimetic biomaterials and diamond-like carbon applications.

1115 Molecular basis of biofilm formation by *Staphylococcus epidermidis*

CHRISTINE HEILMANN

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Biofilm formation on polymer surfaces which involves initial attachment and accumulation in multilayered cell clusters (intercellular adhesion) is the major pathogenicity factor in *Staphylococcus epidermidis* foreign-body-associated infections. We characterized two distinct classes of biofilm-negative Tn917 mutants in *S. epidermidis* affected in initial attachment (class A) or intercellular adhesion (class B). Class A mutants were complemented by a DNA-fragment encoding the 148 kDa surface-associated autolysin AtlE. AtlE has bacteriolytic activity and also binds vitronectin, suggesting that AtlE not only plays a role in binding of the cells to polystyrene, but also to host proteins deposited on the polymer after implantation. The importance of AtlE in pathogenicity has been demonstrated in a catheter-associated infection model. Recently, we cloned the gene *aaE* encoding another multifunctional autolysin/adhesin. The 35 kDa AaE possesses adhesive properties such as binding to Fibrinogen, Fibronectin, and Vitronectin.

Class B mutants lack the polysaccharide intercellular adhesin (PIA) which is a linear β -1,6-linked N-acetylglucosaminoglycan. The genes (*icaADBC*) which mediate cell clustering and PIA synthesis were cloned and sequenced. IcaA was shown to carry out the N-acetylglucosaminyltransferase activity. However, IcaCD are required for the synthesis of long-chain oligomers. In a model of foreign body infection, the PIA-negative mutant was significantly less virulent than the wild type.

A protein, AAP, is also involved in the accumulation phase of biofilm formation. Cloning and DNA sequence analysis revealed that the 132 kDa AAP has features typical of gram-positive surface proteins such as a N-terminal signal peptide, multiple repeat domains and a C-terminal cell wall anchor.

1150 Antibiotic sensitivity of biofilms

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Current proposed mechanisms do not offer a satisfactory explanation for the massive resistance of microbial biofilms in general to diverse agents compared to their planktonic

counterparts. These will be described and include lack of penetration, assisted by enzymatic antibiotic degradation; efflux systems; resistance due to slow or zero growth rate; an attachment specific, resistant phenotype. An unrecognised mechanism is the general stress response (GSR), influenced by density and partly under the control of quorum sensing signals. GSR will be described and involves protecting cell structures in a process gradually resulting in quiescence and resistance to numerous physical and chemical stresses. Cell density will be relatively high in a population of cells growing on a surface and could demonstrate density-related effects at a stage when their planktonic counterparts of equivalent population numbers would not. Other situations will be compared where high density occurs with relatively low cell numbers such as in biomasses or within intra-cellular vacuoles. Thus, biofilm growth can lead to an early accumulation of density-dependent signals and to an early GSR relative to planktonic growth and possibly to a more complete expression. It is speculated that hydrophobic signals may form aggregatess within biofilms leading to an early and persistent availability of signal.

Oral biofilms

1400 Microscopic structure of oral biofilms

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Dental plaque, the biofilm found on tooth surfaces, is associated with the commonest diseases affecting man in the developed world, i.e. dental caries and periodontal disease. In the case of caries, disease results from acid production by acidogenic flora at particular sites in the mouth. We have investigated the development of pH gradients in biofilms of oral bacteria grown *in vitro*, using a novel combination of two-photon excitation microscopy (TPEM) and fluorescence lifetime imaging (FLIM). TPEM relies on simultaneous absorption of two (infra-red) photons by a fluorophore to excite fluorescence. TPEM gives far better depth penetration into highly scattering samples such as biofilm. FLIM uses ultra-fast electronic detection (1-10 ns) to allow measurement of the fluorescence decay of the fluorophore. In the case of carboxy-fluorescein, the decay time can be correlated with pH. This information can be used to generate a lifetime-contrast (pH) image. The studies show that there are extreme heterogeneities in pH within oral biofilms following exposure to sucrose or glucose. This heterogeneity is similar in both the lateral (*x-y*) and sagittal (*x-z*) planes. The heterogeneity may reflect differences in species distribution, as well as metabolic fluxes through the biofilms. These differences result in the generation of a kaleidoscope of pH micro-environments. These findings have important implications for the co-existence of different bacterial species in biofilms, and for the approaches to be taken to control the diseases associated with biofilms.

1435 Control of oral biofilms

M. WILSON, J. PRATTEN, J. EMBLETON, I. ALLAN and T. MORGAN

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Oral biofilms are responsible for some of the most prevalent infectious diseases of man - caries, gingivitis and periodontitis. They are also the source of the causative organisms of diseases with a high mortality such as infective endocarditis and coronary artery disease. A number of approaches have been used to prevent or treat these oral biofilm-associated infections and these fall into five main categories: (a) preventing biofilm formation, (b) removing/disrupting existing biofilms, (c) altering biofilm physiology, (d) controlling the bacterial composition of the biofilms (e) killing bacteria in the biofilms. The specific techniques involved in these approaches include the use of anti-adhesive compounds, antimicrobial agents, sugar

substitutes, pH regulators, enzymes, surfactants, oxygenating compounds and various devices for the mechanical disruption of biofilms. Laboratory evaluation of these techniques is an essential pre-requisite to clinical trials which are expensive, time-consuming and often difficult to interpret. A variety of model systems are available for such *in vitro* evaluation including biofilm-generating devices, chemostats and flow cells. Using these systems, the formation, growth, composition, structure and physiology of mono-species and multi-species biofilms, as well as microcosm dental plaques, have been investigated and methods for interfering with these processes have been evaluated. The use of these laboratory models in the evaluation of methods for controlling oral biofilms will be discussed.

1540 *Candida* biofilms and their susceptibility to antifungal agents

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The pathogenic *Candida* species are capable of causing a range of superficial infections as well as life-threatening, systemic disease. Many of these infections involve biofilm formation on implanted devices such as a denture or indwelling catheter. *Candida* biofilms can be formed *in vitro* using several model systems. In the simplest of these, biofilms are grown on the surfaces of small discs of catheter material or denture acrylic. Growth is monitored quantitatively by dry weight measurements, colorimetric or radioisotope assays, and can be visualized by scanning electron microscopy. Biofilms of *C. albicans* prepared in this way consist of a dense network of yeasts, hyphae and pseudohyphae, arranged in a bilayer structure. The cells are surrounded by a matrix material whose synthesis is increased when developing biofilms are subjected to a liquid flow. *C. albicans* biofilms are resistant to a range of antifungal agents in current clinical use, including amphotericin B and fluconazole. The mechanisms of resistance are unknown. The biofilm matrix material does not appear to constitute a barrier to drug penetration. Similarly, drug resistance is not simply attributable to a low growth rate, since biofilm organisms are drug resistant over a range of growth rates. Drug susceptibility studies with mixed fungal/bacterial biofilms indicate that the presence of bacteria can affect antifungal activity and that conversely, fungal cells can modulate the action of antibiotics.

1615 Gene expression in oral biofilms

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The majority of oral bacteria, and in particular those that have been associated with caries and periodontal diseases, have the mouth as their only natural habitat. Thus, these organisms can be considered "obligate biofilm bacteria" and they have evolved specialized mechanisms for responding at the genetic and physiologic levels to the constantly changing environment in the oral cavity. Of the environmental factors known to have major influences on the composition and pathogenic potential of tooth biofilms, pH and carbohydrate availability seem to be the most critical. Applying a combination of reporter gene fusion technology coupled with the use of mutants that have become deregulated in responses to specific environmental stimuli, the regulation of key factors that modulate virulence or biofilm community stability have been explored in *in vitro* single-species biofilms. Additionally, using the recently available sequence from the *Streptococcus mutans* genome project, genes encoding products that were predicted to modulate virulence expression in response to environmental stimuli have been isolated and mutated. The resulting mutants have been shown to be impaired in the ability to form stable biofilms in an *in vitro* model system.

This work was supported by Grant #R01 DE13239-01 from the National Institute of Dental and Craniofacial Research.

FRIDAY 15 SEPTEMBER 2000

Biofilms on shedding surfaces

0930 Dissection of the genetic pathway leading to biofilm behaviour in *Salmonella typhimurium* UTE RÖMLING

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Biofilm formation in Enterobacteriaceae is an important cause of nosocomial infections. Several infections caused by *Escherichia coli*, such as urinary catheter cystitis, biliary tract infections and bacterial prostatitis, involve biofilm-forming bacteria. In *E. coli* and *Salmonella* spp. a biofilm-forming morphotype was identified. We use a *Salmonella typhimurium* isolate constitutively expressing the biofilm morphotype as a model system to study the genetic pathway leading to biofilm formation. The biofilm morphotype is positively controlled by *agfD* a putative transcriptional regulator of the FixJ family of response regulators. *AgfD* cells do not adhere to surfaces and are in the planctonic status. Combined genetic and electron microscopy studies showed that the extracellular matrix of biofilm-forming cells contains two substances; thin aggregative fimbriae and an unknown extracellular substance. Thin aggregative fimbriae confer the community behaviour to the cells. The extracellular substance is responsible for elastic cell-cell interactions and loose adhesion to surfaces. Both pathways to extracellular substances are independent from each other and regulated by *agfD* on the transcription level. *AgfD* regulates 1. The *agfBAC* operon encoding for structural components of thin aggregative fimbriae; 2. *adrA* (*agfD* regulated) involved in the production of the unknown extracellular substance. *AdrA* contains an N-terminal putative transmembrane domain and a C-terminal GGDEF motif of unknown function which is present in over 50 proteins from Gram-positive and Gram-negative bacterial species. Currently we are trying to isolate genes by differential colony morphology screening. By this approach we suspect to identify genes which are involved in the production of the unknown extracellular substance, but might not be regulated by *agfD*. Therefore, those genes might link the biofilm behaviour to other cellular functions.

1005 Growth and metabolism of human intestinal bacteria on surfaces

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The human colonic microbiota is usually viewed as being a homogeneous entity, yet the proximal colon and distal bowel are quite dissimilar in their physical and environmental characteristics. Individual species and assemblages of microorganisms exist in a multiplicity of different microhabitats and metabolic niches in the large gut, either in association with the mucosa and mucus layer, or in the gut lumen. Examination of intestinal contents by scanning electron microscopy and confocal microscopy shows that most of the bacteria are not freely dispersed, but occur in clumps, and in aggregates attached to plant cell structures, resistant starch granules and other solids. With respect to the numerically predominant species, bacteria attached to surfaces in the gut lumen appear to be phylogenetically similar, but physiologically distinct compared to non-adherent populations. These adherent organisms are more directly involved in the breakdown of complex insoluble polymers than unattached bacteria, giving them a significant

competitive advantage in the ecosystem. Mucosal populations are more difficult to study in healthy individuals due to the physical inaccessibility of the large gut, and this has restricted studies on these communities. As a consequence, the composition, metabolism and health-related significance of bacteria growing at, or near the mucosal surface, is largely unknown.

1115 *Pseudomonas aeruginosa* biofilms in lung infection

GERALD B. PIER

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Mucoid *P. aeruginosa* is the predominant bacterial pathogen in chronic pulmonary infection in cystic fibrosis (CF). The mucoid exopolysaccharide (MEP) produced by these strains is the major virulence factor of *P. aeruginosa* in CF lung infection: only when mucoid *P. aeruginosa* is isolated from CF patients is there a decline in pulmonary function and associated lung pathology. Elaboration of MEP is thought to be the molecular factor promoting growth of mucoid *P. aeruginosa* as a biofilm in the lung of CF patients. This is likely a major factor in long-term bacterial survival. Biofilm formation in *P. aeruginosa* has been linked to genes involved in quorum sensing and motility. However, the genes controlling MEP production are independent of control by the known quorum-sensing genes of *P. aeruginosa* including *lasR* and *rhlR*. In sputa of infected CF patients there is no correlation between the levels of transcripts for the *lasR*-regulated *toxA* (Exotoxin A) gene and *algD*, the first gene in the MEP/alginate biosynthetic pathway. Thus, while quorum sensing plays a clear role in formation of biofilms in vitro, its role in the pathogenesis of *P. aeruginosa* infection in CF patients is not apparent at the moment.

Therefore, the key question is: how does the growth mode of *P. aeruginosa* in the lung of a CF patient promote bacterial survival and interfere with host immune effectors? Prior work has identified opsonic antibodies specific to MEP as key mediators of acquired immunity. These antibodies protect rodents against chronic lung infection by mucoid *P. aeruginosa* enmeshed in agar beads and are found in high titers only in older (>12 years) CF patients lacking detectable mucoid *P. aeruginosa* infection. However, infected CF patients make opsonic antibodies to mucoid *P. aeruginosa* that are specific to antigens other than MEP. Further work showed the MEP-specific antibodies could kill mucoid *P. aeruginosa* growing in a biofilm, whereas non-MEP specific opsonins did not mediate opsonic killing of mucoid *P. aeruginosa* in a biofilm. More recent work has identified the acetate substituents on the MEP antigen as molecular mediators of bacterial resistance to opsonic killing by complement and leukocytes. Mucoid *P. aeruginosa* mutants unable to acetylate MEP are opsonized and killed by low concentrations of human complement and leukocytes under conditions wherein wild type *P. aeruginosa* resist killing. Protective MEP-specific opsonic antibodies bind to acetylated epitopes on MEP whereas non-opsonic antibodies to MEP bind to non-acetylated epitopes. Overall, both the growth mode and molecular composition of MEP are critical for mucoid *P. aeruginosa* resistance to host immune effectors during chronic lung infection in CF patients.

1150 The use of the sorbarod biofilm system to investigate the interaction of medically important bacteria

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Sorbarod filters consist of a concertina of cellulose fibres in a paper sleeve and are suitable for experimental biofilm work. There are a number of advantages with the system. These include the use of a T connector upstream of the biofilm, making it possible to change the media being delivered to the biofilm without disturbing the growing biofilm itself. Using a 12 channel peristaltic pump, it is possible to set up a sufficient number of filters to cover a suitable range of antibiotic concentrations so that appropriate studies with antibiotics can be performed. The conditions for the growth of a number of bacteria, including *Streptococcus pneumoniae* and *Moraxella catarrhalis* are described. In addition the susceptibility of these bacteria to a range of appropriate antibiotics is discussed. The ability of β -lactamase producing *M. catarrhalis* to protect penicillin sensitive pneumococcus from the effects of certain β -lactam antibiotics is demonstrated. In the Sorbarod system it was shown that pneumococcus reversibly lost its capsule. The significance of this finding is discussed.

POSTERS

CCS/MI 01 Particulate bioglass reduces the viability of bacterial biofilms formed on its surface in an *in vitro* model

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45S5 Bioglass is a bioactive implant material used in the repair of periodontal bone defects. The surface reactions undergone by this material may have potential antibacterial effects. In this study, the Constant Depth Film Fermenter was used to examine the formation of mixed species oral biofilms on particulate Bioglass. These were grown under conditions which modelled the use of Bioglass subgingivally i.e. an anaerobic atmosphere (5% CO₂, 95% N₂) and a serum-containing medium (40% horse serum in RPMI, with or without hemin and vitamin K). The antibacterial activity of Bioglass was assessed by comparison of the viability of the biofilms grown on this substratum, to those grown on inert glass particulates. Bioglass was found to significantly reduce the total viable anaerobic counts of the biofilms for 48 hours in the medium with hemin and vitamin K, and for 96 hours in the unsupplemented medium. Thus, Bioglass was found to have the potential to reduce biofilm formation on its surface.

CCS/MI 02 Identification of stress-inducible genes in *Streptococcus mutans*

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Differential gene expression in response to environmental stress was analyzed by fingerprinting of RNA using arbitrarily primed PCR (RAP) in *Streptococcus mutans*, which is a principal pathogen in oral biofilms causing human dental caries. The exposure of the bacteria to acid, high salt and plasma components is hypothesized to induce proteins that are essential for survival and virulence inside the host. To identify stress responsive genes, total RNA from treated and untreated bacterial cultures were RAP fingerprinted with a panel of 11mer primers designed for prokaryotic differential display RT-PCR. Different RAP products ranging from 200 to 500-base that were differentially amplified were subsequently cloned and sequenced. Dot and Northern blot confirmed that the RNA designated ASP-185 (acid specific protein) and GSP-781 (general stress protein), were significantly increased when bacteria were treated with acid stress. GSP-781 also responded positively to high salt and temperature. Cloning and functional characterization of these genes will be presented.

CCS/MI 03 The viability of biofilm bacteria following exposure to contact lens case disinfectants J.V. EMBLETON¹, J. DART², L. MCLAUGHLIN-BORLACE³ and M. WILSON¹

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This study was designed to assess the bactericidal activity of lens case disinfectant solutions towards biofilms. The biofilms were grown from organisms isolated from contaminated contact lens cases of asymptomatic contact lens users in a clinical study. Single and mixed-species biofilms were grown on polypropylene discs in a thin film fermenter. The isolates chosen for the generation of these biofilms reflected the common isolates and commonly identified consortia of organisms that had been found in the clinical study. Single species biofilms were composed of a *Micrococcus* spp. and the mixed-species community comprised of a *Micrococcus* spp., a *Staphylococcus* spp. and *Pseudomonas maltophilia*. Biofilms were grown for eleven days, at 25°C, in a medium containing salt concentrations similar to that of human tears. The solutions used in the study were Complete Comfort, Opti-free express (OE), Oxysept 1 (OX) and Renu Multiplus (RM). Biofilms were incubated with the solutions for the minimum use time (MUT), as quoted by the manufacturers, and for 12 h. Growth medium was used as a negative control. Viable counts were performed on the biofilms post-treatment. After incubation for the MUT, there was a significant decrease ($p < 0.01$) in the viable count (CFU per biofilm) of single species biofilms treated with OE ($\bar{x} = 8.0 \pm 4.1 \times 10^4$), OX ($\bar{x} = 2.5 \pm 1.7 \times 10^5$) and RM ($\bar{x} = 1.1 \pm 0.2 \times 10^5$) compared to the control group ($\bar{x} = 2.7 \pm 1.3 \times 10^6$); although the decrease was significant statistically, there was still large numbers of organisms present that were probably of clinical significance. After twelve hours treatment, the viable count in all test groups was significantly lower than the control. The treatment of mixed-species biofilms for the MUT did not significantly reduce the total viable count. After twelve hours, the viable counts of biofilms treated with OX ($\bar{x} = 3.2 \pm 2.2 \times 10^7$) were significantly lower than controls ($\bar{x} = 4.9 \pm 5.2 \times 10^8$).

In summary, the viability of single-species biofilms was significantly reduced by treatment with contact lens case solutions for the MUT. In contrast, the total viable counts of the mixed-species were unaffected after treatment for the MUT.

CCS/MI 04 *Staphylococcus aureus* accessory regulators: expression within biofilms and effect on adhesion

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Many of the genes encoding the virulence factors of *Staphylococcus aureus* are controlled by the accessory gene regulator (*agr*) and staphylococcal accessory regulator (*sar*). This regulation may be affected by the environment in which the organisms are grown. In the majority of ecosystems, bacteria grow attached to surfaces and form biofilms. We have used *S. aureus* strains containing mutations inactivating *agr* and *sar* to determine whether the presence of these genes influences the attachment of the organism to a surface. We have also used strains harbouring reporter constructs of the

agr and *sar* operons to determine their expression in biofilms. The attachment study results showed that the *sarA*⁻ mutant strain adhered better to glass than the *agrA*⁻ mutant or the wild-type. There was an increased adherence to fibronectin-coated glass for all three strains compared to glass. However, after 8 h the *agrA*⁻ mutant strain adhered better than the *sarA*⁻ mutant or wild-type of *agr* and *sar*. Thus, these adhesion studies demonstrate that *agr* and *sar* have pleiotrophic effects on the surface expression of molecules responsible for binding to different substrata. In the biofilms, higher numbers of bacteria, and the greatest expression, were observed at the base, possibly as a result of quorum signalling or environmental factors or through the interaction with other regulons involved in biofilm formation.

