society for general

Microbiology

AUTUMN MEETING
6–9 September 2010

University of Nottingham
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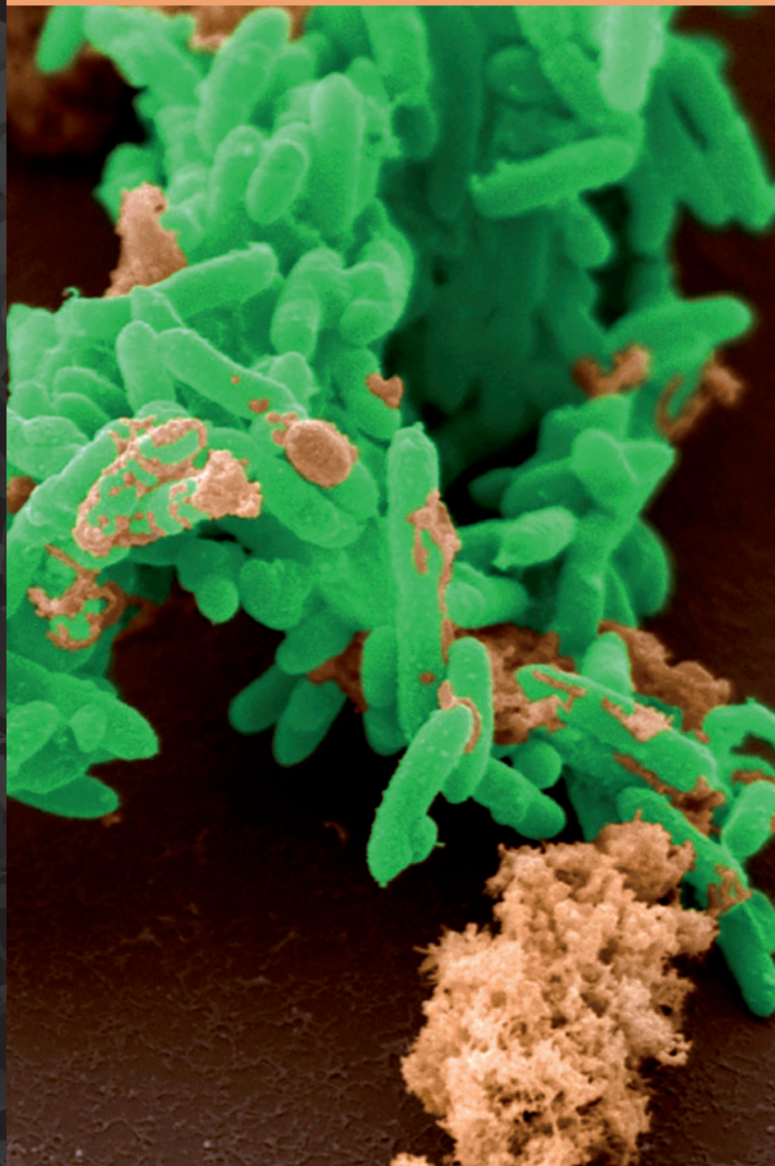
ABSTRACTS



society for general microbiology

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Autumn Meeting 6–9 September 2010

University of Nottingham – www.sgmnottingham2010.org.uk

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Hot Topic Lecture

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Building a cell controlled by a synthetic genome

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Starting from a bacterial genome sequence stored as a computer file, we made intentional design changes to the sequence, chemically synthesized it, and brought it to life by transplantation into a recipient cell, to create a new strain of bacterial cells, *Mycoplasma mycoides* JCVI-syn1.0. These cells are capable of continuous replication, and have expected phenotypic properties. The only genetic information they contain is the synthetic chromosome, including all designed identifying watermark sequences, gene deletions, and polymorphisms. After approximately 20 doublings, every macromolecule in a progeny cell will be a product specified by the synthetic genome. For this reason we like to refer to a cell controlled by a synthetic genome as a 'synthetic cell'. In the course of this work methods have been developed for the construction, cloning, and installation of large DNA assemblies. It is possible to assemble segments of natural or synthetic DNA up to several hundred kilobases enzymically *in vitro*. Natural and synthetic DNA greater than 1 Mb can be assembled and cloned by natural recombination in yeast. These methods will facilitate experimental definition of the minimal genetic requirements for cellular life, and should aid in the design of new bacterial strains with useful properties.

Outreach Prize Lecture

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More than a gut feeling

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From flatulence to bloating, constipation to diarrhoea, everyone can relate to gut feelings – but how many people know the science behind what is happening? Previous approaches of gut microbiology informing have included flatus collecting cowboys in Colorado and artichoke eating children in Essex. This presentation will look at ways of communicating microbiology to non-scientists, the use of media for getting messages across and also for challenging existing perceptions.

Peter Wildy Prize for Microbiology Education Lecture

↑CONTENTS

How the mushroom got its spots and other stories

Sue Assinder

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Talking to the public about science was once an activity conducted by a few committed eccentrics under the radar of the normal academic 'day job'. It is now a legitimate enterprise supported by a framework of national and international science centres and festivals, and is endorsed by funding bodies and university management alike. When I first became involved around 15 years ago, few people outside of a lab had heard of DNA and science communication was working to a 'deficit model' (with the unfortunate acronym of 'PUS') of increasing public understanding of science by imparting information. Now DNA is part of everyday vocabulary and the focus is on engaging the public as active participants. My enthusiasm for communicating science has taken me from primary schools and draughty village halls to the Royal Show and the House of Lords. It has affected whole aspects of my life – I can no longer cut up an onion without wanting to extract its DNA, nor can I think of body glitter as anything other than a model for the cold virus in mucus. In my talk I will recall the fun I have had as one of the early eccentrics and give a personal perspective on the changing agenda for science communication.

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NT01 Metals & microbes

Uptake and reduction of iron in *Escherichia coli*

Nadia Abed, Mohan B. Rajasekaran, Kim A Watson, Helen A. Goodluck, Jieni Cao, Ana Rangel & SIMON C. ANDREWS

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The EfeUOB system is a new type of bacterial iron transporter that is relatively common within bacteria (561 bacterial EfeU homologues in the Pfam database) and is clearly distinct from previously identified systems. In *E. coli*, it is active aerobically, utilizes ferrous iron as substrate but is cryptic in the K-12 strain. As expected, the *efeUOB* operon of *E. coli* K-12 is induced by iron restriction, but its expression responds more strongly to acidity than to iron. Thus, EfeUOB acts as a low pH, ferrous transporter. We have identified an extracellular reductase (Efr) that is an inner membrane cytochrome. Efr activity is repressed by iron and Fur, and the *efr*-promoter region is associated with an Fur-binding site. It is possible that Efr provides ferrous iron for uptake by EfeUOB. However, *efr* is strongly induced by alkalinity (rather than acidity) and indeed the *efr* mutant exhibits sensitivity to alkalinity. These findings suggest a potential role for Efr in maintenance of the reduction status of the cell envelope at high pH.