CCS/MI 05 Composition and antibiotic resistance of microcosm dental plaques induced by the addition of tetracycline

D. READY, A. ROBERTS, J. PRATTEN, M. WILSON and P. MULLANY

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The aim of this study was to investigate the effects of tetracycline administration on the viability and antibiotic resistance patterns of microcosm dental plaques. A Constant Depth Film Fermentor was used to generate multi-species oral biofilms under conditions similar to those which would exist *in vivo*. The composition of the microcosm plaques was determined by viable counting on selective and non-selective media. The prevalence of antibiotic-resistant genera was determined on antibiotic-containing media. Before administration of tetracycline, the biofilms had a total viable anaerobic count of 1×10^8 CFU per biofilm, and contained 7 % lactobacilli, 19 % streptococci and 0.6 % *Actinomyces* spp. Immediately after pulsing with tetracycline, the composition changed and now consisted of 22 % lactobacilli, 1.5 % streptococci and 0.5 % *Actinomyces* spp. with a total anaerobic count of 1×10^7 CFU per biofilm. The prevalence, and composition, of the antibiotic-resistant flora also changed dramatically after the addition of tetracycline, with the proportion of the microflora displaying resistance to tetracycline increasing from 6 % to 45 %. Corresponding changes in the proportions of the microflora displaying resistance to other antibiotics were as follows: 5 % to 28 % (erythromycin), 1 % to 5 % (vancomycin) and 0.4 % to 3 % (ampicillin). The results of this study have shown that the addition of tetracycline to a model system alters both the composition of the bacterial microflora and its antibiotic-resistance profile.

CCS/MI 06 Transfer of conjugative transposons within oral biofilms

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Conjugative transposons (eg. Tn916) are probably the most promiscuous mobile elements discovered to date. They usually confer antibiotic resistance, (particularly to tetracycline) and they transfer to different species and genera of bacteria. To investigate their role in the dissemination of tetracycline resistance in oral biofilms, microcosm dental plaques were grown in a constant depth film fermentor from an inoculum of human saliva. The tetracycline resistance profile of the microcosm dental plaques was determined before and after a single pulse of tetracycline, (similar to a single oral dose *in vivo*), was added to the microcosm system. Tetracycline resistance was present in three morphologically-

distinct species of bacteria in the initial inoculum, before the tetracycline pulse. After addition of tetracycline, additional species were identified which were resistant to the antibiotic. Molecular analysis on all of the tetracycline-resistant species of bacteria showed the presence of a Tn916-like element. The fact that the number of tetracycline-resistant species increased during the course of the study strongly suggests that this element is mobile. The results of this study have shown that Tn916-like elements are present in saliva and can transfer themselves to other hosts in oral biofilm environments. The results also show the importance of these broad host range elements with respect to the spread of antibiotic resistance.

CCS/MI 07 Characterisation of *tet(W)* and the elements involved in its conjugal transfer

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The widespread use of antibiotics has resulted in the rapid emergence of antibiotic resistance in both human and veterinary pathogens. However little work has been done on the incidence of resistance in the commensal gut flora which may play an important role in onward transfer to pathogenic bacteria. A previously undescribed tetracycline resistance gene, *tet(W)*, was isolated by our group from the rumen anaerobe *Butyrivibrio fibrisolvens*. This new ribosome protection type gene is less than 65% homologous to *tet(M)* and in the *B. fibrisolvens* strain 1.230, is carried on a 50 Kb mobile chromosomal element, TnB1230, which is unrelated to known conjugative transposons.

Here we wish to present 9 Kb of the sequence of TnB1230. We have also isolated a gene > 99% homologous to *tet(W)* from the human colonic bacteria, *Fusobacterium prausnitzii* and *Bifidobacterium longum*. In each of the human isolates *tet(W)* is present on differently sized chromosomal fragments which seem unrelated to TnB1230 from *B. fibrisolvens*, providing evidence that *tet(W)* is not always carried by the same mobile element.

CCS/MI 08 Evaluation of the population genetic structure of coagulase-negative staphylococci associated with carriage and disease in preterm infants using pulsed-field gel electrophoresis

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Coagulase-negative staphylococcal (CNS) infection is the commonest cause of late-onset sepsis (onset after 48 hours of life) in neonates. CNS infection in this population is associated with increased morbidity, prolongation of hospital stay and cost. The relative roles of host factors and bacterial determinants in the pathogenesis of infection are poorly understood. The aim of this study was to determine whether disease-causing strains are drawn randomly from the bacterial carriage population, or whether they represent hypervirulent clones.

A library of coagulase-negative staphylococci associated with bacteraemia (37 isolates), blood culture contamination (47 isolates) or skin carriage (35 isolates) from patients with bacteraemia and 36 isolates from well controls) was assembled from pre-term infants in the Oxford Neonatal Unit over a 12 month period using strict clinical criteria. The population genetic structure and genetic relatedness of these isolates was evaluated using pulsed-field gel electrophoresis, and analysed using BioNumerics software.

CCS/MI 09 Adherence of *S. aureus* to fibrinogen after growth in used peritoneal dialysate: loss of a putative virulence determinant

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Staphylococcus aureus causes 10–20% of cases of continuous ambulatory peritoneal dialysis-related peritonitis. *S. aureus* adhesins such as fibronectin- and fibrinogen-binding proteins are considered to play a central role in the pathogenesis of prosthetic material-related infection. We evaluated the interaction of *S. aureus* cell wall-associated adhesins with host proteins following growth in used peritoneal dialysate rather than standard culture media.

Growth in PD fluid was associated with moderately reduced adherence to fibronectin and markedly reduced adherence to fibrinogen compared with growth in Todd Hewitt broth. Scanning electron microscopy demonstrated that the bacterial surface became coated with host derived extracellular material following growth in used PD fluid. Western immunoblotting confirmed the presence of host fibronectin, fibrinogen and immunoglobulins (Ig) in PD fluid, and that the bacterial coat contained fibronectin and Ig. Adherence to fibrinogen did not differ between the FnBP-deficient mutant and wild-type parent following growth in PD fluid, but adherence of a mutant defective in protein A was more than 2 fold greater than the isogenic parent.

We conclude that adherence of *S. aureus* to host proteins after growth in ideal culture conditions is not equivalent to that following growth in used PD fluid. This has important implications for the models used to study bacterial adherence as a virulence determinant in CAPD-related peritonitis. This data also indicates that binding of human immunoglobulin to cell wall-associated protein A inhibits bacterial adhesion to fibrinogen.

CCS/MI 10 Is the presence of the *ica* operon and expression of biofilm a virulence determinant for coagulase-negative staphylococcal disease in preterm infants?

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The *ica* operon of *Staphylococcus epidermidis* encodes a beta-1,6-linked glucosaminoglycan termed polysaccharide intercellular adhesin which supports bacterial aggregation and biofilm formation. The presence of the *ica* operon and biofilm formation have been reported to be associated with virulence in studies of *Staphylococcus epidermidis* infection in undefined patient populations. The aim of this study was to evaluate the reproducibility of these findings in a Special Care Baby Unit setting.

A clinically validated collection of coagulase-negative staphylococci associated with bacteraemia (13 patients), blood culture contamination (32 patients) or skin carriage (35 isolates from 13 patients with bacteraemia and 36 isolates from 13 matched well controls) were isolated from pre-term infants in the Oxford Neonatal Unit over a 12 month period. Detection of biofilm was performed using Congo Red agar and a quantitative microtitre plate assay. The presence of *icaA*, *icaB* and *icaC* was examined using the polymerase chain reaction.

A comparison of the presence of the *ica* operon and biofilm formation between the following bacterial groups will be presented: (1) isolates associated with disease, contamination and carriage groups; and (2) infecting and colonising isolates from infants with bacteraemia.

CCS/MI 11 Adherence of *Staphylococcus aureus* 8325-4 to fibronectin is not modified by V8 protease

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Adherence of *Staphylococcus aureus* to extracellular matrix components is mediated by bacterial cell wall-associated adhesins. Remodelling of the bacterial cell surface by bacterial proteases, leading to a shift from an adhesive to a relatively non-adhesive phenotype, may be required during the transition between colonizing and invasive phases of infection. We have examined the effect of V8 protease on the fibronectin binding phenotype of *S. aureus* 8325-4 and *S. aureus* V8 by comparing the parent strains with isogenic mutants defective in V8 protease.

The gene encoding V8 protease of *Staphylococcus aureus* 8325-4 was deleted by allelic replacement mutagenesis and the mutation transduced into strain V8. Mutants were verified by Southern hybridization; polymerase chain reaction; Western immunoblotting of culture supernatant using rabbit polyclonal anti-V8 protease antibody; and quantitative determination of total protease activity.

The production of V8 protease during overnight culture did not affect bacterial adherence to fibronectin. Adherence of 8325-4 and V8 and their respective V8 protease-deficient mutants following overnight culture in the presence of the universal protease inhibitor γ -macroglobulin demonstrated a significant increase in adherence to fibronectin for all strains ($p < 0.05$ in all cases). There was no difference in the intensity of fibronectin-binding proteins on Western ligand affinity blotting between 8325-4 and the isogenic mutant defective in V8 protease. Culture of wild-type 8325-4 in the presence of γ -macroglobulin resulted in a marked increase in fibronectin-binding protein. We conclude that degradation of FnBP during growth of *S. aureus* V8 and 8325-4 is mediated by a protease other than V8 protease.

CCS/MI 12 Inhibition of the growth of *Streptococcus mutans* to a glass surface

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Previous models designed to investigate adhesion and growth of oral bacteria to solid surfaces *in vitro* have tended to show considerable variability between experimental replicates. In this study, we report the development of a new model to compare the effects of commercially available dentifrices with a prototype dentifrice containing a non-antimicrobial compound, on the growth of *Streptococcus mutans* to a glass surface *in vitro*. Statistical differences were observed between pastes containing triclosan compared to a basic paste without triclosan. The presence of a novel test article in a dentifrice containing no other antimicrobial agents was shown to significantly reduce the growth of *S. mutans* on glass. Further, in the presence of both the test article (16% w/w) and 0.3% (w/w) triclosan, the reduction in growth on glass was even more marked, resulting in an observable dose response relative to the concentration of test article. Reductions in growth on glass of upto 53% compared to control pastes containing 0.3% (w/w) triclosan and upto 42% compared to a commercially available paste containing 0.3% (w/w) triclosan and 2% (w/w) PVM/Copolymer (Gantrez). The results of this preliminary study suggest that the non-antimicrobial test article reduces the growth of *S. mutans* on glass and may also increase the retention and/or activity of triclosan on a surface when used in a dentifrice.

CCS/MI 13 Modulation of surface polypeptide expression by *Streptococcus gordonii* in response to specific human proteins

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Streptococci colonizing the human oral cavity demonstrate a marked growth response to type I collagen that enables them to invade tooth root dentine. This response is mediated through the activity of cell surface proteins of the antigen I/II adhesin family. The presence of collagen type I in the growth medium causes an up-regulation of SspA and SspB (antigen I/II family) polypeptide adhesins in *Streptococcus gordonii*. This appears to be just one effect of a complex transcriptional regulatory response to environmental collagen. Cells of the wild-type strain DL1 (Challis) grown in the presence of acid-soluble collagen type I, type IV collagen or collagenase-digested type I collagen also demonstrated a number of phenotypic changes in addition to increased antigen I/II production. These included induction of a novel cell-surface protein of approximate molecular mass 100 kDa and of a proteinase with gelatinase activity. These effects were not observed in the presence of human serum albumin or fibrinogen, although antigen I/II production was up-regulated in

the presence of salivary glycoproteins. These results provide evidence for an environmental sensing system responsive to specific human tissue proteins that may modulate invasion and virulence of *S. gordonii*.

CCS/MI 14 Altered surface protein expression by biofilm-grown *Streptococcus sanguis* cells

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Streptococcus sanguis is a primary colonizer of the clean tooth surface and is numerically abundant in plaque biofilms. Successful colonization and growth depends, at least in part, on the array of surface proteins and adhesins expressed by the bacterium. The aim of this work was to identify proteins differentially expressed by biofilm and chemostat derived cells grown at the same mean specific growth rate. Biofilm cells were grown in a glass packed tube device (GPT) that comprised a length of silicone tubing filled with glass beads (diameter 1mm) through which was pumped a mucin-containing artificial saliva. Planktonic cells were obtained from a chemostat culture. Cells were harvested 5 days after inoculation and their cell surface proteins were extracted by mutanolysin digestion. Surface associated proteins from GPT and chemostat grown cells were compared by SDS-PAGE and by 2D-gel electrophoresis. Numerous proteins ranging in size from 25 kDa to >175 kDa were differentially expressed including several that appeared specific to biofilm grown cells. Rabbit polyclonal antibodies were raised to glutaraldehyde-fixed whole biofilm or chemostat cells. By differential screening of an *S. sanguis* expression library we intend to clone and identify surface proteins specific to or up-regulated in the biofilm mode of growth.

CCS/MI 15 An *in vitro* model to evaluate microbial microleakage around restorations in teeth

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Microbial microleakage at the junction between the tooth and restorative material is an important cause of pulpal disease yet it is poorly understood. The aim of this study was to develop a polymicrobial biofilm to mimic the clinical ecosystem sufficiently to replicate events at a tooth/restoration interface using a Constant Depth Film Fermenter (CDFF). The flora beneath amalgam restorations exposed to such a biofilm was examined by culturing,

scanning electron microscopy and vital staining. The amalgam restorations were placed in standardised cavities prepared in bovine dentine cylinders. The cylinders were housed in the CDFF, inoculated with saliva to produce a multi-species biofilm (representative of micro-organisms found in supra-gingival dental plaque) over the restoration and cylinder. The biofilm was grown for four weeks at 37°C in artificial saliva. Cylinders were removed, surface disinfected, split and samples of the cavity wall and amalgam restoration were taken. The samples were (i) cultured to determine total aerobic/anaerobic counts, (ii) prepared for SEM analysis to determine biofilm morphology and spatial arrangement and (iii) vital stained to determine bacterial viability. Biofilms were observed at the tooth restoration interface using SEM and vital staining. On further investigation a diverse and complex microflora was revealed. The results indicate that the CDFF is an effective system to study the dynamics of microbial microleakage.

CCS/MI 16 Mutational analysis of *staphylococcus aureus* fibronectin binding protein and bacterial invasion of endothelial cells

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Staphylococcus aureus fibronectin-binding proteins (FnBPs) mediate invasion of a range of cell lines *in vitro*. Bacterial uptake of *S. aureus* by a human embryonic kidney cell line has been shown to occur via fibronectin bridging between FnBPs and host cell integrin $\alpha_5\beta_1$. Such cellular invasion may be important in the pathogenesis of invasive staphylococcal disease which is frequently characterised by seeding from the bloodstream to distant sites.

S. aureus has two cell wall-anchored fibronectin-binding proteins FnBPA and FnBPB. The FN-binding activity has been localized to the C-terminal portion (D repeat) consisting of three consecutive repeats of ~40 amino acid residues and one incomplete repeat (D1-D4), plus a fifth repeat N-terminal to D1. Region A has fibrinogen-binding activity while the B region has not been assigned a function. This work summarises the effect of deleting one or more regions of FnBPA on bacterial adherence to fibronectin, and on invasion of human endothelial cells *in vitro* following expression in the Gram positive, heterologous host *Lactococcus lactis* and a mutant of *S. aureus* 8325-4 defective in fibronectin-binding proteins A and B.

CCS/MI 17 Detection of broad-host-range plasmids in periodontal bacteria

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Periodontal disease, in which the supporting gingival tissues of the tooth recede, is common within the human population and may result in tooth loss. A diverse population of mainly Gram negative, anaerobic bacteria coexists in the pocket that forms between the tooth surface and the gingiva. Treatment regimes often incorporate administration of either systemic or topical antibiotics and as a result increased levels of resistance in bacteria have been reported in recent years. Broad host range plasmids such as those in Incompatibility (Inc) groups N, P, Q and W have high conjugation frequencies between bacteria within a biofilm, and often carry a battery of

antibiotic resistance genes. Their role in spread of genes between oral bacteria is unknown. In this study, PCR based assays directed to conserved replicative and conjugative gene sequences were used to detect these plasmids in subgingival plaque samples. These assays were augmented by Southern hybridization analyses that increased the sensitivity of detection. DNA was extracted from subgingival plaque obtained from 26 patients presenting at Bristol Dental Hospital with adult periodontitis, of whom 8 had taken antibiotics within the previous six months. Inc N *repA*, *PtrfA*, *QoriT*, and *WoriT* plasmid sequences were detected in 38, 85, 65, and 23% of the samples respectively. Overall, the frequency of detection did not correlate with antibiotic usage by a patient. However, one sample, from a patient who had taken doxycycline prior to sampling, was positive for Inc N, IncP and IncQ plasmid sequences. This sample was analyzed microbiologically, and the IncN plasmid sequence found to originate from an isolate presumptively identified as *Actinomyces odontolyticus* from which a large plasmid could be extracted. Laboratory mating experiments indicated that type plasmids from these Inc groups could transfer from *E. coli* into many of the bacteria implicated in disease aetiology. IncP plasmids, in particular, were able to replicate stably in *Actinobacillus actinomycetemcomitans*,

Eikenella corrodens, *Fusobacterium nucleatum*, *Porphyromonas gingivalis*, and *Prevotella intermedia*, following conjugation from *E. coli*. In conclusion, our results suggest there is considerable potential for plasmid-mediated gene dissemination between periodontal bacteria.

CCS/MI 18 Phagocytosis and defence against invasive *Staphylococcus aureus* infection

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Opsonisation and neutrophil phagocytosis play important roles in host defence against invasive *Staphylococcus aureus* (*S. aureus*) infection. We investigated host genetic variability in three functionally polymorphic genes implicated in these processes in 427 patients with severe *S. aureus* infection and 676 neonatal cord blood controls. We found no significant disease associations with coding mutations in the mannose binding lectin and the Fc gamma receptor IIIb (CD16) genes. However the presence of a common mutation in the Fc gamma receptor IIa (CD32) gene was associated with protection against infection (mean odds ratio (95% CI) 0.80 (0.70 to 0.92) between mutant homozygotes and heterozygotes and between heterozygotes and wild type homozygotes, $p=0.008$). *In vitro*, neutrophils expressing the mutant receptor are more efficient at phagocytosis of *S. aureus* opsonised with IgG2 than neutrophils with wild type receptors. The results of our case-control study confirm that this experimental finding is clinically relevant, and that host factors play a role in determining susceptibility to invasive *S. aureus* disease.

CCS/MI 19 Adhesins in hypervirulent clones of *Staphylococcus aureus*: a study of putative virulence determinants using a candidate factor approach

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A large number of putative virulence determinants have been described for *Staphylococcus aureus*, including bacterial surface proteins implicated in adhesion to host matrix

molecules. However their relevance to the pathogenesis of severe disease is poorly understood.

Using multi-locus sequence typing we compared the genetic population structure of *S. aureus* isolates causing community-acquired disease with that of nasal carriage isolates drawn from the same population, and demonstrated the presence of ecologically successful hypervirulent clones circulating in the community. This finding allows us to identify bacterial groups which are putatively virulent and compare candidate virulence factors with nasal carriage isolates which are not part of a hypervirulent clone.

We selected 50 virulent and 50 putatively non-virulent isolates and assessed bacterial adherence to fibronectin, fibrinogen, collagen and human myeloma IgG. Genotyping for *fnbA*, *fnbB*, *clfA*, *clfB*, *cna*, *spa*, *sdrC*, *sdrD*, and *sdrE* has been carried out using PCR. We will present the results of these investigations and discuss their pathophysiological implications.

CCS/MI 20 Line-associated *Staphylococcus aureus* sepsis in a dialysis unit: a prospective longitudinal study of bacterial transmission dynamics and virulence using a population genetic approach

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Line-associated *Staphylococcus aureus* infection is a major cause of morbidity in dialysis units. The extent to which risk of infection is defined by bacterial as opposed host susceptibility factors is undefined. We have prospectively collected clinical information, serial nasal carriage isolates, and invasive disease isolates from all consenting patients over a period of one year. So far we have studied 144 patients, obtaining in total 107 nasal carriage isolates and 37 invasive isolates. In addition we have obtained through the microbiology laboratory a further 28 invasive isolates from renal patients not in the study. To define the transmission dynamics within the Unit we have characterised these bacteria using multilocus sequence typing and pulsed-field gel electrophoresis. This enables us to assess the link between transmissibility and virulence (demonstrated previously in natural populations using a cross-sectional survey) using a prospective longitudinal approach. These results have major implications for the investigation of bacterial pathogenesis in this setting.

CCS/MI 21 Photosensitisation of *Salmonella minnesota* Re595 by controlled delivery of liposomal zinc(II)-phthalocyanine to the bacterial envelope

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Gram-positive bacteria are often sensitive to photoinactivation following exposure to tetrapyrrolic dyes such as porphyrins, phthalocyanines and bacteriochlorins. However, Gram-negative species appear to be relatively insensitive to photosensitisation unless the permeability properties of the outer membrane (OM) have been altered. There is good but circumstantial evidence that activated lipophilic photosensitisers mediate the killing of Gram-negative bacteria by causing damage to the cytoplasmic membrane (CM) and that photosensitivity is determined by the capacity of dyes to gain access to this target prior to photoactivation. Zinc(II)-phthalocyanine (Zn-Pc) is an extremely hydrophobic second-generation photosensitiser that is completely water insoluble. We have delivered, in a controlled manner, small quantities of Zn-Pc into the CM of the deep rough mutant *Salmonella minnesota* Re595 by

fusion of Zn-Pc-containing liposomes with the cell envelope and studied the effect of photoactivation at 670nm on the viability of sensitised bacteria. Fusion was monitored by quantification of the dye in OM and CM fractions and by determination of mixing of bacterial and liposomal lipid using the self-quenching fluorescent probe octadecyl rhodamine B chloride (R18). Localisation of Zn-Pc within the CM appears to be a prerequisite for efficient killing using low doses of light generated using a diode laser.

CCS/MI 22 Attachment of *Staphylococcus epidermidis* RP62A to chemically modified cellulose derivatives

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Coagulase negative staphylococci, most notably *Staphylococcus epidermidis*, have been identified as a predominant cause of cardiovascular implant infection, which begins with the colonization of the device by the bacteria. One possible approach to reduce this event is to understand how the physicochemical properties of bacterial surface influence attachment to biomaterials.

In the present study, the attachment of coagulase negative *Staphylococcus epidermidis* expressing capsular polysaccharide/adhesin (PS/A), the most common etiological agent of colonization of implantable medical devices, was assessed *in vitro* to cellulose diacetate (CDA), to CDA chemically modified by de-acetylation (CDA-D) and by phosphorylation (CDA-P), as well as to reference Low Density Polyethylene (LDPE).

The quantification of *S. epidermidis* attached to cellulose diacetate (CDA) in phosphate buffer saline (PBS) elicited information regarding the interaction between the bacterial strain and the polymeric biomaterial. There was a significant difference in the adhesion of RP62A to CDA, compared to LDPE. Chemical modifications of CDA by de-acetylation and by phosphorylation were effective in lowering bacterial attachment. These chemical treatments increased the acidic parameter of the surface energy and decreased the acid-base interactions with acidic sites of the capsular PS/A. In other terms, these treatments also promoted a decrease in hydrophobicity that linearly correlates with a decrease in the number of attached cells.

CCS/MI 23 Biofilm:morphogenesis parallel control mechanisms in bacteria and moulds

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Cell-cell signalling is increasingly recognised as being critical to morphogenesis in virtually all living systems. N-acyl homoserine lactones have been recognised as general sensors of cell density in Gram-negative bacteria and are implicated in the structural development of bacterial biofilms. Roles for similar lactone-based molecules in the regulation of phenotype in *Streptomyces* and certain filamentous fungi, whilst long established, have been largely overlooked. We have investigated the possible role of extracellular signal molecules in the morphogenesis and differentiation of a range of filamentous fungi *Aspergillus nidulans*, *Aspergillus niger*, *Cladosporium cladosporioides* and *Penicillium chrysogenum*. Preliminary data suggested that a water-soluble signal molecule responsible for the induction of conidiation. Each of the organisms was extracted with water

and concentrates prepared. These, when placed on developing lawn cultures of the moulds were found to markedly enhance the rate of formation and extent of conidiation, not only in the producer strain but also to a lesser extent in the other fungi. The extracts were fractionated by Gel permeation chromatography and tested for induction potential. Two distinct fractions corresponding to 6500-4500 daltons and 600-250 daltons were detected. The higher molecular weight fractions were protease resistant and heat stable, these fractions were polygamous in their induction of conidiation in the range of fungi used The lower molecular weight fractions were protease resistant and heat labile this material was species specific. We suggest that such molecules would diffuse away from a colony formed on an aqueous substrate, yet be concentrated within the more hydrophobic mycelial mat. In such a fashion both the asymmetry and the radial expansion of conidiation in fungal colonies might be explained.

CCS/MI 24 Effect of environmental conditions on secreted proteins of biofilm-grown *Actinobacillus actinomycetemcomitans*

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Little is known concerning the expression of proteins in bacterial biofilms or the effects of environmental variables on such expression. The purpose of this study was to determine the effects of a number of environmental factors on the profile of proteins secreted by *Actinobacillus actinomycetemcomitans*, an organism which grows as a biofilm on tooth surfaces and which is implicated in the pathogenesis of various forms of periodontitis. Biofilms were grown on agar plates under different conditions (a CO₂-enriched aerobic atmosphere, an anaerobic atmosphere, presence of serum, presence of blood, iron depletion) and the secreted proteins extracted and subjected to 2-Dimensional polyacrylamide gel electrophoresis. Differential expression of a number of the secreted proteins was observed under the different growth conditions and some of these were subjected to sequencing by Edman degradation. Homology searches were carried out using the Fasta3 algorithm and EMBL database. Three of the proteins (those up-regulated by growth in the presence of blood) were found to have a high degree of sequence homology with proteins produced by the closely-related organism *Haemophilus influenzae*. These included a triose phosphate isomerase, a thiol peroxidase and a superoxide dismutase. An ability to adapt to prevailing environmental conditions may facilitate the survival of the organism in the changing microenvironment of its habitat and may modulate its pathogenic potential.

CCS/MI 25 The impact of genetic code ambiguity in the cell wall structure and adhesion of *Saccharomyces cerevisiae*

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Genetic code ambiguity in *Saccharomyces cerevisiae* has a major impact on gene expression, cell size and shape and adaptation to severe stress conditions, namely osmotic, oxidative and drug induced stress. In order to investigate the molecular mechanism of stress resistance and elucidate how genetic code ambiguity alters cell shape and size we carried out a detailed study of the cell wall of *S.cerevisiae* expressing ambiguity at the leucine-CUG codon. For this, a *Candida albicans* ser-tRNA_{CAG} which decodes the leucine CUG codon as serine, was expressed in *S.cerevisiae* and the cell wall

properties of transformed and non-transformed strains grown at 23 and 30° C were compared. Cell hydrophobicity was determined by measuring the contact angles, and the chemical cell wall composition was determined by X-Ray Photoelectron Spectroscopy (XPS). The ability to colonise acrylic surfaces was measured in a parallel plate flow chamber.

The cell wall of *S. cerevisiae* expressing CUG ambiguity displays a severe decrease (50%) in its phosphorous content, is less hydrophilic and is more adherent to acrylic surfaces. Both strains were less hydrophilic and more adherent when grown at 30°C than at 23°C. The data suggests that genetic code ambiguity which is characteristic of *C. albicans* and other *Candida* species might modulate the cell wall structure and adhesion properties of *C. albicans*. This hypothesis is currently under investigation.

CCS/MI 26 Differential sensitivity of *Trypanosoma congolense* to Tri-phenyl tin salicylate and Tri-phenyl silicon salicylate

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In vitro and *in vivo* trypanocidal properties of the organometallic compounds Tri-phenyl-tin salicylate (TPT-S) and Tri-phenyl silicon salicylate (TPS-S) were tested for trypanocidal effects on Procyclic and blood stream forms of *Trypanosoma congolense*. Both compounds at 5- 100 µg/ml exhibited cytotoxic effects against the parasites at varying levels.

Pre-incubation of *T. congolense* 10⁷/ml with 2 µg/ml of TPS-S or TPT-S killed about 50 % of the parasite population. When the mixture was inoculated into healthy mice, there was no development of parasitemia for upto 60 days. Also thin layer chromatography (TLC) of parasite lysates treated with the TPS-S and TPT-S showed the absence of Myristic acid indicating a counter effect on Lipid incorporation.

In the *in vivo* experiments, Balbc mice infected with *Trypanosoma congolense* responded to treatment with 20-250 mg/kg TPT-S. All the animals were cured of the parasites within 10 days of treatment. However, infected mice treated with 20-250 mg/kg TPS-S showed initial regression in parasitemia which subsequently remained stable and failed to respond to any further treatment. High density lipoprotein (HDL) analyzed for both groups of animals treated showed a diminishing tendency for the mice that responded to TPT-S. Our results strongly suggest the trypanocidal efficacy of TPT-S is linked to lipid metabolism.

CCS/MI 27 Evaluation of antimicrobial treatments in a laboratory model of dental unit water systems (DUWS)

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Dental Unit Water Systems (DUWS) are used to irrigate the oral cavity during dental treatment. A number of studies have demonstrated that DUWS become heavily contaminated with biofilms that may contain pathogens. The aim of this work was to establish a reproducible laboratory model system to simulate microbial contamination of DUWS, and to use this system to develop practical, cost-effective, and evidence-based methods for decontamination. Samples of water from 20 DUWS units were pooled and aliquots stored in liquid nitrogen as a reproducible inoculum for a continuous culture-based model system. The chemostat was maintained at 20°C in filtered (0.2µm), potable tap water. The pH was monitored, but not controlled, and the dilution rate was 0.05 h⁻¹. Viable counts (TVC) were assessed on dilutions of culture plated onto R2A agar, with biofouling of DUWS tubing assessed using microscopic/image analysis techniques. The chemostat culture contained a TVC of 6.0 x 10⁴ cfu.ml⁻¹. Chemostat culture fluid was used to contaminate six parallel lines of

DUWS tubing (polyurethane) to develop biofilms. Tubing samples were analysed on a weekly basis and substantial biofilm developed within two to three weeks. Disinfection studies were carried out using sodium hypochlorite. Whilst 0.3ppm was found to eliminate the planktonic bacteria, the biofilm was entirely resistant to 3ppm. Some viable cells were recovered from the biofilm following treatment at 30ppm. A robust, reproducible laboratory model DUWS system has been developed. Biofilms in DUWS systems may be entirely resistant to disinfection regimes which appear to be highly effective against planktonic bacteria. [Alternative treatments for DUWS are being pursued.](#) [Supported by the NHS Primary Dental Care R & D Programme.](#)

CCS/MI 28 Conjugal transfer of erythromycin-resistance in clinical isolates of *Streptococcus pyogenes*

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The aim of the present study was to demonstrate transfer of erythromycin-resistance determinants from Ery^R *Streptococcus pyogenes* clinical isolates to Ery^S strains by a conjugative event, followed by restriction pattern analysis of both transconjugants and parental strains.

A total of 14 *S.pyogenes* Ery^R donors were mated with 4 Ery^S recipients strains using cell-to-cell contact on a filter bed (Shoemaker's method). Tetracycline resistance has been used as a character for counterselection.

Transfer frequency resulted low (10⁻⁷) with phenotype C (MLS resistance) donors, but was very high with phenotype I and phenotype M (efflux system) strains (10⁻³ – 10⁻⁴). These results were independent from growth phase of conjugants.

Phenotyping, T-serotyping and DNA fingerprinting of transconjugants indicated that strains derived from C donors showed the same phenotype of donors, while transconjugants from I phenotype were I and C about equally; M donors always originated M transconjugants, but a part of them (from 12.5% to 62%) showed a sub-phenotype M which could be inhibited only by a MIC of >32 mg/l of Em. Haemolysin production was quantitatively variable.

T-serotyping showed a constant selection in transconjugants of few preferred antigens of recipients.

Restriction pattern analysis of *Sma*I digested bacterial genomic DNA evidenced that, for each conjugation experiment, transconjugants obtained had identical DNA pattern of his parental donor.

Stability of Ery^R determinants in transconjugants and their possibility to be re-transferred to other strains were assessed. Conjugation experiments were done using some transconjugants strains as donors and strains belonging to other species of *Streptococcus* as recipients.

A high frequency of transfer (10⁻⁵) resulted with spontaneous mutants in streptomycin resistance of *Streptococcus gordonii* Challis, while no conjugative events have been revealed in matings with *Streptococcus pneumoniae*.

[Supported by the NHS Primary Dental Care R & D Programme.](#)

THURSDAY 14 SEPTEMBER 2000

1405 Why are we so scared of mathematics?

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University of Exeter

Every individual leaves school with at least eleven years of formal mathematics education and many will receive more through further, higher or continuing education. Nevertheless research consistently shows a widespread problem with mathematics and a pervasive fear of things mathematical. In one major UK survey, more than half the people originally identified refused even to take part in the study. Those that did revealed the extent of the feelings of anxiety, helplessness, fear and guilt in people's emotional response to mathematics, their inability to understand simple percentages such as 10 per cent tips or sales tax and the common perception that a fall in rate of inflation should cause a fall in prices. People do cope, but by organising their lives so that they make virtually no use of mathematics. They remove the need for quantitative skills by developing alternative strategies. This avoidance and dislike of mathematics is prevalent throughout society.

But mathematics is arguably crucial to the life chances of the individual. It holds a 'gatekeeper' role to many professions, is the 'language' of science and is a necessary ingredient of active citizenship in a democratic society.

This session will examine various reasons why mathematics, though arguably so useful and certainly valued so highly by society, is loved by a few, hated by some and feared by so many.

1440 Approaches to developing numeracy skills by non-mathematical scientists

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Abstract not submitted

1545 The development of a diagnostic package to evaluate entry level numeracy skills

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In the Faculty of Science at the University of Ulster we have developed a computer based package designed to evaluate and improve the numeracy skills of undergraduate students. The package has three elements:

- Diagnosis: to evaluate numeracy skills during induction
- Tutorial: to support student learning
- Assessment: tailored to suit individual modules

The topics required for successful completion of a module were identified. During induction, students take a diagnostic test in each of the topics appropriate to their course of study.

The use of a diagnostic package to evaluate entry level numeracy skills, allows us to

- identify common areas of weakness
- compare cohorts from differing educational backgrounds
- emphasise the importance of numeracy to the course of study

Following the initial evaluation students are directed to tutorial material, where appropriate. The importance of numeracy skills is again emphasised through the use of

assessment packages which have been developed for specific modules.

It is hoped that as the package is integrated across the faculty, students will realise the importance of numeracy skills and gain confidence in approaching numerical problems.

1620 Developing numeracy in biologists via a workbook

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In 1992 a half-module was devised with the aim of improving numeracy as staff recognised that undergraduate students were particularly deficient in this area, apparently unable to carry out simple calculations, e.g. on dilution of solutions, yet this was a problem that was largely ignored.

Initially the numeracy module was taught in a conventional manner with lectures and worked examples, a new topic being introduced each week. This did not seem to be very effective as students had very varied abilities and did not necessarily reach an appropriate level of understanding of one topic before the next was introduced. It was decided to alter the approach to allow students to identify any particular areas of mathematics with which they had difficulties, and to provide them with instruction and examples to work with at a pace that suited them.

Students were given a short test at the beginning of the module, consisting of applied examples that require the use of a variety of different mathematical concepts. They were also given a diagnostic test consisting of non-applied examples, in sections that dealt with different mathematical concepts, e.g. decimals, proportions, logs. The results from these tests demonstrated that for most students their main difficulty was in applying their mathematical abilities to practical problems, but some had little understanding of basic mathematical ideas covered at GCSE.

Students were provided with an extensive workbook (now published) of eight chapters. The chapter contents were sequential in the development of concepts. Each started with an explanation of the mathematical concepts covered, followed with examples that were purely mathematical, and ended with examples that were applications of the mathematics in biology. Students were asked to work through the book at their own pace and attend a tutorial session every week at which they were offered individual attention. Over succeeding years a variety of ways of assessing the students has been used, all showing that on average students make significant improvements.

1655 Developing a first-year numeracy module

ALAN J. CANN

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"The best way to predict the future is to invent it" - Alan Kay
Based on seven years experience of using the World Wide Web for teaching and research, this talk will consider the past and future of teaching via the internet. Along the way, I will describe how to present a lecture live from the surface of Mars (and why you shouldn't), how to deliver a paperless course (nearly), and the revenge of the nerds. For the past two years, I have been developing WWW-based resources for numeracy teaching and learning for non-mathematicians. This work uses biological examples to illustrate mathematical principles, rather than vice versa, which is how the subject has traditionally been taught to non-mathematicians. A major aim of this approach to learning is to make the subject matter attractive in its own right. Most maths teaching initiatives have originated from "well-meaning" mathematicians who nevertheless experience difficulties in communicating their enthusiasm to other subject areas and hence fail to co-ordinate aspects of common provision across degree programmes. A major strength an integrated approach to numeracy and I.T. teaching is that it combines the two areas, using one to reinforce the other (e.g. online submission of numeracy assessments over the WWW). Looking to the future, I will try to predict where teaching may be headed in an era of learning machines, peripatetic electronic teachers, life-long learning and global mega-universities.

TUESDAY 12 SEPTEMBER 2000

WORKSHOP ONE - Formation and control of biofilms

Short presentations:

Transposon mutagenesis to identify genes effecting adherence of *Listeria monocytogenes* to a variety of food-processing materials

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Listeria monocytogenes is a facultative intracellular pathogen and the causative agent of listeriosis. It is a human and animal pathogen which can cause septicaemia, encephalitis and meningitis and also an inhabitant of the environment, for example sewage, where it can survive for up to 8 weeks, vegetables, and soil. This widespread occurrence of the organism means it is an important threat to the food industry (Rijpens et al 1998). The ability of microorganisms to attach to surfaces has been demonstrated by many research groups (Costerton et al 1978, Busscher and Weerkamp 1987). This ability is aided by the production of extracellular material.