Comparative genomics of trace element utilization

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Trace elements are used by all organisms. They provide proteins with unique coordination, catalytic and electron transfer properties and are used in a variety of cellular systems. Although many trace element-containing proteins are well characterized, comprehensive sets of such proteins in organisms are not known. Also, little is known about the utilization of some trace elements by organisms as well as general trends in trace element utilization. We carried out comparative genomic analyses of trace elements in hundreds of sequenced organisms at the level of (i) transporters and transport-related proteins, (ii) cofactor biosynthesis traits, and (iii) trace element-dependent proteins, providing a first glimpse on the utilization of trace element at the genome-wide scale. These analyses also characterized trace element metabolism in common model organisms and suggested new model organisms for experimental studies of individual trace elements. Mismatches in the occurrence of user proteins and corresponding transport systems revealed deficiencies in our understanding of trace element biology. Biological interactions among some trace elements were observed; however, such links were limited, and trace elements generally had unique utilization patterns. Finally, we developed a database that features transporters and user proteins for five trace elements in sequenced organisms from the three domains of life. Overall, these data provide insights into the general features of utilization and evolution of trace elements.

Ancient and modern environmental pressures shape biological metal utilization

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The fundamental chemistry of trace elements dictates the molecular speciation and reactivity both within cells and the environment at large. All of life exhibits the same size-dependent scaling for the number of metal-binding proteins within a proteome. This fundamental evolutionary constant shows that the selection of one element occurs at the exclusion of another, with the eschewal of Fe for Zn and Ca being a defining feature of eukaryotic proteomes. Early life lacked both the structures required to control intracellular metal concentrations and the metal-binding proteins that catalyze electron transport and redox transformations. The development of protein structures for metal homeostasis coincided with the emergence of metal-specific structures, which predominantly bound metals abundant in the Archean ocean. This promoted the diversification of emerging lineages of Archaea and Bacteria through the establishment of biogeochemical cycles. Structures binding Cu and Zn evolved much later, providing further evidence for environment influencing metal utilization. The late evolving Zn-binding proteins are fundamental to eukaryotic cellular biology, and Zn bioavailability may have been a limiting factor in eukaryotic evolution during the Proterozoic. In the modern ocean, Fe isdrawn down to vanishingly low levels, and Bacteria have numerous methods for replacing or minimizing Fe utilization.

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Redox-dependent copper trafficking: from eukaryotes to bacteria

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Copper is an essential but potentially harmful trace element required in many enzymatic cellular processes. Several copper binding proteins are part of a complex cellular machinery devoted to correctly deliver and insert copper ions into the copper enzymes. The characterization of the structural and metal binding properties of these accessory proteins as well as of their copper exchange mechanisms is a key element to understand the enzyme assembly process at the molecular level in both eukaryotic and prokaryotic organisms. Thanks to this comparative analysis, we found that, at variance with what it occurs in the cytoplasm, cysteine-based redox chemistry can be operative in the intermembrane space of mitochondria as well as in the periplasm of bacteria during the copper exchange mechanisms between these proteins. Living organisms can have developed efficient and specific mechanisms of copper incorporation into copper enzymes, capable of handling more complex redox scenarios of the cell.

Maturation of multicopper oxidases in the Golgi – the chloride connection

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Ion gradients across intracellular membranes contribute to the physicochemical environment inside compartments. CLC anion transport proteins that localize to intracellular organelles are anion/proton exchangers involved in anion sequestration or vesicular acidification. By homology, the only CLC protein of *Saccharomyces cerevisiae*, Gef1, belongs to this family of intracellular exchangers. Gef1 localizes to the late Golgi/prevacuole and is essential under iron limitation. In the absence of Gef1, a multicopper oxidase involved in iron uptake, Fet3, fails to acquire copper ion cofactors. The precise role of the exchanger in this physiological context is unknown. Here we show that the Gef1-containing compartment is adjusted to a more alkaline pH under iron limitation. This depends on the antiport function of Gef1 since an uncoupled mutant of Gef1 (E230A) results in the acidification of the lumen and fails to support Fet3 maturation. Furthermore, we found that Gef1 antiport activity correlates with marked effects on cellular glutathione homeostasis raising the possibility that the effect of Gef1 on Fet3 copper loading is related to the control of compartmental glutathione concentration or redox status. Mutational inactivation of a conserved ATP-binding site in the cytosolic cystathione β synthetase domain of Gef1 (D732A) suggests that Gef1 activity is regulated by energy metabolism.

The role of Mzm1 in maintenance of mitochondrial zinc and complex III biogenesis

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Zinc is essential for function of mitochondria as a cofactor for several matrix zinc metalloproteins. A labile Zn(II) pool exists in the mitochondrial matrix of yeast for metallation reactions. We identified a series of proteins that are important in the maintenance of the matrix zinc pool, one of which is a novel matrix protein designated Mzm1. Mutant *mzm1* null cells have reduced total and labile mitochondrial zinc and these cells are hypersensitive to perturbations of the labile pool. In addition, *mzm1* cells have a destabilized cytochrome *c* reductase (Complex III) without any effects on Complexes IV or V. The mutant cells are blocked in the biogenesis of Complex III and accumulate an assembly intermediate containing all subunits except the Rieske Fe/S subunit. Cells lacking a second Complex III assembly factor Bcs1 stall at a similar stage of Rip1 insertion. The *mzm1* mutant cells show a dramatic enhancement in the accumulation of the Bcs1 AAA ATPase and the accumulated protein is complexes to the Complex III assembly intermediate. Thus, Mzm1 functions in conjunction with Bcs1 in a final step of Complex III assembly with the insertion of Fe/S subunit Rip1. A functional Complex III is necessary for the maintenance of the labile Zn(II) pool in mitochondria. The link between Complex III and mitochondrial Zn(II) will be discussed.

Don't shoot the messenger! Metal compounds as bearers and targets of carbon monoxide

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Metal centres in biology are classical targets for carbon monoxide (CO), the most familiar examples being terminal respiratory oxidases and haemoglobins. CO is an anthropogenic poison with severe health effects on Man. However, CO is also generated endogenously by haem oxygenases, and its extraordinary biological activities are now recognized. Recent advances in CO biology and chemistry have

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opened up exciting possibilities for understanding and exploiting its biological consequences. Microbiological interests in CO have been mostly restricted to CO oxidation, CO sensing mechanisms, and the use of CO as a haem ligand in microbial biochemistry but, more recently, CO toxicity and the microbial stress responses. An important advance in recent years has been the development of CO-releasing molecules (CORMs) for experimental administration of CO as an alternative to the use of CO gas. CORMs have therapeutic potential in vascular disease, anti-inflammatory effects, CO-mediated cell signalling in apoptosis, applications in organ preservation, and as antimicrobial agents, particularly in the control of bacterial infections. Many CORMs are metal carbonyl compounds, including CORM-3 [Ru(CO)$_3$Cl(glycinate)]. Here, recent experimental data on the antimicrobial activities of CORM-3 are presented. CORM-3 is a potent inhibitor of bacterial growth and respiration; the compound penetrates bacterial cells and delivers CO to biological targets. The effects include not only the binding to ferrous respiratory oxidases, but global changes in the transcriptome affecting genes for respiration, metal homeostasis and other functions. Future prospects are suggested and unanswered questions posed.