The aim of the investigation is to identify and characterise any genes involved in the adhesion of *L. monocytogenes* to a variety of materials commonly used in food-processing environments. This was initially done by screening a transposon Tn917 insertion library of *L. monocytogenes* using transposon Tn917. Mutants that were less able to adhere to glass coverslips were identified by a simple cellular staining assay. Twelve mutants from a total of 2500 screened mutants adhered to glass in low numbers. Consequently, a quantitative estimate of the number of cells adhered to glass (and seventeen other materials), was enumerated by viable counts following removal of adherent cells by sonication. The seventeen materials selected are all commonly found in food-processing environments and comprise three major groups: metals, thermoplastics and rubbers. The genes disrupted by Tn917 in these mutants will be described. Identification of genes involved in the adhesion of microorganisms to materials involved in food contact could revolutionise the effectiveness of sanitizers and detergents and general treatment of food.

Use of ^1H NMR to visualise and characterise the structure and diffusional properties of methanogenic granular sludge

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By using NMR imaging, the structure of a range of anaerobic granules was imaged. These images were correlated to back scattering electron microscopic images, that give a distribution of the metal rich and EPS rich regions within a granule. Moreover, maps of the diffusive properties of mesophilic methanogenic granular sludge were made as well. These were correlated to diffusion analysis by relaxation time separated pulsed field gradient nuclear magnetic resonance (DARTS PFG NMR) spectroscopy measurements.

NMR measurements were performed at 22°C with 10 ml granular sludge at a magnetic field strength of 0.5 T (20 MHz resonance frequency for protons). Spin-spin relaxation (T_2) time measurements indicate that three ^1H populations can be distinguished in methanogenic granular sludge beds, corresponding to water in three different environments. The T_2 relaxation time measurements clearly differentiate the extragranular water ($T_2 = 1000$ ms) from the water present in the granular matrix ($T_2 = 40-100$ ms) and bacterial cell associated water ($T_2 = 10-15$ ms). Self-diffusion coefficient measurements

at 22 °C of the different ^1H -water populations as the tracer show that methanogenic granular sludge does not contain one unique diffusion coefficient. The observed distribution of self-diffusion coefficients varies between 1.1×10^{-9} m²/s (bacterial cell associated water) and 2.1×10^{-9} m²/s (matrix associated water).

Biofilm competition and resistance

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Biofilms in natural and industrial environments often consist of complex communities of microorganisms. The interactions that occur between mixed populations of bacteria in biofilms is currently of considerable interest. Synergistic communities, in which interactions are mutually beneficial to each species, can be more resistant to antimicrobial agents than bacteria in isolation (Skillman et al, 1999, Karthikeyan et al, 1999). However, little is known of the interactions occurring in competitive biofilms, or their resistance to disinfectants. We have studied a competitive biofilm system composed of *Enterobacter* spp; *Enterobacter agglomerans*/*Ent* and *Enterobacter gergoviae*/1.15. The production of bacteriocins by the strains 1.15 and *Ent* had a marked effect on the thickness and stability of a 1.15/*Ent* dual species biofilm. The dual species biofilm was less resistant to the disinfectants triclosan, benzalkonium chloride and sodium hypochlorite than single species biofilms. The competitive biofilms apparently failed to gain the advantages of stability and increased recalcitrance to disinfectants seen in synergistic biofilms. However, examination of the biofilms produced by 1.15 as the strain colonised a surface with *Ent*, and when 1.15 invaded a stationary phase biofilm of *Ent*, showed marked differences in the competition strategies used by 1.15. When compared to single species biofilm controls, the biofilm produced by the simultaneous colonisation of *Ent* and 1.15 contained higher amounts of bacteriocin activity. However, bacteriocin production ceased in the invaded biofilm 6h after the introduction of 1.15. As the strains were no longer antagonistic, the invaded biofilm could be re-defined as 'neutral', resulting in a biofilm which was thicker, more stable and more resistant to disinfection when compared to the competitive biofilm.

Control of biofilms of MRSA

S. JONES, T.J. HUMPHREY and H.M. LAPPIN-SCOTT
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Abstract not submitted

Biocide resistance in biofilms: intrinsic, extrinsic or homoserine lactone-mediated?

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That bacteria demonstrate enhanced resistance to antimicrobials when grown as biofilms as opposed to planktonic suspensions is now a well-documented phenomenon. While such resistance has been attributed to a number of biofilm properties that embrace phenotype, physiological and physico-chemical features specific to biofilms, none provide a unifying explanation for biofilm resistance. More recently *N*-acyl homoserine lactone (HSL) mediated quorum sensing has been reported to influence biofilm accumulation, differentiation and physiology. These perhaps also cause changes in biofilm that may be associated with biocide susceptibility. We examined the susceptibility to several biocides differing in mode-of-action and chemical structure, of an HSL-wild-type strain of *Pseudomonas*

aeruginosa (PAO1) and two quorum sensing mutants (C-4 and C-12 defective). No general trend existed between HSL-deficiency and susceptibility in the planktonic status. We then exposed these strains along with other *Pseudomonas putida* and *Ps. fluorescens* strains to a regime of chronic exposure to each biocide. The response was monitored throughout the exposure in terms of changes in susceptibility to (1) the stressing biocide and (2) subsequent cross-exposure to the other biocides and antibiotics. Observed differences in these responses prompted a study of the Pseudomonads grown as biofilms in poloxamer-hydrogel biofilm-constructs. The responses of the biofilm itself (within the construct) and the individual biofilm cells (dissolved construct) were recorded following acute exposure to a single biocide and subsequent cross-exposures to other biocides. This enabled a picture to be sketched as to the role of the biofilm as a whole, the biofilm components and quorum sensing in the resistance of biofilms to antimicrobials.

Evaluation of *Salmonella* phage as biocontrol agents in the poultry processing environment

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The aim of poultry processing, in terms of bacteriological control, is to eliminate bacterial growth and restrict the spread of undesirable organisms between carcasses. This study aims to examine the use of bacteriophage to control *Salmonella* surface contamination. Work has centered upon the issues of phage host range, resistance and the assessment of application to surface contamination. A bacteriophage with rapid lysis characteristics (BP) was isolated from chicken meat and 67 *Salmonella* strains (chicken isolates) were tested against it. Forty seven (~70%) were found to be susceptible. Of the remaining twenty, twelve (~18%) were found to be semi-resistant and 8 (12%) fully resistant to BP. The resistance/semi-resistance of eleven strains was due to the existence of a restriction modification system. Hybridization with labelled BP DNA suggested that some of the remaining nine were resistant due to lysogenisation by BP or a related bacteriophage. It was presumed that the remaining *Salmonella* strains were resistant due to a lack of cell wall binding sites.

In order to assess the applicability of BP in the poultry processing environment, its ability to act on attached cells was established. Importantly, it was found that that BP had the ability not only to prevent attachment of *Salmonella* to stainless steel by killing susceptible cells in liquid culture, but also to kill cells once attached to a metal surface. In addition, BP was able exclusively to kill attached salmonellae in the presence of *Pseudomonas aeruginosa*. Introduction of protein into the assay had a negligible effect on BP, but reduced the efficacy of chlorine. BP was therefore more effective than chlorine (commonly used as a disinfection agent in the poultry industry) in the presence of chicken protein. These findings suggest that the phage could be applied as part of the chlorinated wash and could help prevent the establishment of salmonellae in biofilms. For effective treatment of all cells present, however, a mixture of different phage would be needed.

Digital analysis of protozoan colonisation of biofilms

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Microbial grazers are abundant in natural biofilms and yet their impact on the development and subsequent stability of these systems is largely unknown. This project addresses the

question of how bacteria and one of their potential grazers, protozoa, interact during the initial stages of biofilm formation, i.e. surface colonisation. A flow cell is employed whereby two chambers allow the simultaneous observation of bacterial colonisation in the presence (test) and absence (control) of various protozoa. Colonisation of surfaces is filmed with a CCD (charge coupled device) video camera and the footage is then digitised to yield both a general picture of bacterial colonisation in the presence/absence of protozoa together with more detailed information on the colonisation and feeding behaviour of protozoa themselves.

WEDNESDAY 13 SEPTEMBER 2000

WORKSHOP TWO - Teaching the topic of biofilms

CHAIRS: J. LENNOX (Penn State Altoona College, Pennsylvania, USA) and J.W. COSTERTON (Montana State University, USA)

Intensive research over the past three decades has brought to light a growing understanding of the structural and physical nature of biofilms and of their importance in medicine, industry, and environmental microbiology. Nevertheless, an examination of widely used undergraduate textbooks, in the USA and Europe, reveals only a cursory and fragmented treatment of this topic. The purpose of this workshop is to attempt to reach consensus on at least three questions in order to prepare materials that may influence the undergraduate curriculum in microbiology.

The questions are:

1. What elements of the biofilm story should be included in the undergraduate curriculum?
2. What properties of biofilm microbiology can be introduced into the undergraduate microbiology laboratory experience given the equipment constraints of the undergraduate laboratory.
3. What strategies of information dissemination are most likely to be successful in getting the word about biofilm biology to curriculum developers, textbook authors, planners of educational symposia and teachers.

POSTERS

EM 01 Establishment of *P. Aeruginosa* and *B. Cepacia* binary culture biofilms

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ALLISON

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The leading cause of death in Cystic Fibrosis (CF) patients is brought about by co-infections with *Pseudomonas aeruginosa* and *Burkholderia cepacia*. Characteristically, both organisms can grow as biofilms within the lungs of CF patients. *B. cepacia* infections, however, rarely precede those of *P. aeruginosa*. Subsequent colonisation might, therefore, be due to a conditioning effect of *P. aeruginosa* on the lung tissue or through mechanisms of cell-cell co-operation. In this study pseudo-steady-state monoculture biofilms (ca. 10⁸ cells/pan) of clinical strains of *B. cepacia* and *P. aeruginosa* derived from the same CF patient were established in constant depth film fermenters (CDFF) with a pan depth of 0.1mm after 5d. When 7d old steady state biofilms of *B. cepacia* were challenged with planktonically grown *P. aeruginosa* (20 mls, 5 x 10⁸ cfu/ml), a stable, mixed population was formed. By contrast, established biofilms of *P. aeruginosa* resisted challenge by *B. cepacia*. Following establishment of pseudo steady-state biofilms, 10 of the 15 pans in the *P. aeruginosa* CDFF were removed and replaced with 5 from the *B. cepacia* CDFF, forming a *P. aeruginosa*: *B. cepacia*: blank pan alternating sequence. This was repeated for the *B. cepacia* CDFF. Whilst one CDFF acted as control, the other fermenter was treated with antibiotics. In the control CDFF, binary

populations comprising similar levels (ca. 1×10^8 cfu/pan) of both organisms developed in each pan. This included the blank pan which was subject to co-inoculation with biofilm derived cells from each of the two primary cultures. Co-treatment of the mixed-pan fermenters with gentamycin (1mg/L) following pan swap-over did not affect binary biofilm development. Similarly, pre-treatment of a steady state *P. aeruginosa* primary culture for 6d with tobramycin (0.3mg/L) before pan swap-over had little effect on the development of the resulting steady state binary populations. These results suggest that in CF patients different parts of the lung are initially colonised by *P. aeruginosa* and *B. cepacia*, thereby presenting a continuous co-challenge to the lung by both organisms.

EM 02 Electrokinetic assembly of biofilms containing artificial structured microbial consortia

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Biofilms are highly structured materials, which play an important role in different areas, such as environmental technology, food industry, and medicine. We are in the process of developing new techniques for the *de novo* assembly of structured biofilms containing different species. In this study, microbial cells of different species were directed towards specific places on a microelectrode array using dielectrophoresis. After pattern formation of cells under the electric field, the artificial consortia were stabilised using UV-polymerisable hydrogels. We will discuss the effect of the dielectrophoretic properties of the cells on the immobilisation procedure and the optimisation of the hydrogel stabilisation of the consortia.

EM 03 The application of Atomic Force Microscopy in the study of primary colonisation events during biofilm formation

W. RICHARD BOWEN, ROBERT W. LOVITT and CHRIS J. WRIGHT

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Adhesion of biological cells and proteins to surfaces has far reaching consequences in medicine, dentistry, the natural environment and biotechnology. A great potential of atomic force microscopy (AFM) is found in its force measurement capability within a relevant aqueous environment. The forces of interaction between proteins or cells and surfaces are important determinants of bacterial colonisation or protein adsorption. In the present study cell probes, spore probes and coated colloid probes were constructed to allow the direct quantification of interaction forces found in bioadhesion processes using AFM.

A *Saccharomyces cerevisiae* cell was immobilised on the end of an AFM cantilever (cell probe) to study the adhesion of cells to surfaces relevant to biochemical engineering and medicine. The force distance curve measured when the cell probe was retracted from the surface was seen to have a distinctive adhesive force component and also suggested a degree of cell stretching. The degree of cell adhesion increased with time of surface contact.

Individual spores were attached to the end of an AFM cantilever, to construct a spore probe, and brought into contact with a mica surface in different electrolytes. *Aspergillus niger* spore adhesion was influenced by both the ionic strength and pH of the solution. Measurement of zeta potentials and the total force incident on the spore as it approached the surface suggested that the spore-surface interactions were not dominated by double layer interactions and that specific interactions were operating. The adhesion of individual *Aspergillus niger* spores to freshly cleaved mica in air, a system which is relevant to the removal of spores by filtration, was also investigated. It was found that adhesion

was reproducible at a relative humidity of 64%, but that it showed substantial variability at a humidity of 33%, findings which are consistent with capillary forces playing a dominant role in the adhesion process.

The present study used the coated colloid probe technique to investigate protein-protein interactions at different electrolyte concentrations. The measured force-distance curves were in good quantitative agreement with predictions based on the DVLO theory using zeta potentials calculated from an independently validated site-binding-site dissociation surface model.

We have also investigated the adhesion of colloids to surfaces relevant to process engineering. A model system for the adhesion of small particles to stainless steel surfaces of different finishes was defined. The performance of membranes used in biotechnology was also studied in terms of fouling by measuring the adhesion of a BSA coated probe and a yeast cell to membranes. AFM provides a useful means of studying primary colonisation events during biofilm formation and has great potential for the screening of innovative materials produced for their low cell adhesion properties.

EM 04 Surface analytical techniques and surface hygiene

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For food preparation surfaces it is very important to prevent the accumulation of microorganisms occurring. There is a strong relationship between the adhesion, removal and retention of microorganisms and the surface chemistry and physical surface structure of the underlying substrate and on the chemical nature of any conditioning film adhered onto the surface. In order fully understand this relationship the surface roughness characteristics of representative stainless steel samples were determined by atomic force microscopy and were related to the retention of bacterial and conditioning film material. X-ray photoelectron spectroscopy and static secondary ion mass spectrometry have been used to characterise the surface chemistry of conditioning film material. The overall aim of this research work is to provide quantitative information on the hygiene quality of materials used in the food industry.

EM 05 Control of biofilms of a nosocomial pathogen

A.K. BRODZIAC, S. JONES and H.M. LAPPIN-SCOTT
University of Exeter

Abstract not submitted

EM 06 A novel fermentation system to model biofilms in the human large intestine

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An anaerobic fermentation system intended to represent the physiochemical environment of the human gut was used to initiate and support a gut biofilm community. It incorporated a fixed mucin bed enclosed within a dialysis membrane. Dense biofilm growth was promoted by the removal of toxic fermentation by-products via diffusion into a solute system. The fermenter was inoculated with a faecal slurry and fed a media containing the typical exogenous substrates reaching the colon. After the system had reached equilibrium samples were taken from various regions and the bacterial flora characterised using *in situ* hybridisation with fluorescently labelled oligonucleotide probes designed to be specific for

common genera of gut bacteria. Samples were also cultured on selective agar to enumerate the predominant bacterial species. These studies showed that the microflora community structure was generally similar to that in luminal contents. However, certain populations were detected in the biofilms which could not be recovered from the lumen. Current studies are being directed towards the identification of these strains which probably represent novel gut diversity.

EM 07 Interactions within biofilm of *Listeria monocytogenes* with strains belonging to the resident microflora of food industries open surfaces
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Listeria monocytogenes in pure culture does not have a high biofilm development capability and does not form microcolonies. Published works showed that association with strains that belong to the genus *Pseudomonas* allowed better colonization of surfaces by *L. monocytogenes* or by *L. innocua*. We searched whether other strains were able to modify *L. monocytogenes* settlement on stainless steel. Among the strains that reduced its settlement Gram⁻ (*Pseudomonas fluorescens*) as well as Gram⁺ (*Bacillus* sp., *Cellulomonas* sp.) were found. Strains that increased *L. monocytogenes* settlement were Gram⁻ (*Stenotrophomonas maltophilia*, *Comamonas* sp.) or Gram⁺ (*Kocuria varians*, *Staphylococcus capitis*), in this case *L. monocytogenes* formed microcolonies. For one of the strains used (*Comamonas* sp. CCL 24), the filter sterilized supernatant of a biofilm culture, added to a pure culture of *L. monocytogenes* led to an increase of *L. monocytogenes* (3 strains) biofilm population and formation of microcolonies. This phenomenon did not occur with a supernatant of a planktonic culture of *Comamonas* CCL 24. Only one of the 3 *L. monocytogenes* had an increased growth (assessed by count of planktonic and biofilm CFUs) when supernatants were added. It can be concluded that the supernatant of a biofilm culture of CCL 24 led to a trapping of *L. monocytogenes*.

One of strains that increased *L. monocytogenes* settlement (*Bacillus* sp.) led to a decreased growth and a decreased proportion of the CFUs of the sessile phase. This last observation allows to hypothesize that this biofilm produced an anti-adhesive component. Those two effects led to an average logarithmic CFU difference of 4 between the pure and mixed culture of *L. monocytogenes*.

EM 08 Outer membrane structure, desiccation tolerance and environmental survival in *Salmonella* spp.

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Isolates of *Salmonella* spp. have been shown to be capable of extreme persistence in the farm, factory and domestic environment, particularly when they are in protective materials such as blood or egg. Strains with normal RpoS expression survive significantly better than those isolates that do not have a normal *rpoS* allele. In general, isolates that survive well on surfaces are also capable of forming convoluted colonies on a variety of agar types. Such colony morphology is largely associated with the presence of SEF17 but the removal of these surface structures has little or no effect on the survival profiles of the *Salmonella* isolates on surfaces. In contrast, LPS mutants survived very poorly.

Survival of surfaces is poor if cells are suspended in culture broth. If populations are cycled through repeated drying and re-hydration, however, marked and apparently

permanent improvements in survival are seen. Data on this and other matters will be presented.

EM 09 Characterisation of biofilm formation and fouling in paper manufacturing

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Paper manufacturing is a water-based process, employing a range of raw materials that introduce a vast array of organisms into a warm, nutritious environment. Whilst micro-organisms in the process stream are not a direct problem to the manufacturer, the presence of microbial slime presents a direct physical challenge to process continuity by falling onto the wire mesh upon which paper-fibre is deposited. When dried, rolled and polished the presence of slime causes breaks, holes and discoloration in the paper. Slime formation in paper mills is controlled through the widespread use of biocides (slimicides). Samples of white-water and microbial slime were collected at various times from a Lancashire paper mill. These were enumerated on R2A and TSA medium and further characterised by conventional culture and microscopy. Individual morphological types were identified by Vitek analysis. Significant differences were noted between the white-water micro-flora and that comprising the slime community. The latter were predominately *Sphingomonas*, *Agrobacterium*, and *Acinetobacter* spp. These differences could be replicated by inoculation of an in-vitro, perfusion model of biofilm (Sorbarod) with freshly collected white-water and continuous perfusion with a synthetic white-water medium. Under such circumstances the slime-forming community actively colonised the Sorbarod fibres as discreet macro-colonies whilst the residual white-water flora was only transient. A multiple Sorbarod model was constructed in stainless steel and instrument with pressure and oxygen sensors. This device was able to monitor the build up of the slime community with respect to its biofouling potential and metabolic oxygen demand. The model is currently being evaluated for its potential to monitor the use and effectiveness of paper-slimicides.

EM 10 Nitrifying biofilms: structure, co-operation and performance

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An expanded bed, nitrifying bioreactor designed to remove ammonia from effluents has been in operation for several years. It was inoculated with a mixed microbial population from a nitrifying trickling filter, and it took several weeks for visible biofilms to form on the support material, coke. This biomass support material is non-toxic, hard-wearing, cheap and porous. Its porosity makes it ideal for the attachment of adhesive microorganisms, which first colonise the pores and then overgrow the particles, to form biofilms approximately 0.5 mm thick. This results in a biomass concentration of up to 40 g dm⁻³ (dry weight estimated from volume). Transmission electron microscopy of detached biofilms revealed a mixed population, mainly of bacteria, occurring primarily as colonies of similar cells.

The use of Response Surface Methodology to simultaneously optimise the temperature and pH of the nitrification process revealed these optima to lie in the range 14-16°C and pH 7.8-8.0. When operated at these optima, the biofilms were capable of oxidising a 140 mg dm⁻³ ammonia feed at a maximum rate of 120 mg dm⁻³ h⁻¹; which equated to achieving complete nitrification at a dilution rate of 0.85 h⁻¹. This was equivalent to a residence time of less than 1.2 h, a recirculation ratio of 70 and the removal of 2.0 mg dm⁻³ per pass. With a 280 mg dm⁻³ ammonia feed, the maximum rate of

nitrification was $145 \text{ mg dm}^{-3} \text{ h}^{-1}$; which equated to achieving complete nitrification at a dilution rate of 0.5 h^{-1} . This was equivalent to a residence time of about 2 h, a recirculation ratio of 120 and the

removal of 2.4 mg dm^{-3} per pass. The amount of ammonia oxidised per pass was limited mainly by ammonia-limitation and the insolubility of oxygen in water, as the bioreactor relied on aeration during recycle of the effluent.

The nitrification capacity of the biofilms was found to be equivalent to an ammonia removal rate of up to $5.5 \text{ kg NH}_3\text{-N m}^{-3} \text{ d}^{-1}$.

EM 11 Detection of different functional groups from subsurface environments

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Abstract not submitted

EM 12 The potential harbouring of pathogenic bacteria by protozoa in biofilms

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Protozoa have been nicknamed "Trojan Horses of the Microbial World" since certain species of pathogenic bacteria, for example *Legionella* sp. and *Vibrio cholera*, have been proven to survive and replicate within protozoan hosts. Others have been identified as surviving in protozoa but not replicating, for example coliform bacteria and others such as *Pseudomonas* spp. and *Salmonella typhimurium*. In the natural river environment coliform bacteria, of human and animal origin, that can be potential human pathogens can integrate into biofilms and considering that there are a number of bacterivorous protozoa also existing in biofilms, this project aims to examine protozoan-coliform interactions within them. The River Conder, Lancashire has been chosen as the experimental site as it shows a continuum of faecal coliform levels, from pristine sites to those which are heavily contaminated. Naturally occurring coliforms have been isolated and identified, and their fate within protozoan species is being evaluated, i.e. whether they are digested or survive within the protozoan, with or without replication.

EM 13 The effects of constant and exponentially decreasing concentrations of antibiotics on biofilms of susceptible and resistant *Staphylococcus aureus*

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The effects of linezolid and four other comparator antibiotics (vancomycin, teicoplanin, ciprofloxacin and quinupristin/dalfopristin) against biofilms of methicillin and vancomycin susceptible, methicillin resistant and vancomycin resistant *Staphylococcus aureus* were assessed for bactericidal activity. The concentrations of the antibiotics used corresponded to peak serum levels. The bacteria were exposed to the antibiotics at a constant concentration, representing administration as an infusion and also at an exponentially decreasing concentration, representing parenteral administration.

Significant differences between the two methods of administration, constant and exponentially decreasing were not generally seen. Linezolid and quinupristin/dalfopristin produced similar levels of bactericidal activity and were particularly active against the vancomycin resistant strains. The two glycopeptides exhibited a surprising lack of activity against the susceptible strains. Overall ciprofloxacin

appeared to give the best results, in most cases three doses of the drug appeared to sterilise the biofilm cells. In conclusion the results of this study suggest that the bactericidal effect of the antibiotics tested does not differ

significantly whether administered at a constant or an exponentially decreasing concentration. This approach provides a further dimension to the in vitro assessment of novel anti-infectives.

EM 14 Attachment, resuscitation and biofilm formation of starved SRBs

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The presence of mesophilic sulfate-reducing bacteria in oil bearing rock formations and associated pipelines has been proven to have detrimental effects, including reservoir souring and corrosion. These bacteria have low nutrient requirements and respond to stress, such as starvation, by maintaining a state of low metabolic activity. Following nine months nutrient deprivation, an isolate from produced oil field fluids was shown to be capable of resuscitation in planktonic media. It was also capable of attachment and biofilm growth which was monitored using microscopic image analysis. Growth rates were compared with those of non-starved cultures of the same isolate.

EM 15 Analysis of intestinal bacterial populations in different age groups

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Although the colonic microbiota in adults has been studied extensively, little information is available with respect to changes that occur with ageing. These may have consequences in elderly people, who are more susceptible to intestinal dysbiosis. The aims of this study were to characterise the major faecal bacteria in different age groups (children 16 months - seven years, n = 10; adults 21 - 34 years, n = 7; geriatric patients 68 - 73 years, n = 4 diagnosed with *Clostridium difficile* diarrhoea (CDAD); healthy elderly people 67 - 88 years, n = 5) using cultural and molecular approaches. Children had higher numbers of enterobacteria than adults, as determined by viable counts ($P < 0.05$) and 16S rRNA ($P < 0.01$) measurements, while a greater proportion of children's faecal rRNA was hybridised by the three probes (bifidobacteria, enterobacteria, bacteroides-porphyrromonas-prevotella) used in the study, due to their less developed microbiotas. Species diversity was also low in the CDAD group, which was characterised by high numbers of facultative anaerobes and low levels of bifidobacteria and bacteroides. The results showed that significant structural changes occur in the microbiota with ageing, and this was especially evident with respect to putatively protective bifidobacteria. Reductions in these organisms in the large bowel may be related to increased disease risk in elderly people.

EM 16 Protozoan participation in microbial food webs of epilithic biofilms in upland streams

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Very little information exists as to the potential complexity of trophic interactions occurring within biofilms since most research to date has been directed at only two of the microbial components, i.e. bacteria and algae. This study was thereby initiated to examine the abundance of a number of microbial groups in developing biofilms over a one year period. Those microbes enumerated included bacteria, cyanobacteria,

chlorophytes, diatoms, autotrophic flagellates, heterotrophic flagellates, ciliates and amoebae, together with larger invertebrate grazers such as rotifers and nematodes. Results clearly showed a significant correlation between bacteria and heterotrophic flagellates in biofilms and that the latter were

present throughout the year, whereas other predators were intermittent members of the biofilm. Thus, heterotrophic flagellates could be the most important grazers of bacteria within biofilms, as they have already been shown to be in planktonic systems.

EM 17 Characteristics of rugose *Vibrio cholerae* biofilms at air-liquid and solid-liquid interface

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Vibrio cholerae serotype O1 and more recently O139, have been causative agent of the diarrheal disease, cholera. *V. cholerae* forms two different colony morphologies, the smooth and after long term starvation a rugose colony morphology appears. The rugose variant synthesizes different exopolysaccharide that aids in biofilm formation, and confers increased resistance to chlorine treatment and oxidative stress. We have compared the biofilms formed at the solid-liquid and air-liquid interfaced of both serotypes, O1 (TSI4) an O139. Static cultures of both smooth and rugose variants were grown for 3 days at room temperature in LB broth inoculated with a 10^5 cfu/ml. The smooth variant grew as planktonic cells in suspension while the rugose variant appeared to grow primarily in biofilms at both interfaces. Biofilm at the solid surface appeared first after 1 day. The air-liquid biofilm was visible after 2 days however after 3 days this biofilm became very thick, strong and hydrophobic. Bacterial populations of both biofilms ranged from 10^7 - 10^9 cfu/cm², although the biofilm at the air interface appeared thicker. To determine if the biofilm sequesters other bacteria, *E. coli* was added at 10^4 cfu/ml to the initial culture and after 3 days the cells were found at the air interface. *E. coli* does not normally produce air interface biofilms, however substantial population of 10^7 cfu/ml were located within the biofilm. Localization of the *E. coli* within the *Vibrio* biofilms by fluorescent microscopy is underway.

EM 18 Biocide sensitivity of transient and resident skin bacteria in a biofilm model and attached to hand squames

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Hand-carriage of pathogenic transient bacteria has long been identified as a mode of transmission for infection in medical and food production environments. As a result the use of antimicrobial handwashes and soaps is an important tool in reducing nosocomial infections and food poisoning outbreaks. Ideally, such disinfection products would kill the transient bacteria whilst leaving the benign resident, mostly Gram positive flora, intact. No information is presently available comparing the biocide sensitivity of freshly isolated transient and resident skin bacteria when present as individual cells and as microcolonies. Bacterial growth attached to the surface has been shown to confer increased resistance to antimicrobial agents. It would be expected that resident bacteria, which can attach and multiply as microcolonies on the skin, would be more resistant to biocides in situ than transient bacteria, which are unable to colonise the skin. In this study a range of freshly isolated transient and resident bacteria, together with a clinical isolate and a laboratory strain, were tested for their susceptibility to parachlorometaxylenol (PCMX) and triclosan. The minimum inhibitory (MIC) and minimum bactericidal concentrations (MBC) of planktonically grown bacteria were compared to the

biofilm inhibitory (BIC) and biofilm eradicating concentrations (BEC). In the absence of an established microcolony model, biofilms were generated in polystyrene microtitre wells for 65h then challenged with varying biocide concentrations in a regrowth assay. There appeared to be no difference in the sensitivity of the resident and transient bacteria. Both groups were found to be up to 8 times more resistant to biocides when grown as biofilms. A model system attaching bacteria to hand skin squames is now in use to determine the biocide sensitivity of transient and resident strains when attached and grown as microcolonies on the skin surface. Attachment to hand squames did not confer an increased PCMX resistance for a representative transient organism *Pseudomonas veronii* BL146, although starvation of bacteria did increase resistance of both planktonic and attached calls. PCMX sensitivity of the resident bacterium *Staphylococcus epidermidis* will be presented.

EM 19 Colonisation of mucin by bacterial communities isolated from the rectal mucosa

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The inflammatory bowel disease ulcerative colitis (UC) is of unknown aetiology, however, it is well established that there is a strong connection with the intestinal microflora. The aims of this investigation were to study the establishment, structure and function of mucosal bacterial populations, since they are putative initiating factors in the development of UC. Because the disease starts in the distal gut, rectal biopsies obtained from healthy people and UC patients were taken with a view to identifying and comparing their resident microbiotas. Individually and collectively, rectal populations (*E. coli*, *Streptococcus* spp., *Bifidobacterium adolescentis*, *Bacteroides vulgatus*, *B. ovatus*, *B. tectis*, and *Prevotella bivia*) were tested for their cytotoxic effects on HT29 cells. The abilities of these defined rectal communities to colonise artificial mucin-coated surfaces were studied in chemostats. Viable counting was done, and *in-situ* hybridisation using fluorescently-labelled oligonucleotide 16S rRNA probes in combination with confocal laser scanning microscopy was used to visualise the development of community structure and microcolony formation. All bacteria in the planktonic phase colonised mucus surfaces, and enterobacteria proliferated most rapidly. While colonisation by *Bif. adolescentis* was slow, the organism achieved comparable cell population densities to *E. coli*. Streptococcal populations also developed slowly, but these bacteria did not establish in high numbers, reflecting their occurrence *in vivo*.

EM 20 Antimicrobial effects of Chitosan, Carvacrol and a hydrogen peroxide based biocide against foodborne microorganisms adhered to surfaces

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The ability of natural compounds to inactivate biofilms of food-borne organisms was investigated with a view of replacing synthetic biocides by more environmentally friendly, natural biocides. The antimicrobial effects of chitosan, carvacrol and Spor-Klenz (hydrogen peroxide, 0.8% w/w; peroxyacetic acid, 0.06% w/w) against *Listeria monocytogenes*, *Salmonella typhimurium*, *Staphylococcus aureus* and *Saccharomyces cerevisiae* in suspension and adhered to stainless steel were determined using modified European suspension and surface tests at 20°C. Chitosan concentrations of 0.5, 1.0 and 2.0% inactivated planktonic counts of the four microorganisms by 1.0 - 1.7 log cfu/ml. Adhered cells were reduced by 2.4, 1.8, 2.3 and 0.9 log cfu/test surface (t.s) for *L. monocytogenes*, *S. typhimurium*, *Staph. aureus* and *Sacch. cerevisiae* respectively. Concentrations of carvacrol above 1.25mM produced at least a 2 log cfu/t.s reduction of adhered *L. monocytogenes*, *S. typhimurium* and

Sacch. cerevisiae but reduced *Staph. aureus* by only 0.85 log cfu/ t.s. Spor-klenz showed a similar level of inactivation as carvacrol against the microorganisms but was more effective than the natural antimicrobials at the highest concentration tested (2.0%). It was concluded that carvacrol has potential for use against microbial biofilms.

EM 21 Environmental signals and regulatory genes involved in microbial adhesion

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We have developed a system to investigate the effects of different environmental signals and the roles of genes involved in microbial adhesion to solid substrates, the first stage of biofilm formation, by measuring bacterial adhesion to glass bead (a hydrophilic substrate) and sand (more hydrophobic) columns. In this communication we show that different laboratory strains of *Escherichia coli* display very different abilities to adhere to glass beads and sand. In particular strain MV1161, a non-motile K-12 strain, is remarkably capable to adhere to both glass beads and sand, compared to other laboratory strains. Different growth conditions, such as temperature and the use of different carbon sources did not affect adhesion levels of MV1161.

However, growth in anaerobic conditions led to a considerable reduction of adhesion to glass beads, but did not affect significantly adhesion to sand columns.

Inactivation of the *csgA* gene, encoding the curlin subunit of curli, an extracellular structure involved in adhesion to plastic, resulted in reduction of adhesion to glass beads, but not to sand. In order to gather information on the genes involved in microbial adhesion and in the mechanisms of their regulation, we tested derivatives of MV1161 in which global regulatory genes had been inactivated. Despite the fact that MV1161 cells in exponential and stationary phase bind to glass and sand columns with comparable affinity, we found that inactivation of the *rpoS* gene, which encodes a transcription factor produced during the stationary phase, strongly affected MV1161 adhesion to both glass and sand. On the other hand, inactivation of the *hns* gene significantly increased adhesion, but only when MV1161 was grown in oxygen-limited conditions. We propose that both *rpoS* and *hns* directly regulate genes involved in adhesion of *E. coli* to solid substrates, and this regulation is modulated by an environmental signal related to the availability of oxygen in the growth media.

EM 22 Biofilm development for a nitrification process

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Ammonia released into the aquatic environment causes a series of problems, including toxicity to fish and oxygen depletion through nitrification, with an increased risk of eutrophication through consequent nitrate production. Sources of ammonia include industrial, agricultural and sewage treatment effluents; and its removal requires an industrial nitrification process. A key feature of nitrifying bacteria is their slow growth rate (*Nitrosomonas* $t_d \sim 7$ h; *Nitrobacter* $t_d \sim 13$ h), which means that to retain them in conventional suspension culture systems, the residence time must exceed their doubling time. This would result in processes of excessive volume, unless the cells are retained in some way.

Cell retention by natural immobilization has advantages over other methods, in terms of cost and long-term stability. Immobilization onto small support particles provides a large surface area for cell attachment ($3,620 \text{ m}^2 \text{ m}^{-3}$ for 1 mm spheres) and these cells can multiply to form thick biofilms. It also allows for expansion of the bed of particles, so that each one is suspended and surrounded by the upflowing

aqueous medium. This reduces any substrate or product transport delays to diffusional limitation within the biofilm, by minimising the boundary layer. Immobilization onto a particulate support material and operation as an expanded bed allows the dilution rate to exceed the maximum growth rate of the cells without them being washed out.

Previous work has shown that coke is a suitable support material for microbial colonisation, although the best type for immobilization of nitrifying bacteria is not known. In this study, five types of coke were evaluated by feeding them from a common reservoir of artificial effluent, which was recirculated through the system. This "effluent" was fed separately to five columns (2.5 x 50 cm), each containing 50 cm^3 of coke, so that 50% bed expansion was achieved. The system was inoculated with a mixed culture of nitrifying bacteria, obtained from an expanded bed bioreactor, which had been running for six years. The system was fed at a low rate, with a growth medium containing ammonium sulphate (420 mg dm^{-3} $[\text{NH}_3\text{-N}]$) and trace elements in a phosphate buffer. One type of coke was colonised at a much higher rate than the others, with a maximum increase of over 1mm per day in the static bed height, which equated to 1% of the original bed height per day.

EM 23 Methanogenic population structure in anaerobic wastewater treatment biofilms

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Anaerobic treatment of municipal and industrial wastewater is a well-established technology with a wide range of applications. Although the past few decades has seen the development of more efficient novel reactor types, relatively little is known about the structure and function of the microbial biofilm communities involved in the process. The emergence in recent years of molecular methods to describe microbial populations in natural communities has attracted considerable interest. In particular, the direct amplification of 16S rRNA genes from environmental samples by using Polymerase Chain Reaction (PCR) has proven to be an attractive technique for characterizing members of complex microbial communities. The objective of the present study was to carry out a culture-independent study of the bacterial diversity present in six different anaerobic sludge samples, collected from a variety of wastewater treatment plants. Total DNA was isolated from each of the samples using an SDS-based chemical lysis method. A PCR protocol for the amplification of 16S rDNA using domain *Archaea*-specific oligonucleotide primers was optimized for each of the sludges. 16S rDNA clone libraries were generated by cloning PCR products into *E. coli* using the plasmid vector pCR 2.1-TOPO. Subsequently, unique rDNA types were identified by Restriction Fragment Length Polymorphisms (RFLPs) using a tetrameric restriction endonuclease (*Hae* III). The resulting DNA fragments were separated by gel electrophoresis and the banding patterns compared by visualization and grouped into Operational Taxonomic Units (OTUs). The 16S rDNA sequences from the dominant OTUs from each sludge sample were sequenced and phylogenetically classified. Considerable methanogenic diversity was observed in the anaerobic biofilm samples.

EM 24 Biofilm: morphogenesis parallel control mechanisms in bacteria and moulds

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Abstract not submitted

EM 25 Biofilm formation of *Salmonella enterica* serovar *enteritidis* under refrigerated temperatures

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EM 26 Anti-adhesive and antibacterial properties of a proprietary denture cleanser

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The antimicrobial efficacy and anti-adhesive attributes of a proprietary denture cleanser were evaluated. To determine cleanser antimicrobial efficacy, *Streptococcus mutans* was grown on heat-cure denture acrylic strips which were then exposed to the cleanser.

To evaluate anti-adhesive efficacy, the strips were treated with the cleanser and then placed in the *S. mutans* suspension. Following incubation, adhered bacteria were removed and enumerated by viable counting.

Treated denture acrylic plates were also placed in a parallel-plate flow chamber and then exposed to *S. oralis*. Images of adhered bacteria were analysed to determine biofilm coverage. Biofilm removal force was quantified by increasing the flow rate between the acrylic plates.

The cleanser exhibited a 100% kill against *S. mutans* adhered to the acrylic surface and inhibited attachment of cells by 66%.