Metal-induced oxidative stress and iron–sulfur proteins in yeast

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The molecular targets of metal toxicity are generally not well characterized. Oxidative stress is a commonly cited cause of metal action. Therefore, it might be anticipated that metals and oxidants share common toxicity mechanisms. The redox activity of metals like Cu, Cr and Fe can account for their catalysis of oxidative stress. Other metals like Cd may perturb antioxidant defence systems, lowering the cell's capacity to deal with reactive oxygen species. The reversal of methionine oxidation by methionine sulfoxide reductases (MSRs) is a well conserved antioxidant defence mechanism. MSRs protect cells from oxidative stress and, we show in the yeast model, Cu toxicity. This action of yeast MSRs centres on a novel role in helping to preserve the integrity of Fe–S clusters, major targets of oxidative stress. Thus, Cu toxicity in yeast can involve an action on Fe–S proteins. Furthermore, this action centres on an extra-mitochondrial Fe–S protein that is also susceptible to stress associated with other metals and pro-oxidants. The work highlights novel and common mechanisms involved in the toxic action of diverse pro-oxidants.

Oxidative stress and metalloenzymes

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The adventitious autoxidation of redox enzymes ensures that aerobic organisms continuously form intracellular superoxide and hydrogen peroxide. The endogenous superoxide is constrained to non-toxic levels by the actions of superoxide dismutases and reductases, while hydrogen peroxide is scavenged by peroxidases and catalases. However, when hydrogen peroxide is present in microbial environments, it flows across membranes and into the cell, where it may accumulate to concentrations that exceed the capacity of basal defenses. When *E. coli* encounters this situation, the OxyR stress response activates several strategies that protect the organism. Hydrogen peroxide is especially hazardous for iron-containing enzymes, and a key element of the OxyR response is the Suf system, which takes over the synthesis and repair of iron-sulfur enzymes. Peroxide also threatens mononuclear iron enzymes. They are protected by the concerted import of manganese and sequestration of iron, which together ensure that the vulnerable iron atoms in these enzymes are replaced with non-reactive manganese.

Iron and copper acquisition in the fungal pathogen *Candida albicans*: an intimate partnership

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Candida albicans is an opportunistic pathogen of humans, existing in yeast and hyphal forms. Both copper and iron are essential for *Candida* to grow, but levels of these metals are tightly regulated in order to avoid toxic effects to the cell. We have identified surface reductase genes, *CaFRE7 and CaFRE10*, both of which code for iron and copper reductase activities. There is a copper transporter, encoded by *CaCTR1* and an iron transporter, enoded by *CaFTR1*. The FTR1 transporter is complexed with multi-copper oxidase proteins, with copper being essential for iron uptake. Therefore the processes of iron and copper acquisition are inextricably linked. We have also identified a gene *CaMAC1* , that encodes a transcription factor, and demonstrated that this transcription factor regulates several genes in response to copper levels. The CAMAC1 regulon includes genes involved in both iron and copper uptake (eg. *CaFRE7, CaFRE10, CaCTR1*); it also regulates itself. This emphasizes the intimate relationship between the processes involved in the acquisition of these two metals. In order to elucidate CaMAC1-mediated regulation we are investigating the protein/DNA and protein/protein interactions involved.

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Nickel, iron and urease: the acid-resistance triangle of pathogenic *Helicobacter* species

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The acidic gastric environment of mammals can be chronically colonized by pathogenic *Helicobacter* species (with *Helicobacter pylori* as the best known example). Nickel plays a key role in gastric colonization by *Helicobacter* species, as nickel cofactors the urease and hydrogenase enzymes. Both enzymes are essential colonization factors, with urease conferring acid resistance by urea hydrolysis, and hydrogenase allowing usage of microflora-produced hydrogen for energy production. Iron is also an essential metal for *Helicobacter* species, both for metabolism and for systems protecting against oxidative damage. Hence it is no surprise that *Helicobacter* species have intricate systems to both acquire sufficient nickel and iron to allow growth, while simultaneously preventing toxicity by uncontrolled nickel- and iron-acquisition. One interesting aspect is that nickel availability in the GI-tract is low, being mostly restricted to vegetarian dietary sources, and while this is no problem for *Helicobacter* species colonizing omnivores, it does present a challenge for *Helicobacter* species colonizing obligate carnivores. We have recently shown that these carnivore-colonizing *Helicobacter* species have adapted by expressing a second urease enzyme lacking nickel, demonstrating the intricate link between host, diet and pathogen evolution. In this overview the recent developments in *Helicobacter* urease, nickel- and iron-metabolism will be discussed.

Interplay between manganese and iron during pneumococcal pathogenesis

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Variation in transition metal ion concentrations may strongly influence pneumococcal virulence. The nasopharynx has a high level of available manganese, relative to iron, while in blood the opposite is the case. The response regulator RitR appears to be central to the response to iron in *S. pneumoniae*. A *ritR* mutant was defective in growth in medium with a high Fe/Mn ratio but was not affected when the ratio of Mn/Fe was increased. In a mouse model system the *ritR* mutant was able to colonize the nasopharynx but was unable to survive in lung or blood. Our results show that RitR is essential for the adaptation of pneumococcus to a high Fe/low Mn environment found in the lung and blood.

Antimicrobial metals – back to the future?

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Metals such as mercury, arsenic, copper and silver have been used in various forms as antimicrobials for certainly hundreds – if not thousands – of years. The discovery of antibiotics and new organic antimicrobial compounds during the twentieth century saw a general decline in the clinical use of antimicrobial metal compounds, possibly with the exception of silver for burns treatments. These 'new' antibiotics and antimicrobials were regarded as generally being safer to the patient and more effective antimicrobials than the metal-based compounds they supplanted. However, increasing concerns about antibiotic and multidrug resistance (MDR) in emerging and re-emerging pathogens, and new, and rediscovered uses for antimicrobial metals have promoted a recent upsurge in interest in their use in both clinical and non-clinical products. Metal ion resistance mechanisms have already been characterized in a wide range of bacteria, but how widespread are these resistances in emerging and re-emerging pathogens? This talk will examine the evidence for antimicrobial metal ion resistances in recent genome sequence data from emerging pathogens – suggesting that the resistance genes are already present in some of these organisms – and will address some of the possible consequences of resistance to antimicrobial metals in these bacteria.

Structure and function of Fur proteins from *Mycobacterium tuberculosis*

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The ferric uptake regulator (Fur) proteins form a diverse family of metal-dependent transcriptional regulator. Fur was originally identified in *E. coli* but homologues have been found in many Gram-positive and negative bacteria. Members of the Fur family control a wide

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range of genes not only associated with metal homeostasis but also involved in acidic and oxidative stress response. *Mycobacterium tuberculosis* the causative agent of tuberculosis expresses two Fur orthologues, FurA and FurB that share approximately 25% sequence identity. Over the last years these proteins have been characterized using a wide range of biological and biophysical techniques to unravel their biological function. Whereas FurA appears to be a local Fe-dependent regulator involved in oxidative stress response, FurB has been identified as the genuine Zn-dependent zinc uptake regulator (Zur). Recent biological, biochemical, biophysical and crystallographic data will be summarized and compared to other members of the Fur family to illustrate our current understanding of mechanisms and function.

References 1. Lee, J.W., Helmann. J.D. *Biometals* (2007) 20:485–499; 2. Lucarelli, D. *et al. J Biol Chem* (2007) 282:9914–9920; 3. Riccardi, G. *et al. FEMS Microbiol Lett* (2008) 287:1–7.

Metal ion homeostasis: a virulence determinant of streptococci?