The flow chamber study found cleanser-treated denture acrylic allowed the formation of a multilayer biofilm which was easily removed by a slight increase in flow rate.

EM 27 The use of Periopaper strips for sampling subgingival staphylococci

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Prosthetic valve endocarditis (PVE) is a serious complication of replacement heart valve surgery with a high mortality rate. The microbial aetiology of PVE is dominated by staphylococcal species, the majority of which are thought to originate from the skin. However, staphylococci have also been isolated from the oral cavity, where they are in a prime position to enter the bloodstream through the gingival crevice or periodontal pockets and cause PVE by haematogenous spread. Periodontal pockets not only provide a site where non-specific adherence can occur and bacteria can be retained within the oral cavity, but the ulcerated and damaged tissue also facilitates access to the bloodstream. Consequently periodontitis patients were chosen as study subjects for investigating subgingival staphylococci.

Curettes and paper points are the most common devices currently used for sampling subgingival bacteria, but both techniques have the disadvantage that they have the potential to cause the gingival tissues to bleed. Consequently the use of thin paper strips, such as Periopaper was investigated as an alternative to these techniques for the collection of subgingival bacteria. In a pilot study Periopaper was successfully used to isolate several species of staphylococci from the gingival crevice of patients with chronic adult periodontitis and from healthy controls. In both of these groups *S. epidermidis* was the most frequently isolated species. Other staphylococcal species isolated included *S. aureus*, *S. capitis*, *S. lugdunensis*, *S. warneri* and *S. hominis*.

EM 28 Growth of biofilms at oil:water interfaces: spoilage of sorbitan ester stabilised oil-out macroemulsions by *Pseudomonas aeruginosa* sp1

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P. aeruginosa SP1 was isolated from a spoiling formaldehyde-preserved oil-out proprietary macroemulsion. Spoilage was not

due to an intrinsic formaldehyde resistance by the organism. The aim of the study was, therefore, to determine the cause and manner of spoilage. This was performed using a test emulsion consisting of a 1:9 ratio of oil (0.1% sorbitan monooleate in hexadecane) and aqueous phase (10% dextran). Samples comprising whole culture, filtered supernate, autoclaved filtered supernate, washed cells and autoclaved washed cells were incorporated into the aqueous phase and emulsified by shear in the oil phase. Spoilage was monitored by a reduction in optical density as a function of time for 125µl aliquots in 96-well microtitre plates. All preparations tested were capable of spoilage with the exception of autoclaved filtered supernate, thereby implicating an extracellular factor. Whilst the active component was sensitive to autoclaving and protease treatment, extracted lipids, phospholipids and lipopolysaccharides did not induce spoilage. In addition, SP1 was able to utilise various sorbitan esters as sole carbon sources but was unable to utilise long and short chain triacylglycerols. Growth of SP1 on sorbitan esters resulted in ca. a ten-fold increase in cell supernate related spoilage activity per colony-forming unit. As such we believe that an extracellular proteinaceous factor is responsible for emulsion spoilage and that this factor has a role in uptake of sorbitan esters by SP1.

EM 29 The development of a method for determining protozoan grazing rates on surface-associated prey

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This poster describes the progress made in the development of a method for determining the potential grazing impact of protozoa on surface-associated prey. It employs a GFP-expressing *Escherichia coli* strain as a marker prey, which can be incorporated into biofilms at a known concentration. Upon ingestion by a protozoan, the *E. coli* is subjected to a combination of acidification and digestion within the food vacuole and this leads to an irrecoverable loss of GFP fluorescence. Thus, as protozoan grazing proceeds over time, a decrease in fluorescence is observed and this is recorded by an automated multi-task plate reader (Victor 1420). The rate of fluorescence loss equates to a grazing rate and values obtained for different protozoa show that a significant fraction of surface-associated bacteria can be removed by protozoan grazing over a short period of time.

EM 30 Microbial populations of marine biofilms and their role in the settlement of *Enteromorpha* zoospores

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Microbial biofilms play an important role in the marine biofouling process. Aspects of the relationship between bacterial biofilms and zoospores of the green macroalga *Enteromorpha* are being investigated. Zoospore attachment to glass slides was enhanced in the presence of a natural marine bacterial assemblage, and the number attaching increased with the number of bacteria present. Spatial microscopic analysis showed that zoospores attached preferentially to clumps of bacteria. The effect of single

strains of bacteria isolated from rock and vegetative plants of *Enteromorpha*, on the settlement of zoospores will be described. A molecular approach is being used to characterise the biofilm populations, through DGGE of PCR-amplified 16S rDNA sequences. Seasonal variation in the biofilm community is also being investigated.

EM 31 Expression of the multidrug efflux gene *acrAB* during growth of *Escherichia coli* in nutrient-limited conditions

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The influence of nutrient limitation, and hence slow growth rate of various harmful bacteria has been extensively studied in chemostat culture and also within biofilms. It has been unequivocally demonstrated that a decrease in growth rate of bacteria is accompanied by rapid alterations in cellular structure and composition associated with resistance to a wide variety of chemicals.

Multiple drug efflux pumps, sometimes with unusually broad specificity, act as major factors to create the general intrinsic resistance of Gram-negative bacteria to natural and synthetic antibacterial agents. One such efflux system encoded by *acrAB-tolC* operon expels a range of antibiotics including (β)-lactams, fluoroquinolones, novobiocin, tetracycline, erythromycin, and fusidic acid. The precise nature of the molecular event(s) that control intrinsic tolerance under slow growth conditions largely remains unknown.

We will show that strains of *E. coli*, which can produce ppGpp, and ^S have a greater intrinsic resistance to a wide range of antimicrobial compounds, compared with strains that are impaired in ppGpp and ^S production. Expression of the multi-drug efflux gene, *acrAB*, is regulated by the specific growth rate during growth in a defined minimal medium in batch and continuous culture. Expression of *acrAB* is inversely related to growth rate. Under certain conditions, growth rate regulation of *acrAB* does not appear to require the presence of ppGpp. Expression of *acrAB* can be correlated with the presence of ^S.

EM 32 Expression of the coaggregation phenotype by aquatic biofilm bacteria after growth at different interfaces

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Abstract not submitted

EM 33 Monitoring planktonic cells in a mixed community biofilm

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Abstract not submitted

EM 34 The effects of wear on fouling and cleanability of food contact surfaces

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The hygienic status of food contact surfaces may deteriorate as they wear, resulting in enhanced fouling and greater difficulty in cleaning. As harsher cleaning regimes are used to recover an acceptable level of cleanliness the abrasion and deterioration of the surface may be accentuated.

An *in-situ* longitudinal study using atomic force microscopy (AFM) and impression material technology assessed the wear patterns found in industrial food processing sites. The AFM with a stand-alone attachment

(Quesant) was applied directly to the food contact surfaces, and the image compared with negative representations made by impression materials. This information was used to validate the roughness characteristics of abraded stainless steel test surfaces produced for studies of bacterial retention and surface cleanability *in vitro*.

The test surfaces used were of roughness ranging from Ra 23 nm (unabraded, mirror finish) to 900 nm. Unidirectional or multidirectional abrasion was achieved using either a quartz paste or silica paper. Fouling of these surfaces with a *Pseudomonas aeruginosa* cell suspension demonstrated that following a simple rinse there was no significant difference in cell retention on surfaces with different roughness characteristics and Ra values at or below 0.8µm (the industrial roughness standard of a hygienic surface). Neither was there an increase in coupon surface area with abrasion sufficient to increase the total cell attachment. Despite this there is an obvious association of micro-organisms with the surface features of stainless steel; surface topography and hydrophobicity will affect the pattern of cell retention.

The cell load per unit area was directly related to the initial cell inoculum, although it was independent of surface finish. A ten-fold increase in the inoculum size led to a ten-fold enhancement in cell attachment, within the inoculum range of 10(5)- 10(8) CFU/ml.

The relative cleanability of components of a combined microbial and food soil may be assessed by dual fluorescence staining and UV microscopy coupled with image analysis. Cells tagged with the viability stain acridine orange can be detected against a simple food soil of modified starch stained with fluorescein. Thus any preferential cleaning of components of the soil may be detected following a mechanical cleaning regime which could incorporate a range of detergents or sanitizers. The effect of the degree and type of surface roughness may also be discerned.

Acknowledgements: This work has been supported by a grant from the Ministry of Agriculture Fisheries and Food within the LINK Advanced and Hygienic Food Manufacturing Programme in collaboration with Unilever Research Ltd., British Nuclear Fuels, Zeneca plc., John L. Lord & Sons, Woolliscroft Tiles Ltd./Pilkingtons, and Campden & Chorleywood Food Research Association.

EM 35 Physiological factors associated with the attachment of *Escherichia coli* to surfaces during biofilm development

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The sequestration by *Escherichia coli* biofilms of isogenic strains of *E. coli* and their dispersal from stainless-steel coupons was investigated. Chemostat cultures of closely matched, yet genetically marked strains of *E. coli* (*E. coli* JM101/S, Lac-deleted and *E. coli* HMS/S, Lac-positive) were established, at various growth rates, within which test coupons were submerged for varying times (x – y hours) in order for biofilms to develop. Tiles were removed from the vessels and switched to the alternate chemostat. The appearance of Lac- cells within the preformed Lac+ biofilms and *vice versa* were monitored by plating onto MacConkey agar. Likewise the fate of each engineered strain within the planktonic phases was monitored. Such data was related to the steady-state population dynamics (attachment, detachment and growth) of the developing biofilm.

Media were designed in which the growth of the cultures ceased through nitrogen-limitation at an optical density (E470nm) of 1.0 and where there was either a 50% excess (CDM1) or a 200% excess of carbon substrate (CDM2). SDS-PAGE of the organisms, cultured in batch and continuous

culture over a range of growth rates using both CDM1 and CDM2, indicated cell envelopes of these strains were identical. *E. coli JM101/S* had a greater μ_{max} than did *E. coli HMS/S* (ca. 15%). This advantage translated into a selective advantage of *E. coli JM101/S* over that of *HMS/S* in mixed cultures. Whereby *E. coli JM101/S* would dominate *E. coli HMS/S* in both batch cultures and chemostats, at a dilution rate of $0.125h^{-1}$, within a period of 8 to 10h, dependent upon the relative numbers on inoculation.

In batch and in continuous culture, the density of colonization by cells of stainless steel surfaces was highly dependent upon the growth rate and growth phase. Increased colonization was observed for cells grown in the higher carbon strength medium. Cross-over experiments, involving the translocation of biofilm coupons from one specific cell type into planktonic suspensions of the other type ($D=0.125h^{-1}$), confirmed that during this period the cells were reversibly bound to the surface and could freely exchange with planktonic cells. After the quiescent phase then not only did the strength of attachment of the cells to the substratum increase, but there was a rapid outgrowth in the numbers of attached cells. This outgrowth was significantly greater than could be accounted for by growth and division of the original biofilm and occurred more rapidly at faster dilution rates. Data generated for mature biofilms of *E. coli HMS/S*, transferred to chemostat cultures of *E. coli JM101/S*, suggested an active displacement of the *HMS/S* strain. *HMS/S* biofilms were rapidly (1 to 2h) over-grown by *JM101/S* cells when transferred to the *JM101/S* fermenter. In contrast *JM101/S* biofilms were more stable in the *HMS/S* culture than when transferred after only 10h of primary colonisation. Increased retention of the *E. coli JM101/S* cells by the biofilm can be interpreted in terms of a change in the association between cells and surface from reversible to irreversible attachment.

EM 36 Biofilm formation and control: natural wetlands and reed beds used for treating stream water polluted with lead and copper

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Heavy metal pollution in urban streams is a problem in historic mining areas. Passive treatment with natural wetlands is gaining importance. The efficiency of commercially used reed beds can theoretically be improved by introducing additional layers of adsorption filter media including granular activated carbon, charcoal and burned clay. The aim of this study was to investigate biofilm formation during the set-up phase of wetlands dominated by *Phragmites australis* for the treatment of lead and copper and to develop a mathematical control model based on indicator micro-organisms. The main indicator groups include free swimming and flagellated protozoa, diatoms, actinopoda and invertebrates. The interactions between environmental control variables, microbial composition on different filter media and microbial influence on the reduction of biochemical oxygen demand, lead and copper were investigated. The model belongs to a predictive saprobic scheme used to optimise the overall filtration performance. It was found that low copper and lead concentrations do not inhibit microbial growth as much as expected due to the formation of an active biofilm which functions as a buffer during toxic shocks. Furthermore, a one-way analysis of variance revealed that additional adsorption filter media have no significantly positive effect on the filtration performance of mature filters.

EM 37 The influence of homoserine lactones on biofilm formation and detachment

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There is much debate about the involvement of *N*-acyl homoserine lactones (HSLs) in regulating bacterial biofilm physiology. In this study we have examined the role of cell-cell signalling on the attachment and detachment of *Pseudomonas fluorescens* and *P. aeruginosa* to glass surfaces. Biofilm formation was quantitatively assessed by vertically clamping glass coverslips, through the air-liquid interface of cultures (55ml) held within 600 ml beakers. Slips were periodically removed, washed in saline (0.9%), dried, fixed in cetylpyridinium chloride (10mM), air dried, stained with Coomassie Blue R-250 (0.1%) and transmission images obtained using a BioRad GS-670 densitometer. Analysis of the images enabled quantification of the extent of biofilm formation across the interface. *P. fluorescens* biofilms were maximal ca. 20-50h after inoculation and contained large numbers of cells enveloped within extensive exopolymetric (EPS) matrices. Prolonged incubation led to reduction in biofilm, which could be related to a loss of EPS. Significantly, biofilm formation was more rapid (10-20h), and occurred to a greater extent, when either cell-free supernatant from 2d cultures was used as the growth media or fresh media was supplemented with *N*-acyl-hexanoyl (C-6) HSL. Similar trends were observed with *P. aeruginosa* PA01 (HSL wild type), whereby maximal attachment occurred after 20-25h and collapse of the biofilm had occurred by 50h. By comparison, attachment of *P. aeruginosa* PA0-JP2, deficient in the C-12 dodecanoyl HSL and C-4 butanoyl HSL was slower (20-40h) and less extensive (67%) than for the wild type. Moreover, whilst biofilm collapse occurred some 50h later, it was not as catastrophic and spontaneously reformed after further incubation. These results suggest that the C-12 and C-4 HSL's are neither primarily or directly involved with attachment events, but may play a significant role in the regulation of detachment.

EM 38 Genetic link between 2,4-D and mecoprop degradation

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Abstract not submitted

EM 39 Inhibition of the growth of *Candida albicans* on denture acrylic and glass following exposure to urea

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An acidogenic denture plaque and an increased proportion of *Candida albicans* have been associated with denture stomatitis. An increase in plaque alkalinity may therefore reduce the potential pathogenicity of denture plaque. Urea is a major source of base production with the oral cavity. In order to determine any effect of increased urea availability on the prevalence of *C. albicans* in plaque, acidogenic and alkaligenic liquid media (containing glucose and urea respectively) were inoculated with whole plaque, to which *C. albicans* (9DH2346) (approximately 1.61×10^7 cells/ml) was added. Sterile samples of denture acrylic or glass were added to three tubes of each type of inoculated medium and after 48 hours anaerobic incubation, pH values of the media were determined. Adherent cells were removed from the glass or acrylic via vortexing and sonication for culture on sabourouds dextrose agar to obtain counts of *C. albicans*, 10 % blood fastidious anaerobe agar (FAA) for plaque total count, and FAA containing filter sterilised glucose and bromocresol purple as the pH indicator to enable determination of the acidogenic ratio. The end pH of 48 hr urea broth was >

pH 6.7 and the end pH of 48 hr glucose broth was < pH 4.2. Counts of *C. albicans* were expressed as a ratio of the total count and a lower percentage of the total count consisted of *C. albicans* following exposure to urea than sucrose for both acrylic and glass ($P < 0.01$). However subcultured plaque was more acidogenic following incubation with urea than with glucose for both acrylic ($P < 0.01$) and glass ($P < 0.001$). The total plaque and acidogenic counts from glass following exposure to glucose was significantly lower than following exposure to urea ($P < 0.1$) but there was no significant difference for denture acrylic. Difference in glass total counts could be as a result of exposure to the low pH of the sucrose broth. Although with subsequent carbohydrate challenge acidogenicity appears to be increased results warrant further longitudinal investigation. Results indicate a possible inhibitory role of alkalinity on the presence of *C. albicans* in plaque *in situ*.

EM 40 The role of fimbriae in the adherence of Salmonella serotypes to inanimate surfaces

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It may be argued that pathogens that pass down the food chain to cause human infection elaborate a number of adaptive responses to withstand and survive environmental stresses. A number of *Salmonella enterica* serotypes continue to be significant threats to human health and, whilst much is understood of the biology of these bacteria in the farmed animal host, adaptation to environmental stress is a theme of current active research. We are interested in the adherence and survival of *Salmonella* serotypes to both animate and inanimate surfaces. Recent studies showed that Enteritidis adherence to inanimate surfaces was mediated in part by two fimbrial types, SEF17 (curli) at ambient temperatures and SEF14 at 37°C. Curli are ubiquitous amongst *Salmonella* serotypes and the question arose whether this fimbrial type, and possibly other fimbriae, mediated adherence by other serotypes. All *S. Dublin* isolates tested were genetically competent to elaborate both curli and SEF14 but, with rare exceptions, failed to elaborate either and did not adhere to inanimate surfaces. Elaboration of curli fimbriae orthologues by *S. Typhimurium* and *S. Montevideo* was variable. Those isolates that expressed curli adhered to inanimate surfaces whilst isogenic non-curliated mutants did not. The role of other fimbriae was investigated by creation of knock-out mutants. Type-1 fimbriae have been cited as mediators of adherence to inanimate surfaces but *S. Montevideo* mutants unable to this fimbrial type adhered better than wild type. Type-1 mutants tended to elaborate more curli fimbriae which supported the concept of co-regulation of these fimbrial types.

EM 41 Single-strand-conformation polymorphism for comparison of PAH-degrading communities derived from enrichment and biofilm producing continuous-flow slide culture

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Microbial communities degrading polycyclic aromatic hydrocarbons were grown using a standard enrichment method and a novel continuous-flow slide culture (CFSC) method. In both cases, minimal salts medium (MSM) supplemented with naphthalene and phenanthrene was used and the same soil was the source of microorganisms. The standard enrichment procedure was conducted for 10 d. The CFSC biofilm was developed from an upstream flow-cell containing soil, allowing continuous inoculation for 7 d, prior to 14 d to allow for the formation of a PAH-degrading

biofilm. The resulting communities were isolated by serial dilution on solidified MSM supplemented with yeast extract and sodium salicylate, with naphthalene provided under constant vapour pressure. Genomic DNA was prepared from every single colony for both culture systems. Phylogenetic analysis was conducted using Com1 and Com2 universal primers, encompassing the V4 and V5 regions of 16S rDNA. SSCP profiles showed that there were 12 distinct bacterial species in the enrichment culture community and 36 in the CFSC community. Two fungal species were also contained in the CFSC community. Whole community single-strand-conformation polymorphism (SSCP) profiles (generated from RNA directly extracted from soil) were used as markers in phylogenetic analyses of the two communities. SSCP-based functional gene diversity, based on *nah*-like catabolic genes; naphthalene dioxygenase reductase; *nahAa*; naphthalene dioxygenase ferredoxin; *nahAb*; 2-hydroxynaphthalene-2-carboxylate isomerase; *nahD*; and naphthalene chemotaxis protein *nahY*, has been investigated and the results will be presented.