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The genus *Streptococcus* includes several different pathogenic species that cause a diverse range of superficial and invasive infections in humans and animals. During colonization and infection, streptococci are exposed to different body fluids and tissues, and different concentrations of essential nutrients such as trace metals. The ability of streptococci to buffer the intracellular environment against fluctuations in metal ions is critical for their success as pathogens. Recently, a great deal of progress has been made in characterizing selective metal ion import and export systems of streptococci *in vitro*. Metal ion-sensing regulators play a key role in controlling metal transport, and appear to be indispensible in at least some types of invasive infection. However, the precise role of metal ion transporters and regulators during colonization and infection of the host remains far from clear. Colonization involves the incorporation of streptococci into biofilms, often containing other species of bacteria. The influence of a biofilm matrix or juxtaposed non-streptococcal cells on metal ion acquisition is not well understood. The development of appropriate models for studying streptococcal metal ion homeostasis in the natural environment promises to yield new insights into the biology of this important group of bacteria.

Mechanisms of nickel recognition in transport and regulation

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USA Bacteria and archaea require nickel for a variety of enzymatic processes. Specific transporter proteins are used to import nickel. These transporters are frequently under the transcriptional control of the nickel-responsive NikR repressor. Many bacteria encode a second nickel responsive regulator (RcnR) that regulates expression of the nickel/cobalt efflux protein RcnA. NikR and RcnR are structurally distinct metalloregulator proteins. Studies of the nickel binding properties of *E. coli* NikR and RcnR reveal that nickel binding affinity and coordination geometry correlate with function. We have also identified the molecular requirements for nickel recognition by the *E. coli* NikABCDE transporter. RcnR is largely insensitive to nickel transport by this pathway. These observations suggest a mechanistic basis for distinguishing essential vs excess intracellular nickel ions by NikR and RcnR.

Transition metal homeostasis in *Cupriavidus metallidurans* CH34: systematic deletion analysis to complete the inventory

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Cupriavidus metallidurans CH34 serves as model system for cellular transition metal homeostasis. Backbone of this process is a controlled flow equilibrium mediated by uptake and efflux systems. Members of more than three protein families catalyze efflux of Zn(II) but also of Cd(II), Co(II) and Ni(II). P-type ATPases (TC#3.A.3) and CDF proteins (TC#2.A.3) export surplus cations from the cytoplasm to the periplasm. Individual roles of several proteins will be addressed. RND proteins (TC#2.A.6), as parts transmembrane protein complexes, export predominantly periplasmic cations across the outer membrane directly to the outside. In contrast to export much less is known about the uptake of cations by *C. metallidurans*. In a systematic deletion analysis, the genes for putative secondary Zn(II) uptake system were taken into account. The target genes encoded three members of the MIT (TC#1.A.35) protein family, one ZIP (TC#2.A.5), and one PiT (TC#2.A.20) protein. Unfortunately, deletion of the listed five genes did not hamper growth of *C. metallidurans* even in absence of Zn(II). However, it could be demonstrated that ZupT was essential to supply Zn(II) to the cells under conditions of zinc sequestration. Individual function could also be assigned to the MIT protein CorA1 and the metal phosphate uptake system PitA.

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The role of chelatases in tetrapyrrole biosynthesis

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Modified cyclic tetrapyrroles, such as haem, chlorophyll and vitamin B_{12}, are characterized by the presence of a centrally chelated metal ion, which is inserted into the macrocycle by a specific chelatase. There are two broad general classes of chelatase, which differ in subunit composition and requirement for ATP. The class I enzymes, exemplified by the magnesium chelatase of chlorophyll biosynthesis and the cobaltochelatase of the aerobic cobalamin (vitamin B_{12}) pathway require three distinct subunits and ATP for activity. In contrast, the class II chelatases, such as the ferrochelatase of haem synthesis and the cobaltochelatase of the anaerobic cobalamin pathway, are single subunit enzymes that require no external energy input. Through recent developments in metabolic engineering to allow the production of suitable substrates, we are now able to compare and contrast the activities of the class I and II cobaltochelatases and provide molecular detail on aspects of substrate discrimination. In particular, with reference to the class II enzyme, we are able to provide an insight into its evolution via gene duplication, fusion and the introduction of active site asymmetry. Moreover, evidence is provided to show how some of these enzymes appear to have evolved a new function in metal transport.

Defense against copper toxicity by *Lactococcus lactis*

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In *Lactococcus lactis* IL1403, fourteen genes are under the control of the copper-inducible CopR repressor. This so called CopR regulon encompasses the CopR regulator, two putative CPx-type copper ATPases, a copper chaperone, and ten additional genes of unknown function. Genes of the CopR regulon are induced by copper, cadmium and silver. We addressed the function of tow of these genes, *lctO* (lactate oxidase) and *cinD* (Copper-Induced Nitroreductase). LctO was shown to encode a NAD-independent lactate oxidase. It catalyzes the conversion of lactate to pyruvate *in vivo* and *in vitro*. Induction of LctO by copper represents a novel copper stress response and we suggest that it serves in the scavenging of molecular oxygen. *CinD* encodes an NADH-dependent nitroreductase. A knock-out mutant of *cinD* was more sensitive to oxidative stress exerted by 4-nitroquinoline-N-oxide and copper. Purified CinD is a flavoprotein and reduced 2,6-dichlorophenoindophenol and 4-nitroquinoline-N-oxide with k_{cat} of 27 and 11 s^{-1}, respectively, using NADH as a reductant. CinD also exhibited significant catalase activity *in vitro*. The X-ray structure of CinD was resolved at 1.35 Å and resembles that of other nitroreductases. CinD is thus a nitroreductase which can protect *L. lactis* against oxidative stress exerted by nitroaromatic compounds and copper.

The complexities of bacterial copper detoxification proteins

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Copper is essential for a wide range of biochemical processes. Its usefulness in nature is largely based on its ability to cycle between +1 and +2 oxidation states and to form tight complexes with amino acid residue side chains; however, these properties also make copper potentially highly toxic. Therefore, organisms of all types have evolved complex trafficking pathways that involve the transfer of the metal, as Cu(I), from one trafficking protein to the next, with the result that 'free' copper is kept at a very low level and the metal is delivered only where it is required. Work in my laboratory has focused on the copper trafficking proteins CopZ (an Atx1-like chaperone) and CopA (a Menkes-like P-type ATPase transporter) from *Bacillus subtilis*, which are important for Cu(I)-detoxification [1]. Here, I will discuss the Cu(I)-binding properties of these proteins, including the ability to Cu(I) to promote their self association, their ability to bind multiple Cu(I) ions, and the transfer of Cu(I) between them.

Reference [1] Radford, D.S., Kihlken, M.A., Borrelly, G.P.M., Harwood, C.R., Le Brun, N.E. & Cavet, J.S. (2003) *FEMS Microbiol Lett* 220, 105–112.

Nickel homeostasis in the pathogenic yeast *Cryptococcus neoformans*

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Cryptococcus neoformans is a fungal pathogen that can cause life threatening disease in immunocompromised individuals and is a significant health issue in regions of the third world that have a high incidence of HIV/AIDS. The enzyme urease generates ammonium for nitrogen metabolism and is essential for the virulence of *C. neoformans* and other fungal pathogens. We are using *C. neoformans* as

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a model to understand the molecular mechanisms that underlie the role of urease during infection. Although much is known about the regulation and maturation of bacterial urease, an equivalent understanding of the fungal enzyme is lacking. Essential for urease function is a nickel cofactor that forms the active site of the enzyme. Of interest is how *C. neoformans* is able to acquire the nickel it needs during infection. To address this, we are identifying those proteins that are involved in nickel uptake and delivery to urease in *C. neoformans* and determining how these processes are controlled. This will establish to what extent known fungal metal homeostatic mechanisms also apply to nickel.

Iron and zinc homeostasis in *Bacillus subtilis*

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USA Limitation for essential metal ions induces complex and multifaceted adaptive responses. Using *B. subtilis* as a model organism, we have defined the processes of adaptation to limiting iron and zinc. These two stress responses are controlled by the paralogous regulatory proteins Fur and Zur. In response to iron limitation, the Fur regulon is derepressed leading to expression of numerous Fe uptake pathways. In addition, a Fur-regulated small RNA, FsrA, mediates a remodeling of the proteome to prioritize iron usage. Numerous iron-containing enzymes are down-regulated, and in several cases this is due to experimentally demonstrated direct RNA pairing interactions. Zinc limitation leads to derepression of the Zur regulon which includes a high affinity zinc uptake ABC transporter, a zinc metallochaperone, and an alternative (zinc-independent) FolE enzyme for folate biosynthesis. Several members of the Zur regulon function as ribosomal proteins. These function either to mobilize zinc bound to small, ribosome-associated proteins (L31, L33) or to allow continued ribosome synthesis even when zinc is severely limited (S14). Detailed structure-function studies of Zur and Fur are beginning to provide insights into how these two paralogs distinguish between their cognates metal ions.

Iron conservation by reduction of metalloenzyme inventories in the marine diazotroph *Crocosphaera watsonii*

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The marine nitrogen fixing microorganisms (diazotrophs) are a major source of nitrogen to open ocean ecosystems and are predicted to be limited by iron in most marine environments. Using global and targeted proteomic analyses on a key unicellular marine diazotroph *Crocosphaera watsonii,* we observed large scale diel changes in its proteome, including substantial variations in concentrations of iron metalloproteins involved in nitrogen fixation and photosynthesis. The daily synthesis and degradation of enzymes in coordination with their utilization results in a cellular metalloenzyme inventory that requires significantly less iron than if these enzymes were maintained throughout the diel cycle. In a global numerical model of ocean circulation, biogeochemistry and ecosystems, the competitive advantage of this lower iron requirement, even with its energetic cost, allows *Crocosphaera* to grow in regions scarcer in iron and to higher biomass than another important diazatroph *Trichodesmium,* as well as a hypothetical control microbe unable to conserve iron. With rising atmospheric CO_2 predicted to cause increases in both marine nitrogen fixation and the cellular iron content of diazotrophs, this capacity for reduction in iron metalloenzyme inventory may be an important characteristic of *Crocosphaera's* oceanic niche.

Oxidative stress responses in iron-limited *Chlamydomonas*

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Photosystem I with 12 iron atoms per monomer is a prime target for damage in iron-limited heterotrophic cells, resulting in the generation of reactive oxygen species in the chloroplast. Multiple responses to counter the stress are induced, including increased accumulation of ascorbate via up-regulation of *VTC2*, encoding a key enzyme in the biosynthesis of ascorbate, and *MDAR1*, encoding an enzyme involved in regeneration of ascorbate. In addition, there are two pathways that work to increase the capacity of the chloroplast to detoxify superoxide. In one pathway, the *MSD3* gene, encoding a plastid-specific MnSOD (one of 5 MnSODs in *C. reinhardtii*) is dramatically upregulated, up to 1000-fold, at the transcriptional level, in response to iron limitation. In a second pathway, the plastid FeSOD is preferentially retained over cytochrome *f*, the CTH1 di-iron Mg-PPMME cyclase, and ferredoxin, which are degraded during iron limitation. Maintenance of FeSOD occurs, after an initial phase of degradation, by *de novo* resynthesis in the absence of extracellular iron, suggesting a mechanism for recycling and for prioritized allocation of iron.

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NT01 Workshop: metals & microbes

↑CONTENTS

Offered paper **Inactivation of *fecD* and *ceuE* affects nickel acquisition and urease activity in *Helicobacter mustelae***

JEROEN STOOF[1], Ernst J. Kuipers[2], Gerard Klaver[3] & Arnoud H.M. van Vliet[4]

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The genome sequences of *Helicobacter* species colonizing the mammalian gastric mucosa contain a large number of genes annotated as putative iron-uptake genes, but only few nickel-uptake genes, which contrasts with the central position of nickel in urease-mediated acid resistance of these pathogens. In this study we have investigated the transcription and roles of the putative iron ABC transporter genes *fecD* and *ceuE* in the ferret pathogen *Helicobacter mustelae*. Transcription of *fecD* and *ceuE* was iron- and Fur-independent and their inactivation did not affect cellular iron levels. Surprisingly, inactivation of *fecD* and *ceuE* resulted in a strongly reduced urease activity and cellular nickel content as measured by ICP-MS. Inactivation of either *nixA, tonb2* or *nikH* further diminished cellular nickel levels and urease activity of the *fecD* mutant. Inactivation of *fecD* and *nixA* also abolished nickel-dependent regulation of the urease systems. In contrast to NixA, metal acquisition by FecD seemed to be less specific for nickel, since *fecD* mutants also showed reduced cellular cobalt levels and increased cobalt resistance. We conclude that in *Helicobacter mustelae* the ABC transporter system FecDE-CeuE is likely to contribute to nickel and cobalt acquisition, and works independently of the previously described NixA, TonB2 and NikH system.

Offered paper **Purification and characterization of the metalloenzyme arsenite oxidase from *Polaromonas* sp. str. GM1**

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Arsenic is a metalloid toxic to living cells. Its two soluble inorganic forms, arsenite and arsenate, have caused mass poisoning in East Asia. Despite arsenic's toxicity, some prokaryotes have mechanisms to gain energy by either oxidizing or reducing it, and are involved in the global cycling of arsenic. Aerobic arsenite oxidation is catalysed by the arsenite oxidase (Aro), a member of the DMSOR family of molybdoenzymes. *Polaromonas* sp. str. GM1 is a heterotrophic, psychrotolerant, arsenite-oxidizing β-*Proteobacterium*, isolated form a contaminated gold mine in the permafrost of NW Territories, Canada. The organism is capable of oxidizing arsenite at 0°C and does so in the early-exponential phase of growth. The Aro of GM1 is constitutively expressed. It is a periplasmic enzyme and was purified by cation exchange and gel-filtration chromatography. The GM1 Aro is a heterodimer consisting of a large α-subunit (88 kDa) and small α-subunit (15 kDa). ICP-MS and sequence analysis indicate the enzyme contains Mo at its active site, a 3Fe–4S cluster and a Rieske-type 2Fe–2S cluster. Kinetic studies show that the GM1 Aro has the highest V_{max} of any known Aro and its thermolability and stability are compared to the mesophilic homologue from an unrelated arsenite oxidizer.

Offered paper **Investigating the mechanism of inhibition of *Staphylococcus aureus* growth by the human protein calprotectin**

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Previous investigations have demonstrated that the protein complex calprotectin, produced in large quantities by mammalian neutrophils and secreted into abscesses, inhibits the growth of *Staphylococcus aureus* (Corbin *et al.*, 2008). Growth inhibition was observed even when calprotectin was separated from the cells by a dialysis membrane, suggesting that the mechanism of inhibition involves nutrient sequestration. Medium treated with calprotectin is depleted for manganese and zinc. Increased sensitivity of Δ*mntA*/Δ*mntB* mutant strains, defective in high-affinity manganese uptake, to growth in the presence of calprotectin in conjunction with transcriptome analysis lead to the hypothesis that calprotectin chelates Mn^{2+} and inhibits growth through manganese starvation.