EM 42 Protozoan and metazoan microfauna in natural biofilm communities: Predators or players?

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Biofilm, removed from the horizontal pipe section of a domestic kitchen sink harbored a dense and diverse microflora composed of aerobic, facultative and strictly anaerobic bacteria. Microscopic examination revealed substantial populations of protozoa and nematode worms. Microcosm communities were established using constant depth film fermenters (CDFS) which were repeatedly inoculated with drain biofilm material. Pan depths were alternated between 0.3 mm and 5mm. For the first 48 h, anaerobic conditions were maintained by continuous gassing (5: 95: CO₂, N₂). Fermenters were maintained on a feast / famine regime using an artificial dishwasher. Whilst the shallow pans contained some strict anaerobes, such as SRBs, and facultative anaerobes, they were dominated by aerobic species (i.e. aeromonas and micrococci). Analysis of the shallow pans showed considerable variability in microbial composition that was in part attributed to predation by successions of protozoa, nematode worms and bdelloid rotifers (genus *Habrotrocha*). MPN analysis confirmed that each of these higher species achieved climax communities at different times in different pans. Deep-pan samples in the CDFS were reproducible and more closely reflected the species composition of the original samples. With respect to the domestic drain microfauna, preliminary prey-preference studies using bacterial species isolated from the microcosms demonstrated that although protozoa did not exhibit strong prey preference, the rotifers preferentially predated *Klebsiella oxytoca*. *Pediococcus pentosaceus* was a preferred prey for the nematodes although rotifers did not readily utilize this isolate. Investigations into stratification of the microfauna within deep biofilms suggested that although rotifers and protozoa were distributed throughout the depths of biofilm, nematode worms were confined to upper areas. Although these studies are at a preliminary stage, we hypothesise that the microfauna may play key roles in biofilm community architecture more than simply acting as non-specific predators.

EM 43 Impact of acetochlor on surface and subsurface bacterial community structure using Pcr-single-strand conformation polymorphism analysis

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The short term impact of the herbicide acetochlor (2-chloro-(2'-methyl-6-ethyl-N-ethoxymethyl)-acetanilide) on soil bacterial community structure was studied in a sand and a clay soil at three different depths. Over a period of 28 days,

DNA was extracted from soil and conventional plate counts were performed using copiotrophic and oligotrophic media. Universal 16S rDNA primers were used to amplify the extracted DNA. By performing nested PCR, the 16S rDNA PCR products were amplified using Com 1 and Com 2 eubacterial primers and changes in community diversity monitored by single-strand conformation polymorphism analysis. Further analyses of the impact of acetochlor upon distinct phylogenetic groups are being carried out using primers for several bacterial divisions (α -, β - and γ -proteobacteria, pseudomonads and actinomycetes) and shifts in the populations quantified by dot blot hybridisation. These data will be presented.

EM 44 Yeast biofilms: opportunities in nature and industry

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Because of their unicellular nature, yeasts are generally expected to prefer environments with higher moisture content than the filamentous fungi. As a result, yeasts have been extensively studied in aqueous habitats. Most of these studies focussed on the isolation and analysis of planktonic yeasts, and thus relatively little information is available on yeast biofilms in the environment. The importance of surface-associated growth among bacteria is well established; the goal of this study was therefore to assess the significance of biofilm formation by yeasts.

Application of standard culturing techniques to water samples from a natural stream and direct microscopy of stones and other sediments from the stream revealed that the total planktonic yeast count was typically less than 15 cells/ml, while the number of surface-associated yeasts was as high as 386 cells/cm². Eighty six percent of the latter was basidiomycetous red yeasts, a group frequently encountered in aquatic environments. Furthermore, analysis of slide cultures retrieved from the same stream in the vicinity of a local winery revealed >1000 yeast cells/cm². To further explore the ability of yeasts to form biofilms, computer assisted microscopy and flow cells were used in laboratory studies to evaluate biofilm formation by 23 yeast strains representing the genera *Bulleromyces*, *Candida*, *Cryptococcus*, *Debaryomyces*, *Dipodascus*, *Hanseniaspora*, *Lipomyces*, *Nadsonia*, *Phaffia*, *Rhodospiridium*, *Rhodotorula*, *Saccharomyces*, *Schizosaccharomyces* and *Zygozma*. Only two of the strains did not form biofilms. Among those that formed biofilms, great variation was observed in biofilm structure, cell density, susceptibility to high flow rates and biofilm thickness. The growth kinetics of cells growing in the same biofilm also showed great heterogeneity. For instance, the number of *Rhodotorula glutinus* cells doubled every 3 h, while in a neighbouring microcolony the average doubling time was 10 h. These results suggested that yeasts employ a variety of strategies during the formation of biofilms in natural environments. Subsequent observations of biofilm formation in winery effluents revealed extensive biofilm formation. A better understanding of yeast biofilms may therefore be important in degradative processes, such as the treatment of winery effluents.

EM 45 Ageing and the intestinal microflora

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The normal intestinal microflora plays an important role in gut function and the maintenance of host health, through the formation of vitamins and other beneficial substances, metabolic products such as short chain fatty acids, modulation of immune system reactivity and the

phenomenon of colonisation resistance. While development of the microbiota in infants is currently receiving much attention, the natural, and antibiotic-associated changes that occur in the microflora and its metabolic attributes during ageing are not well understood. This investigation compared the gut microflora in young healthy volunteers and elderly people, examining variations in bacterial populations by culturing with selective and non-selective agars and using bacterial cellular fatty acid composition for bacterial identification. These measurements were coupled with the use of fluorescently-labelled (FITC, Cy3, Cy5) 16S rRNA oligonucleotide probes in flow cytometric analysis of stool samples, which is currently being developed as a rapid analytical tool for compositional studies on the microflora. The results showed that while marked inter-individual variations occurred in microbiota composition, both ageing and antibiotic (e.g. amoxycillin, clindamycin, augmentin, flucloxacillin) treatment reduced anaerobic species diversity in the gut. These shifts in anaerobic populations were accompanied by increases in numbers of facultative anaerobes (enterococci, enterobacteria) and lactobacilli, which markedly reduces the functionality of the microbiota.

EM 46 The study of primary colonisation events during biofilm formation

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Abstract not submitted

EM 47 Carbohydrate metabolism by Streptococci: Effect on the growth of anaerobes

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The aim of this study was to investigate whether the end products of streptococcal carbohydrate metabolism had any effect on the growth of selected anaerobes associated with oral malodour. Oral malodour is caused by the metabolism of salivary glycoproteins by gram negative anaerobes found on the tongue, resulting in the evolution of volatile sulphur compounds. The fermentation of sugars such as glucose by oral microorganisms is known to inhibit malodour generation, probably by a combination of pH lowering and Eh raising. Carbohydrate (1%) containing media were inoculated with *Streptococcus mutans*, *Streptococcus salivarius*, *Streptococcus sanguis* and *Streptococcus mitis*. After 48h growth in a CO₂ incubator the surface was overlaid with molten fastidious agar (FAA) inoculated with selected anaerobes including *Porphyromonas gingivalis*, *Prevotella intermedia*, *Fusobacterium nucleatum* or *Peptostreptococcus micros* and plates were incubated anaerobically for 5 days. Inhibition of the growth of anaerobes due to products of streptococcal growth was observed. It is likely that acidic conditions are inhibiting anaerobic growth.

EM48 Stress resistance of *Listeria monocytogenes* and *Escherichia coli* O157:H7 after growth with the use of a new model biofilm: a 2-D electrophoresis study

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Biofilm bacteria, especially in food industries, often lead to serious economic problems due to spoilage or human diseases. Bacteria growing within biofilm differ from those of planktonic cells and are noticeably more resistant to cleaning and disinfection operations. The objective of the present study was to develop a simple and new method based on the use of a glass fibre filter to rapidly obtain biofilms available

for ecological or physiological studies. For biofilm development, the filter was deposited on a nutritive agar medium and incubated in well defined conditions. The resistance to H₂O₂, heat and NaCl, of two pathogenic micro-organisms, *Listeria monocytogenes* and *Escherichia coli* 0157:H7 was investigated during seven days. This model was also used to study bacterial attachment and aspects(SEM) during storage. The results showed that the model is very rapid and easy to set up and that bacteria in biofilms displayed enhanced resistance to stressing agents. 2-D electrophoresis was also carried out to study variation of global proteins expression in biofilm bacteria compared to planktonic cells in order to provide basic informations of global regulation genes. Comparison of proteins patterns obtained from planktonic and sessile cells showed that many spots were found to be new or modified in their level of expression.

TUESDAY 12 SEPTEMBER 2000

09.00 Engineering of efficient biosynthetic pathways to produce chiral pharmaceutical intermediates

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Bacteria have evolved highly efficient pathways for the biosynthesis of chiral molecules such as amino acids. In organisms such as *Escherichia coli* and *Bacillus subtilis* many of the key pathway enzymes and genes have been identified and well characterised. With increasing application of genomic sequencing and PCR methodology it is now possible to construct novel pathways in recombinant organisms, combining enzymes from diverse sources. In such strains, enzymes with appropriate characteristics can be used concertedly to efficiently synthesise commercially important compounds, such as unnatural amino acids.

We have engineered strains of *E.coli* to produce many unnatural or non-proteinogenic amino acids including L-tert-leucine, L-2-aminobutyrate, L-homophenylalanine and L-homoproline. These are synthesised by aminotransferases from keto acid precursors. Substrate biosynthesis, reaction yield and product isolation have been significantly enhanced by enzymes acting in concert with the aminotransferase. In the examples shown, L-tert-leucine and L-2-aminobutyrate are produced in very high yields using strains which combine the action of deaminase, aminotransferase and synthase enzymes. In addition a little chemistry can further augment the efficiency of the artificial biosynthetic pathway.

The use of inexpensive commodity chemicals such as L-threonine and L-lysine as starting materials, the high conversion of substrate to product and the ability to combine all the enzymes in a single immobilised bacterial strain renders this approach extremely competitive for the commercial production of these compounds.

09.35 Biosynthesis of D-phenylalanine in *Escherichia coli*

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Unnatural amino acids (UAAs), of which D-amino acids form a large group, are among the most prominent and diverse of the compound families occurring in the rational design of chiral drugs such as anti-cancer compounds and viral inhibitors. In common with the commercial manufacture of natural amino acids, both chemo-enzymatic resolution approaches and direct single isomer syntheses have been undertaken in the production of UAAs and their derivatives. At NSC Technologies we have developed and scaled up a number of biocatalytic reactions to produce commercial quantities of unnatural amino acids in high yield and enantiomeric excess. Here we will describe our efforts to engineer a fermentative route for the production of D-phenylalanine in *Escherichia coli*. The key enzymes used in this system are L-amino acid deaminase (L-AAD I) from *Proteus myxofaciens*, D-amino acid aminotransferase (DAT) from *Bacillus sphaericus*, alanine racemase from *Salmonella typhimurium* and alanine dehydrogenase from *Bacillus sphaericus*. These enzymes can

form a pathway, which efficiently converts L-phenylalanine into its D-isomer. We will also describe how, due to the broad substrate specificity of the L-AAD I and DAT enzymes, this basic system can be used to synthesise other D-amino acids in high yield and enantiomeric purity.

10.10 Application of fungal expression systems in *Aspergillus*

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Filamentous fungi have been used for production of industrial enzymes for decades due to their high secretory capacity and the successful development of submerged cultivation technology. For more than 10 years *Aspergillus oryzae* has been used at Novo Nordisk also for recombinant expression of fungal enzymes. During that period the host strain used for expression has been improved significantly by deletion of secreted homologous α -amylase and two proteases, leading to higher purity of the product in the fermentation broth and improved stability of a number of heterologous products. Besides, the strength of the applied promoter (TAKA) has been improved, making it easier to obtain high yielding transformants.

The advantages of using a recombinant system for production of fungal industrial enzymes include the possibility of producing individual components of fungal enzyme complexes (e.g. an endoglucanase from *Humicola insolens* is produced for detergent and textile applications), enzymes from fungi that produce unwanted metabolites can be used safely (e.g. a lipase from *Thermomyces lanuginosus*), and the expression level can be increased, making it economically sound to commercialise a broader range of different enzymes.

11.15 Recombinant yeast for production of biologicals

D. MEAD

Delta Biotechnology Ltd, Nottingham

Abstract not submitted

11.50 Prokaryotic biosynthetic fermentations – an overview

WILL COOK

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Metabolic control hormones such as insulin and human growth hormone (hGH) had traditionally been isolated from mammalian glands. However, the projected shortage of animal glands and the real risk of transmittable diseases prompted the development of a biosynthetic route. The first commercial production of a biosynthetic product (human Insulin) was made using a plasmid construct in a prokaryote (*E.coli*). This organism is well understood and has a fast rate of metabolism with simple and economic media requirements. A plasmid construct containing the genetic information for the required product and a suitable promoter is used to control synthesis. Fermentation can proceed in traditional large-scale fermenters but the fast rate of metabolism requires the process to be highly automated in order to achieve consistency and high yields. Appropriate, usually different, control strategies are used in the growth and production phases. It is important not to induce biosynthesis of product until the cell mass in the fermenter is maximised, as undesirable loss of product can occur due to the presence of the heterologous protein interfering with metabolism. Induction is controlled by a variety of chemical and physical techniques and is specific to the type of promoter used.

Quality Control of production is vital to achieve consistent targets of safety, purity and control, also serving to demonstrate to internal company and external Medicine Authorities auditors that the manufacturing process is under control. A Group 1 organism such as *E.coli* containing a plasmid construct has no specific biological safety hazards. However, containment of the organism from the environment has particular production benefits in, for example, the control of contamination. Although the metabolism of prokaryotes is limited to the production of simple peptides and proteins, successful efficacious products are being manufactured.

14.00 Stability of recombinant fermentations

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The advent of recombinant technology heralded a new era where man could selectively overproduce proteins of choice. Initially it was thought that through the use of molecular biology it would be possible to cheaply produce specific proteins on an industrial scale. The reality has been longer in the making due to a number of obstacles, one of which is the stability of such overproduction systems.

It was quickly discovered that these powerful expression systems were also often highly unstable. There was a strong selective pressure for strains which contained such systems to abolish, or down regulate production of the target protein. This became especially apparent as the number of generations the culture was required to go through, increased; for example in large scale production and/or continuous culture.

A number of different strategies (both physiological and genetic) have been developed, to stabilise production of recombinant protein, with varying success. These will be discussed together with their application to specific examples of large scale production.

14.35 Production of a recombinant vaccine to prevent Lyme disease

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With more than 10000 new cases reported every year Lyme disease (or "borreliose") is the most common arthropod-borne disease in the United States. It is also reported in Europe, Japan, China, Russia. The disease is caused by the spirochete *Borrelia burgdorferi* sensu lato that is transmitted via the bite of the tick *Ixodes*.

The Outer surface protein A (OspA) of *B. burgdorferi* has been identified as a candidate antigen for developing a prophylactic vaccine against Lyme disease. The gene was genetically cloned into *E. coli* AR58 strain under the control of thermosensitive lambda promoter. The recombinant strain was cultivated in fermentor using semi-synthetic medium and a glycerol-based fed-batch protocol. This process was showed to consistently result in biomass higher than 50 g DCW/L and OpsA productivity higher than 1 g per liter. The overexpressed antigen was found to accumulate under the form of a lipoprotein. Several aspects such as the feeding strategy, induction conditions, foaming control and process robustness were investigated and will be discussed in the presentation.

Preclinical and clinical studies have demonstrated the efficacy of an Osp-A containing vaccine. The vaccine, called "LYMERix(TM)", has received approval from the U.S. FDA in December 1998 and is now produced at the manufacturing scale and sold in the United States.

1510 The importance of fermentation optimisation in the production of recombinant plasmid DNA (offered paper)

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One of the major challenges in the production of plasmid DNA from *Escherichia coli* is its purification. In particular, host derived contaminants such as chromosomal DNA, RNA and endotoxin have to be reduced to safe levels before use in human gene therapy. The quality and quantity of the plasmid DNA produced may vary depending on the population growth rate and the environment in which cells are grown. These factors have a significant impact on the subsequent processing and purification of the product. Low specific productivity vectors (e.g. plasmids present at low copy numbers per cell) could potentially have relatively higher levels of host organism impurities. The objective of a fermentation optimisation program for plasmid DNA production is therefore to maximise specific yield (or plasmid copy number) not simply to increase the volumetric yield. Three potential fermentation strategies will be discussed, and the relative success of the subsequent downstream processing evaluated.

Firstly, batch culture in rich medium leads to rapid growth, with the risk that the culture's oxygen demand will exceed the aeration capacity of the fermenter. The rapid growth rate leads to an effective dilution of plasmid copy number, as well as eventual depletion of dissolved oxygen. One result of oxygen starvation is uncontrolled cell lysis, which causes problems during downstream processing. Thus the culture is normally harvested just prior to oxygen depletion.

Alternatively, allowing the culture to grow beyond the point of apparent oxygen depletion, using extended batch fermentation, a second, slower growth phase is seen. This slower growth phase allows time for the plasmid copy number to recover, but unwanted multimeric plasmids can be generated. Eventually, the dissolved oxygen is depleted, and the cells stop growing and lyse.

When cells are grown using a fed batch strategy, the growth rate is controlled lower than μ_{max} . This allows the plasmid copy number to keep pace with cell division and the culture's oxygen demand to remain within the aeration capacity of the fermenter. Thus, dissolved oxygen levels are maintained in slow growing cultures and therefore the cell density can continue to increase.

16.10 Increased productivity from industrial recombinant fermentations

JO JONES

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The selection of host and vector and the optimisation of fermentation conditions can have a profound affect on protein expression. There are many factors which can influence the production of soluble protein, both at the genetic level and during the subsequent evaluation process. In an industrial environment, it is this combination of factors that is critical to success, as the ultimate goal is the increased productivity of a high quality product in an ever decreasing time frame.

Our approach involves looking at all aspects of expression, starting at host and vector combinations to work around, for example, any stability or toxicity problems. Fermentation conditions are then optimised using preliminary studies in small scale runs, where many factors are varied, such as temperature and induction conditions. Having established a basic set of criteria that work for the preferred host/vector combination, studies at scale to further optimise the process begin. Online data

of the production conditions and off line analysis is then used to support selection of the final conditions, the aim being to develop a robust process that can be run on any plant efficiently and effectively.

16.35 Recombinant protein production in a pharmaceutical research environment

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The provision and use of recombinant proteins, as drug targets, is vital to the early stages of drug discovery. Recombinant target proteins are used in the drug discovery process for a wide range of purposes, which include low throughput bioassay, high throughput screening and biostructural studies (of drug-target complexes).

It is therefore crucial that these proteins can be produced with sufficient speed and with the required quality and quantity to meet the demand of multiple simultaneous research projects. A key consideration is that expression is not lost on scale up from flask to fermentor. Process optimisation for traditional fermentation tends to focus on abiotic factors eg temperature profiles, pH, agitation and aeration. Increasingly the choice of host strain, promoter system and insert optimisation are important areas for the successful expression of heterologous proteins.

This presentation will give an overview of how Roche Discovery Welwyn undertakes the steps from small scale expression to the production of larger quantities of target proteins.