The mechanism by which Mn-starvation inhibits proliferation of *S. aureus* is not yet known. In order to investigate this, here we present analyses of the abundance of metal in the major manganese and zinc pools of the *S. aureus* cytosol. The major proteinaceous manganese pool is shown to be associated with the two superoxide dismutase enzymes, SodA and SodM, and this pool is reduced in Δ*sodA* and *sodM* mutant strains and eliminated in a Δ*sodA*Δ*sodM* double mutant. A second, large pool of manganese is present as low molecular weight, anionic complexes. In contrast, multiple abundant zinc-proteins are observed in addition to a low molecular weight zinc pool. Data will be presented demonstrating the effect on the abundance of manganese and zinc within these cytosolic pools in *S. aureus* cells cultured in the presence of recombinant human calprotectin.

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Offered paper **Atypical copper homeostasis in *Salmonella typhimurium***

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Bacteria employ a complex array of mechanisms to maintain cellular copper homeostasis. Copper toxicity is prevented by appropriate regulation of proteins which traffic and sequester excess copper. *Salmonella enterica* serovar Typhimurium (*S.* Typhimurium) has two metal-transporting P_1-type ATPases, CopA and GolT, whose actions largely overlap with respect to growth in elevated copper. Mutants lacking both ATPases are hypersensitive to copper and over-accumulate copper relative to wildtype or either single mutant. Such duplication of ATPases is unusual in bacterial copper tolerance. Although CopA and GolT serve to transport copper out of the cytosol, it is not known how copper is removed from the *S.* Typhimurium periplasm. Analyses of periplasmic copper-complexes identified copper-CueP as a predominant metal pool. Copper-CueP is a further atypical feature of copper homeostasis in *S.* Typhimurium and a slight increase in copper accumulation of Δ*cueP* suggests a role in copper export. Elevated copper is detected by a reporter construct driven by the promoter of *copA* in wildtype *S.* Typhimurium during infection of macrophages. Double mutants missing both ATPases also show reduced survival inside cultured macrophages. It is hypothesized that elevated copper within macrophages may have selected for specialized copper resistance systems in pathogenic microorganisms such as *S.* Typhimurium.

Offered paper **How pathogenic *Escherichia coli* use stress hormones to steal transferrin and lactoferrin iron**

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Iron is a key nutritional element required for the growth of all bacterial pathogens; therefore its sequestration by the vertebrate ferric iron-binding proteins (transferrin, Tf, in serum, and lactoferrin, Lf, in mucosal secretions) represents a primary non-specific host-defence mechanism against microbial infection. We have shown that catecholamine hormones (e.g. noradrenaline, adrenaline and dopamine) released during host stress enable bacteria which lack specific Tf or Lf-iron acquisition systems, to access Tf/Lf-sequestered iron. This bacterial-Tf/Lf-stress hormone interaction can result in up to million-fold increases in bacterial growth in normally bacteriostatic host tissues such as blood or serum. The underlying mechanisms by which stress hormones render Tf and Lf iron susceptible to bacterial theft is the subject of this study. We employed EPR spectroscopy and chemical iron binding analyses to demonstrate that catecholamines form direct complexes with the ferric iron within Tf and Lf, resulting in the reduction of Fe(III) to Fe(II) and the subsequent loss of Tf/Lf-complexed iron. Use of wild type *Escherichia coli* and ferric iron uptake mutants showed that both of the Fe(II) and Fe(III) released from the Tf/Lf could be directly used as bacterial nutrient sources. The relevance of these catecholamine-Tf/Lf interactions to the infectious disease process is considered.

Offered paper **The oxidative stress regulator OxyR and iron homeostasis in *Pseudomonas aeruginosa***

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In *Pseudomonas aeruginosa*, the response to oxidative stress is orchestrated by the OxyR regulator, which activates the transcription of two catalases (*katA*, *katB*) and two alkyl-hydropxyperoxidase (*ahpC*, *ahpB*) genes. An *oxyR* mutant is characterized by an extreme sensitivity to H_2O_2 and by an inability to grow in LB medium when inoculated at low cell density. Another striking phenotype is its growth defect in media containing either low ($<1\mu M$) or high ($>10\mu M$) of Fe^{3+}. *P. aeruginosa* produces the siderophore pyoverdine and is able to take up ferripyoverdine, but is unable to grow in the presence of a strong iron chelator. A proteome comparison of wild type and *oxyR* cells grown in an iron-limited medium revealed that the *oxyR* mutant produced reduced levels of the Fur regulator, but increased amounts of two nucleoid binding proteins, IbpA, and the mini-ferritin Dps (DNA protection during starvation [PA0962]). This non-specific DNA-binding protein is composed of 7 to 12 identical subunits organized as a spherical complex in which iron is accumulated to prevent oxidative DNA-damage. Two *dps* mutants were generated by marker-free deletion, one in the wild type and one in the *oxyR* context. The single *dps* mutant did not present any growth defect in LB or CAA medium, but the double *oxyR dps* mutant was even more affected in its growth at low cell density than the single *oxyR* mutant, indicating the importance of Dps under conditions of oxidative stress. The overproduction of Dps probably reflects the need to hide intracellular iron from DNA to prevent Fenton/Haber-Weiss chemistry. In circumstances, in which iron and oxygen cooperate synergistically stimulating ROS formation, the bacterium would benefit, aside from accumulating iron in DNA-associated Dps, from storing iron as much as possible in bacterioferritins and ferritins.

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Offered paper **Characterization of the WhiB1 protein of *Mycobacterium tuberculosis***

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Mycobacterium tuberculosis is a major pathogen and one of the keys to its success is the ability to establish, and emerge from, the persistent state. It has been suggested that nitric oxide signalling is required for establishing the persistent state. Wbl family proteins are associated with developmental processes and *M. tuberculosis* has seven Wbl proteins, including WhiB1, which appears to be encoded by an essential gene. The WhiB1 protein possesses a $[4Fe–4S]^{2+}$ cluster that reacts rapidly with nitric oxide, but not with oxygen. Exposure to nitric oxide switches holo-WhiB1 from an inactive non-DNA-binding form to a dinitrosyl-iron thiol complex-form/apo-form that specifically bind(s) to DNA. *In vitro* transcription reactions showed that binding of apo-WhiB1 to its own promoter repressed transcription. It is suggested that nitric oxide sensing by WhiB1 is likely to be important for reprogramming *M. tuberculosis* gene expression during infection.

Offered paper **Copper homeostasis in *Listeria monocytogenes***

DAVID CORBETT[1], Stephanie Schuler[1], Sarah Glenn[2], Peter Andrew[2], Jen Cavet[1] & Ian S. Roberts[1]

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Copper (Cu) is an essential element to many organisms, being particularly important for enzymes involved in oxidative phosphoryllation due to its ability to undergo redox cycling. However, this property also allows Cu ions to participate in Fenton-like reactions resulting in the generation of deleterious hydroxyl radicals. Furthermore, the ability of Cu ions to bind avidly to sulfur and nitrogen donors results in the displacement of less competitive metals from proteins and enzymes, disrupting key metabolic processes. In order to cope with excessive Cu levels, bacteria employ a range of detoxification mechanisms. *Listeria monocytogenes*, a ubiquitous environmental saprophyte and important human pathogen, must cope with fluctuating levels of copper both in the environment and during infection. We have identified and characterized a Cu-responsive operon in *L. monocytogenes* EGD-e. Transcription of a copper-specific P_1-type ATPase, CopA, which detoxifies cytoplasmic copper, is controlled by the copper-responsive repressor CsoR. The third gene in the operon, a predicted copper chaperone, was shown to have a role in tempering transcription of the operon by reducing the level of copper available to CsoR. Further studies have investigated the role of the copper-responsive operon in the virulence of *L. monocytogenes* in both macrophage and mouse models of infection.

Offered paper **Novel components of iron–sulfur cluster biosynthesis pathways**

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Iron–sulfur (Fe–S) clusters are essential cofactors of proteins involved in a variety of functions and Fe–S cluster biosynthetic pathways are remarkably conserved throughout evolution. Recent genome-wide screens for genetic interactions reported the synthetic lethality of a *grxD* mutation when combined with strains defective in Fe–S cluster biosynthesis (*isc* operon) functions. This data has linked GrxD, an Fe–S containing monothiol glutaredoxin (mono-Grx), to a potential role in Fe–S cluster biosynthesis. Work in higher organisms has demonstrated that mono-Grxs can transfer their Fe–S clusters to apo-proteins *in vitro* while alternative mono-Grxs are linked to Fe regulation in yeast via an Fe–S-containing complex with Fra2p, a homolog of *E. coli* BolA. In contrast to higher organisms, most prokaryotes encode only a sole mono-Grx. We chose to investigate GrxD function to uncover the molecular basis of observed synthetic lethality. Using affinity purification, genetics, biochemistry and phenotypic assays we have: i) confirmed that *E. coli* GrxD can form Fe–S-containing complexes as a homodimer and as a heterodimer with BolA and ii) linked both BolA and GrxD to iron homeostasis. This work will aid our understanding of novel factors that play a role in Fe–S cluster biosynthesis and/or iron homeostasis throughout evolution.

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NT02 Microbial death

Toxin–antitoxin genes can mitigate translational errors and generate persister cells

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Toxin – Antitoxin (TA) loci are a diverse group of genes, present in almost all free-living prokaryotes, often in surprisingly high copy-numbers. TA loci consist of two genes in a transcriptional unit that code for a stable inhibitor or 'toxin' and an unstable 'antitoxin' that combines with and counteracts the inhibitory effect of the toxin. Transcription from TA operons is auto-regulated by the antitoxin, which represses transcription while the toxin acts as a co-repressor of transcription. Interestingly, when [T]>[A], the toxins activate cognate TA locus transcription by conditional cooperativity. Antitoxins are metabolically unstable because they are degraded by Lon. *E. coli* K-12 has ten TA loci encoding mRNases (7 RelE homologs, MazF, ChpB and HicA) that inhibit translation when overexpressed. Here we present evidence that TA loci increase translational fidelity. Amino acid starvation activated transcription of all ten TA loci. Concomitantly, the TA loci reduced the global level-of-translation – consistent with the mRNase activity of the toxins. The multiple TA deletion strain exhibited increased global levels of translational errors and oxidatively damaged proteins. Moreover, endogenous TA loci could be triggered to form high levels of persister cells. Thus TA loci function in translational quality control and/or persister cell formation.

Acetic acid-induced apoptosis in *Saccharomyces cerevisiae*

Susana Chaves[1], Clara Pereira[1], Sara Alves[1], Bénédict Salin[2], Nadine Camougrand[2], Stéphen Manon[2], Maria João Sousa[1] & MANUELA CÔRTE-REAL[1]

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This presentation will provide an overview of the work we have been developing on programmed cell death (PCD) in yeast, with particular focus on acetic acid. We have shown that acetic acid acts as a trigger of programmed cell death (PCD) in *Saccharomyces cerevisiae* exhibiting characteristics of mammalian apoptosis [1]. Biochemical and molecular evidence demonstrating this is a mitochondria-dependent pathway includes the accumulation of mitochondrial ROS, transient hyperpolarization followed by depolarization, decrease in COX activity affecting mitochondrial respiration, and subsequent release of lethal factors like cytochrome *c* [2] and yeast AIF [3]. Additionally, our studies implicate the ADP/ATP carrier (AAC) in the mitochondrial outer-membrane permeabilization and cytochrome *c* release [4]. Finally, our more recent work shows that autophagy is not activated in acetic acid induced apoptosis and points towards a role for the vacuolar protease Pep4p and AAC proteins in mitochondrial degradation [5]. These results will be discussed in the context of a complex regulation and interplay between mitochondria and the vacuole in yeast programmed cell death.

References 1 Ludovico *et al.* (2001) *Microbiology* 147, 2409–2415; 2 Ludovico *et al.* (2002) *Mol Biol Cell* 13, 2598–2606; 3 Wissing *et al.* (2004) *J Cell Biol* 166, 969–974; 4 Pereira *et al.* (2007) *Mol Microbiol* 66, 571–582; 5 Pereira *et al.* (2010) *Mol Microbiol* 1–13.

A radical approach to antibiotics

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Antibiotic drug-target interactions, and their respective direct effects, are generally well-characterized. In contrast, the bacterial responses to antibiotic drug treatments that contribute to cell death have proven to be quite complex, involving multiple genetic and biochemical pathways. Here we discuss how three major classes of bactericidal antibiotics, regardless of drug-target interaction, stimulate the production of highly deleterious reactive oxygen species in bacteria, which ultimately contribute to cell death. This mechanism of reactive oxygen species formation is the end-product of an oxidative damage cell death pathway involving the tricarboxylic acid cycle, NADH, destabilization of iron-sulfur clusters and stimulation of the Fenton reaction. We will also discuss the sequence of events following the initial drug-target interaction that generate an intracellular environment promoting reactive oxygen species formation for different classes of bactericidal antibiotics. We show how the application of sub-lethal levels of bactericidal antibiotics induces mutagenesis that correlates with ROS formation. This results in heterogeneous increases in the minimum inhibitory concentration for a range of antibiotics, irrespective of the drug target. This has implications for the widespread use and misuse of antibiotics.

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Mosquito antimicrobial immune pathways in the defense against eukaryotic parasites and viruses

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The innate immune system of mosquito vectors of malaria and dengue virus represents the main line of defense against these pathogens, is engaged at multiple stages of infection. As part of this defense, three major signaling pathways, the TOLL, IMD and JAK-STAT pathways, transduce the pathogen recognition signal to activate nuclear translocation of two members of the NF-κB Rel family of transcription factors, Rel1 and Rel2, and the STAT transcription factor, respectively. The IMD pathway is a major player in the defense against *P. falciparum* in the three major malaria vectors, *A. gambiae*, *A. stephensi,* and *A. albimanus*, and *Plasmodium* killing is at least partly mediated by the anti-*Plasmodium* factors aTEP1, LRRD7 and FBN9. The *Aedes aegypti* mosquito vector of dengue utilizes the Toll and JAK-STAT pathways to defend against virus infection. Recent advanced in vector biology research has provided new insight on how these antimicrobial immune pathways are involved in modulating the mosquito's capacity to transmit human pathogens.