POSTERS

FB 01 The use of near infrared spectroscopy to monitor key analytes in filamentous and unicellular bacterial fermentations both at-line and on-line

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The ability to monitor chemical and biological parameters within a bioprocess by on-line or *in-situ* means has the potential to produce significant improvements in process control. Near infra red spectroscopy (NIRS) is a rapid, non-destructive technique requiring no sample preparation, which can monitor concentrations of several chemical species simultaneously. Monitoring typical bioprocesses by means of NIRS is more challenging than for simple chemical reaction processes due to the complexity of the chemical matrix and to the pronounced changes in concentrations of reactants, products and by products with time. The application of NIRS for monitoring bacterial cultivations of both *E.coli* and *S.fradiae* was investigated. At-line models for various analytes in both process were successfully built using various mathematical and validation procedures. Spectral acquisition using steam sterilisable fibre optic probes was explored for all on-line work employing similar mathematical and validation procedures as with the at-line work in order to optimise the models. The incorporation of on-line NIRS in a bioprocess setting is highly desirable as it allows real time monitoring of biological parameters and is an important step in the application of this technique within an industrial setting.

FB 02 On-line and at-line monitoring of key analytes in recombinant cho cell and *Pichia pastoris* cultivations using near infra-red spectroscopy

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Monitoring of chemical and biological parameters in bioprocesses by on-line or *in-situ* means is highly desirable since it has the potential to produce significant improvements in process control. Near infra red spectroscopy (NIRS) is a rapid, non-destructive technique requiring no sample preparation, which can monitor concentrations of several chemical species simultaneously by an unskilled operator. Monitoring bioprocesses by means of NIRS is more challenging than for simple chemical reaction processes due to the complexity of the chemical matrix and to the pronounced changes in concentrations of reactants, products and by products with time. The application of NIRS for monitoring recombinant CHO cell and *Pichia pastoris* cultivations was investigated. At-line and On-line models have been built for key analytes within the CHO process while highly successful at-line models have been built for the *Pichia pastoris* system including the product, a heterologous protein, present at low concentrations. Spectral acquisition using novel steam sterilisable fibre optic probes was explored for all on-line work. Various chemometric and validation approaches have been used to optimise these models and assess their performance in practical situations. The incorporation of on-line NIRS allowing real time monitoring of biological parameters within a computer based expert system will also be presented.

FB 03 Physiological responses to nutrient limitation in the actinomycete *Micromonospora echinospora*

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The growth conditions and availability of nutrients strongly determine the physiological response of an organism to its environment. This response has been exploited commercially in the formation of microbial products such as secondary metabolites. Actinomycetes are amongst the most commonly isolated soil microorganisms and are diverse producers of pharmaceutically useful compounds. Many members of this group are able to differentiate morphologically and physiologically in response to nutrient availability. The genus *Micromonospora* is reported to produce representatives of practically every chemical family of antibiotics, yet despite their industrial potential, their physiology is poorly understood when compared with other actinomycete genera such as *Streptomyces*. In this study we have established conditions that are limiting for specific nutrients by adjusting the molar ratio of carbon and nitrogen. We have investigated the response of *M. echinospora* to carbon and nitrogen limiting conditions and present data regarding physiological and morphological responses and product formation in batch culture fermentations.

FB 04 The impact of plasmid pMALp2x on the growth characteristics of *Escherichia coli* with complex and defined media

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Serial batch culture for a total of 72 hours was used to establish the metabolic impact of plasmid replication on the

growth of host cells. Plasmid-free and recombinant *Escherichia coli* HB101 were grown with complex LB and defined M9/01 and M9* media. A consistent number of cell doublings / per growth cycle were observed during the growth of plasmid-free HB101 and recombinant HB101pMALp2x cultures with LB medium. A progressive decrease in cell doubling was observed during the serial growth of recombinant HB101pMALp2x cultures with defined media M9/01 and M9*. No such progressive decrease in cell doubling was recorded with plasmid-free HB101 cultures grown with defined media. This suggested that the replication of plasmid pMALp2x presented a metabolic burden to HB101 host cells. Further studies revealed that the impact of pMALp2x replication was not host cell specific, as a progressive decrease in cell doubling was recorded with several recombinant *E. coli* hosts, TB1, TOP10 and STBL2. Removal of the selection pressure, 100µM ampicillin, from recombinant HB101 cultures during serial batch culture for 72 hours with M9/01 resulted in a decline in plasmid copy number. However, a progressive decrease in cell doubling was observed, comparable to that recorded with HB101pMALp2x cultures grown with ampicillin. This observation suggested that the impact of plasmid replication on growth was not directly related to the plasmid copy number. The metabolic burden of recombinant protein expression on host cells is well documented. Our results indicate that plasmid replication is also a sufficient metabolic burden to alter the nutritional requirements of recombinant cultures.

FB 05 Bacterial community structure and their activity determined by *in situ* hybridization in tropical rivers

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Tropical rivers in South East Asia have been seriously polluted by recent urbanization and industrialization, and microbial ecology in tropical rivers must be studied. However, our current knowledge of bacterial community is limited especially in tropical zones. In this study, molecular biological and fluorescent staining techniques were applied to investigate bacterial community structure and their physiological activity in the Chao Phraya River basin (Thailand). (I) Fifteen to 30% of total cells could be detected by fluorescent *in situ* hybridization (FISH) with the probe EUB338 targeted for domain Bacteria. Combination of FISH with direct viable count approach (DVC-FISH) increased the fraction of bacteria detectable with EUB338 in the Chao Phraya River (73 to 80% of total bacteria). (II) *Escherichia-Shigella* group identified by DVC-FISH with the probe ES445 designed in this study accounted for 1 to 9% of total bacteria in the Chao Phraya River. (III) Certain population detected by fluorescently labelled anti-*Escherichia coli* O157:H7 antibody technique (FA) could be enriched by R2A broth. Simultaneous identification by FISH and FA revealed that a part of this population belongs to *Escherichia-Shigella* group. These methods should be applied to assess risk of tropical waters to public health.

FB 06 On-line monitoring of key analytes in a recombinant *Pichia pastoris* fed batch process using a Virtual Fermentation Biosensor (VFS)

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Real-time information on key biological and chemical variables in a bioprocess can lead to significant

improvements in process control. A novel approach of using existing routinely logged parameters within a bioprocess, to determine the concentrations of other key analytes on-line by neural network technology was investigated using a recombinant *Pichia pastoris* fed batch process. The Virtual Fermentation Biosensor (VFS) is a novel software product giving an on-line reading for key variables in a bioprocess e.g. biomass, substrate and product, whose analyses are normally time consuming, labour intensive and costly. VFS calculates this reading from the available online measurements. To do this, the software uses neural network technology combined with other algorithms. The neural networks are multi-layer perceptrons, trained using various methods including back propagation of errors. The training requires several batches of data from previous runs of the bioprocess to be considered. The available online measurements in the *Pichia pastoris* system were pH, temperature, dissolved oxygen tension, stirrer speed, volume of base and acid additions. Various combinations of these were used as inputs to the system. The VFS output produced an online reading for key analytes within the system. Models developed to date using this technique will be presented.

14.05 Filament formation by *Salmonella* spp. under adverse conditions

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Abstract not submitted

14.20 A single-tube real-time nested PCR for detecting the genomes of human papillomaviruses

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A real-time PCR using the LightCycler[®] was developed for detecting human papillomaviruses (HPVs) of different types with the same sensitivity as in a commonly used nested PCR on a block-based thermocycler. The use of MY09/MY11 (degenerate) and GP5+/GP6+ (consensus) primers, significant adjustments made to the reaction conditions, and the use of anti-Taq antibody in the reaction mix have allowed sequential amplification and detection of PCR amplicons by fluorimetry in a closed single-tube reaction. HPV types 1A, 2, 3, 5-8, 10, 11, 14, 16, 18, 20, 27, 31, 33, 49, 50, 57, 58, 62 and 66 could be detected by both PCR methods. The PCR on the LightCycler[®] was performed in 90 min, whereas conventional PCR, including gel electrophoresis, took more than 10.5 hours. The real-time PCR reduces the risk of contamination often associated with nested PCR, permits a greater throughput of samples and is thus an excellent tool to investigate the molecular epidemiology of HPVs.

14.35 Intracellular activation of the *spv* and *dps* promoters in *Salmonella typhimurium*

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We have studied the activation of two growth phase-regulated and intracellularly-induced promoters, *spv* and *dps*, both during *in vitro* culture and within J774A.1 murine macrophages. Reporter plasmids were constructed from the pQF50 vector which directed co-transcriptional expression of β -galactosidase and green fluorescent protein (GFP) from *spv* (pQF50*spv*) or *dps* (pQF50*dps*). These constructs were independently introduced into wild-type strains *S.typhimurium* SL1344 and HWSH, and into the vaccine strain *S.typhimurium* SL3261 (*aroA*⁻). β -galactosidase expression was induced 20-fold and 100-fold when broth cultures of SL3261 (pQF50*dps*) or SL3261 (pQF50*spv*) respectively, entered stationary phase. Within J774A.1 cells, β -galactosidase expression was induced 3.5-fold with SL3261 (pQF50*dps*), and 7-fold with SL3261 (pQF50*spv*). Expression of GFP by *S.typhimurium* HWSH (pQF50*spv*) was shown to be more heterogeneous amongst a given bacterial population in comparison with *S.typhimurium* HWSH (pQF50*dps*) during stationary phase and within J774A.1 cells. The *spv* and *dps* promoters were used to drive independent expression of the C fragment domain of tetanus toxin (TetC) from plasmids harboured in *S.typhimurium* SL3261. Levels of anti-TetC antibodies were significantly higher in the sera of BALB/c mice perorally inoculated with SL3261 (pSpvtetC) or SL3261(pDpstetC) compared to unvaccinated controls, with higher anti-TetC antibody titres

being recorded for SL3261 (pDpstetC) six weeks post-inoculation.

14.50 Evidence for a genetically stable clone of *Campylobacter jejuni*

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The genetic stability of selected epidemiologically-linked strains of *C.jejuni* during outbreak situations was investigated using AFLP. Strains isolated in 1998 were investigated from chickens and the environment around three broiler houses which were geographically related. There was little similarity in strains between outbreaks. However, the strains from within all three chicken outbreaks, including strains isolated from the house floor, feed lines, and chicken faeces, were identical at over the 95% level of similarity confirming the genetic stability of these strains within the short time courses of chicken flock outbreaks. Strains were also investigated from a human outbreak, in 1981, thought to be due to contaminated water (Palmer *et al.*, 1983). Three AFLP profiles were recognised from this outbreak confirming the serotyping undertaken at that time. The major type (serotype P6;L6) isolated from this outbreak was exemplified by strain 81116. This isolate is a well-characterised laboratory strain. Nevertheless the AFLP profile of strain 81116 was identical with all the other P6;L6 strains from the outbreak indicating that it has remained remarkably stable over almost 20 years despite being subtyped on many occasions. Interestingly the AFLP profiles of the P6;L6 strains from the human outbreak and the strains from one of the chicken outbreaks were identical at the 94% level. This similarity is also remarkable and suggests that some clones of *C.jejuni* remain genetically stable in completely different environments over extremely long periods of time and considerable geographical distances.

15.05 The identification of genes required for growth of *Streptococcus uberis* in milk

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Infection with *Streptococcus uberis* accounts for around 33% of all cases of bovine mastitis in the UK. This bacterium is auxotrophic for a number of amino acids, yet grows in an environment in which the availability of free amino acids and peptides is growth limiting. *S. uberis* is capable of activating bovine plasminogen to the caseinolytic enzyme plasmin, and can utilise peptides derived from bovine caseins by the action of plasmin. The link between the utilisation of these peptides and the ability to grow in milk and/or the bovine mammary gland has not yet been established. A mutant strain of *S. uberis* 0140J, unable to utilise an essential amino acid from a beta-casein derived oligopeptide, was shown to contain a lesion within a gene which showed homology to an oligopeptide permease gene (OppF) of *Streptococcus pyogenes*. Growth yield of the mutant strain in skimmed milk was 10-fold lower than that of the wild-type, but was restored in the presence of amino acids.

To determine further genes required for growth of *S. uberis* in milk, mutant strains were isolated directly by their altered ability to grow in milk. Three strains were found which failed to grow in milk, and a further three isolated which achieved a final growth yield ten-fold lower than that of the wild-type strain. Growth of the latter three strains was restored by the addition of amino acids. The mutant strains

have been characterised at the genetic level and the subsequent role of their mutated genes during growth of *S. uberis* 0140J in milk determined.

15.50 A functional link between the actin cytoskeleton and lipid raft domains during influenza virus budding

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Influenza virus produces virions of two distinct morphologies: pleomorphic spheres with an average diameter of around 100 nm, and filamentous particles of the same diameter but with lengths of up to several μm . The mechanisms underlying the budding process remain largely uncharacterised, but previous work has shown that production of filamentous virions from polarised epithelial cells can be blocked by the actin-disrupting drug cytochalasin D (Roberts and Compans, 1998). In addition, it has been suggested that influenza virions bud from specialised domains of the plasma membrane known as lipid rafts (Schieffele et al 1997). To further understand the mechanism of virus budding we have studied the effect of jasplakinolide, a drug which inhibits actin depolymerisation, on the production and morphology of filamentous virus using atomic force and confocal microscopy in conjunction with a GFP-M1 construct. Jasplakinolide effectively blocked the production of filamentous virus particles, and instead caused the formation of annular structures on the cell surface which contained HA, M1 and NP. Aggregates of β -actin were found underneath and in the interior of these ring-like structures. Consistent with the hypothesis that the virus buds from lipid raft domains, viral filaments and the drug induced ring structures were resistant to extraction with TX-100 at 4°C. Furthermore, both viral filaments and the annular structures contain DAF, a cellular raft-associated protein. Mevastatin, a drug which inhibits cholesterol biosynthesis, also inhibits filament formation. Upon staining for β -actin, we found that changes in the distribution of this protein reflect changes in HA distribution and filament formation. We propose that an intact actin cytoskeleton is necessary to maintain the correct organisation of raft domains for filamentous influenza virion formation.

16.05 HSV-1 Latency in the central nervous system

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Herpes simplex virus type-1 (HSV-1) establishes a lifelong latent infection in nerve cells. During latency a single region of the viral genome remains transcriptionally active, giving rise to the latency associated transcripts (LATs), the expression of which is driven by the latency associated promoter (LAP). The LAP can also be used to drive the long term expression of heterologous reporter genes suggesting that HSV-based vectors may have therapeutic potential as vehicles for gene delivery to the nervous system. However, the kinetics of LAP activity, and its ability to function equivalently in all neuronal cell types and sites within the CNS has not been studied in detail. In order to address these issues, mice were infected via the ear pinna with 2×10^6 pfu of either SC16-L β A, which contains an IRES-linked LacZ gene under the control of LAP, or SC16-C3b, which expresses LacZ under the control of the CMV-IE promoter. 3-5 animals from each group were studied over a time course from 5 days to 1 year post-infection (pi), and brainstem and spinal cord sections were examined histochemically for LacZ expression. CNS neurones were labelled by the CMV-IE promoter from 5-15 days pi only, indicating the regions of the CNS accessed by the virus at acute time points. The LAP was active in identical sites in the CNS from 7-34 days pi, but after 34 days the numbers of LAP-labelled cells decreased and labelling was predominantly detected in facial, hypoglossal and spinal motoneurones. These results suggest that following the establishment of latency in the CNS, the efficiency of long-term LAP activity is influenced by the neuronal cell type in which latency is established.

16.20 The use of site-directed mutagenesis to increase the substrate range of cyanide hydratase

L. NOLAN

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Abstract not submitted

16.35 Detection of Verocytotoxigenic *Escherichia coli* using a PCR/DNA probe membrane based colorimetric detection assay.

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Verocytotoxigenic *Escherichia coli* (VTEC) have emerged as highly virulent food poisoning pathogens capable of causing severe gastrointestinal illness in humans. Human infection is associated with a wide range of clinical manifestations including asymptomatic shedding, non-bloody diarrhoea, haemorrhagic colitis (bloody diarrhoea) and haemorrhagic uraemic syndrome (HUS). Mortality rates associated with HUS vary from 3-5% and patients who recover may have permanent kidney damage requiring dialysis or transplants. Such high morbidity and mortality, along with the number of VTEC cases in Ireland increasing from 8 in 1996 to 76 in 1998 make VTEC a priority for public health. Thus, there is an increasing demand for improved diagnostic procedures for the detection of VTEC. In this study, we have employed a multiplex PCR described by Paton & Paton, 1998, with some modifications for the identification of virulence markers in VTEC. The multiplex PCR comprises two assays; assay 1 utilises four PCR primer pairs and detects the presence of *stx 1*, *stx 2*, *eae A* and enterohaemorrhagic *hly A* genes. Assay 2 amplifies specific regions of the *rfb* (O-antigen encoding) genes of *E. coli* serotypes O157 and O111. Specific DNA probes were designed for each of these genes and were adapted and incorporated into a colorimetric reverse hybridisation membrane based detection format. The specificity of the combined PCR and colorimetric reverse hybridisation assay was established by testing a range of isolates of both clinical and animal origin. The combination of PCR and DNA probe colorimetric membrane based detection avoids the need for post PCR gel based analysis, which can be cumbersome. This multi-parameter detection technology can accommodate the addition of DNA probes for new markers of toxigenic strains as they are discovered enabling the identification of a large number of VTEC types and associated virulence determinants in a single detection reaction.

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EM49 The use of mild detergent blends in the detection of biofilm bacteria

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Hygiene assessment in food processing is currently based on conventional cultivation techniques using swabbing or contact plates. These classical evaluation methods suffer from serious deficiencies in detaching bacteria growing in biofilms on process surfaces. In the cultivation of biofilm cells it is important to detach and mix the sample properly. Excessively vigorous agitation in the detachment of the biofilm from the surface may harm the cells so that they are not able to grow on the agar, whereas insufficient mixing may result in clumps and inaccurate results. Various substances are known to improve the detachment of biofilms. Cations, calcium in particular, may bind to the polymer molecules in the biofilm and strengthen the bonds of the polymer layer. The removal of these cations with chelating agents, e.g. EDTA, can thus increase biofilm detachment. Surfactants are often used in cleaning procedures as they have a good rinsing effect, which means that these agents help in the detachment of particles to be flushed from the surface. Surfactants may also enhance the removal of biofilms by dispersing and flocculating biofilm components so that the various structural components are kept apart and not redeposited back onto the surface. The aim of this study was to investigate whether the detachment of bacteria from surfaces could be enhanced by using mild chemical blends (combinations of chelating agents and detergents) for wetting *Pseudomonas fragi*, *Listeria monocytogenes* and *Bacillus cereus* biofilm surfaces before sampling. The bacteria in the biofilm were quantified using cultivation as well as microscopy before and after swabbing of wetted stainless-steel surfaces.

EM50 Interactions between *Acanthamoeba polyphaga* and the intracellular pathogens *Salmonella typhimurium* and *Listeria monocytogenes*, a study of host-parasite and spatial population dynamics

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Reports of several intracellular bacterial pathogens replicating and/or surviving within protozoa, particularly *Acanthamoeba* spp. are leading to the hypothesis that this type of interaction is widespread in the environment. This study examines interactions of *A. polyphaga* CCAP1501/18 with *S. typhimurium* SL1344 and *L. monocytogenes* DP184, which are respectively intra-vacuolar and intra-cytoplasmic replicators in human macrophages. Infectivity experiments with wild type and *gfp* constructs failed to indicate intracellular replication in either species. A *L. monocytogenes* reporter construct illustrated transient intra-vacuolar *mpl* expression in >0.1% of amoebae. *Mpl* is a bacterial metalloprotease that on acidification processes the proform of bacterial phospholipase C (proPC-PLC) to active PC-PLC, which is used to escape double membrane vacuoles in cell-cell transfer in humans. A study of *A. polyphaga* and *S. typhimurium* biofilm dynamics is currently being undertaken using time lapse photomicrography and image analyses techniques. Preliminary results are discussed.