Protein aggregates: an aging factor involved in cell death

Etienne Maisonneuve, Laetitia Fraysse, Benjamin Ezraty, Daniele Moinier, Sabrina Lignon, Laure Capron & SAM DUKAN
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Protein aggregation is a phenomenon observed in all organisms and has often been linked with cell disorders. In contrast to previous studies and the expected outcome, we observed aggregated proteins in aerobic exponentially growing and 'healthy' *Escherichia coli* cells. These protein aggregates over-represent aberrant or carbonylated proteins. In light of these observations, protein aggregates could be considered as damage to cells that is able to pass from one generation to the next. Based on the assumption that the relative amount of aggregate protein could represent an aging factor, we monitored this amount in a bacterial culture during senescence. In doing so, we observed (i) a significant increase in the relative amount of aggregate protein over time, (ii) a proportional relationship between the amount of aggregate protein and the level of dead cells, (iii) a higher amount in dead cells compared to culturable cells, (iv) a heterogeneous distribution of differing amounts within a homogenous population of culturable cells entering stasis, (v) that the initial amount of aggregate protein within a culturable population conditioned the death rate of the culture. All together, our results suggest that indeed protein aggregates represent one aging factor leading to bacterial cell death.

Programmed cell death in *Candida albicans* following macrophage engulfment

V. Cabezón, L. Monteoliva, V. Vialás, C. Nombela & C. GIL
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Candida albicans is a dimorphic opportunistic pathogenic fungus that grows either in yeast form or as hyphae. It is a commensal resident on the mucosal surfaces and can cause host damage (candidiasis) by mechanisms mediated both by host (predisposing factors) and by fungus (virulence factors). Depending on the underlying host defect, *C. albicans* can cause different types of infections ranging from superficial to systemic candidiasis (SC). This last one remains a leading infectious cause of morbidity and mortality in critically ill and/or severely immunocompromised patients. Clinical outcomes might be improved by early initiation of antifungal therapy. SC diagnosis, however, proves extremely difficult due to the lack both of specific signs and symptoms of invasive disease and of rapid and accurate diagnostic tests. We are studying the *C. albicans*–macrophages interaction, because is the initial step in the development of host immune defenses. An *in vitro* model of phagocytosis that includes a differential staining procedure to discriminate between internalized and non-internalized yeast was developed. Upon optimization of a protocol to obtain an enriched population of ingested yeasts, a thorough genomic and proteomic analysis was carried out on these cells. Both proteins and mRNA were obtained from the same sample and analysed in parallel. We provide evidence of a rapid protein response of the fungus to adapt to the new environment inside the phagosome by changing the expression of proteins belonging to different pathways. The clear down-regulation of the C-compound metabolism, plus the up-regulation of lipid, fatty acid, glyoxylate and tricarboxylic acid cycles, indicates that yeast shifts to a starvation mode. There is an important activation of the degradation and detoxification protein machinery. The complementary genomic approach led us to detect specific pathways related to *Candida's* virulence. Network analyses allowed us generate a hypothetical model of *Candida* cell death after macrophage interaction, highlighting the interconnection between actin cytoskeleton, mitochondria and autophagy in the regulation of apoptosis. We are studying different apoptotic markers in order to confirm the hypothesis of programmed cell death of *C. albicans* after macrophage interaction. In conclusion, the combination of genomic, proteomic and network analyses is a powerful strategy to better understand the complex host-pathogen interactions.

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Conserved cell death pathways in fungi

Mark Ramsdale

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Fungi are of considerable economic importance and cause a range of socially and ecologically devastating diseases. Recent work on both pathogenic and non-pathogenic fungi has highlighted the existence of a variety of cell death responses including apoptosis-like cell death events, autophagy and necrosis. Current research is now focussed on furthering our understanding of cell death events in fungi at the molecular level with the specific aim of identifying the key regulators of fungal cell death responses. Conserved death related signalling pathways in fungi will be outlined, with a focus on the effectors that might be manipulated for therapeutic use. Putative caspase-dependent cell-death related pathways in fungi will be explored further in the context of their evolutionary history and their selective targeting of essential cellular components. Experimental data exploring the nature of cell death events in *Saccharomyces cerevisiae* and *Candida albicans* will be presented, including an evaluation of the roles of *RAS1*, *MCA1* and *AIF1*.

Mutation as a stress response and the regulation of evolvability

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Whereas mutation was long held to be random, growing evidence shows that environmental stresses induce genomic instability in bacteria, yeast, and human cancer cells, generating occasional fitter mutants and potentially accelerating adaptive evolution. The emerging molecular mechanisms of stress-induced mutagenesis vary but share telling common components that underscore two common themes. The first is the regulation of mutagenesis in time by cellular stress responses, which promote random mutations (*i.e.*, those with neutral, deleterious, or adaptive consequences) specifically when cells are poorly adapted to their environments—*i.e.*, when they are stressed. A second theme is the restriction of random mutagenesis in genomic space, achieved *via* coupling of mutation-generating machinery to local events such as DNA repair or transcription. Such localization may minimize accumulation of deleterious mutations in the genomes of rare fitter mutants, and promote local concerted evolution (adaptive evolution requiring multiple mutations). These themes will be illustrated by the example of the *E. coli* Lac system for starvation-inducible mutagenesis, in which mutation is coupled to DNA double-strand-break repair, which switches to a mutagenic mode under stress, controlled by the RpoS, SOS and RpoE stress respones. These general themes are widespread in mutagenesis in many different bacterial experimental systems and natural isolates. Such mechanisms probably fuel many instances of biological evolution, including evolution of microbial pathogenesis and antibiotic-resistance, and human tumor-progression and resistance mechanisms much of which occurs under stress, driven by mutations. The multiple, similar-but-not-identical molecular mechanisms of stress-inducible mutagenesis observed in different environmental conditions, assays, strains and organisms, suggest multiple independent evolutions of stress-inducible mutagenesis, with regulation by stress responses emerging as an overarching common theme.

Bacterial death prevented by antibiotics

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The stationary phase of bacterial growth has been considered as one model enabling the study of adaptation to non-permissive growth conditions. Many microorganisms, including *Mycobacterium tuberculosis* (Mtb), survive prolonged incubation in stationary phase without significant loss of viability. However, *Mycobacterium bovis* (BCG) dies rapidly, retaining less than 0.01% of viable cells after 2–months incubation. Paradoxically we found that the addition of antibiotics, which are generally considered inactive against non-growing cells (ethambutol, cerulenin and isoniazid), to stationary phase BCG cultures dramatically improved their survival. In contrast, rifampicin, doxycycline or streptomycin enhanced bacterial death. The surviving cells remained fully sensitive to all the listed antibiotics with identical MICs for treated and untreated cells. A comparison of transcriptomic profiles between antibiotic-treated and untreated cells revealed differential expression of several genes, in particular, up-regulation of the BCG homologue of Mtb *Rv1219c*, a probable transcriptional regulatory protein, in the surviving treated populations. Over-expression of *Rv1219c* from a tetracycline-regulated promoter significantly improved survival of BCG cells in stationary phase as well as their re-growth in fresh media. A deletion *Rv1219c* mutant of Mtb was attenuated in stationary phase survival. Our findings describe a novel mechanism controlling bacterial survival and may offer new strategies for treatment of bacterial infections. This work was supported by grants from the Wellcome Trust and BBSRC.

NT03 Streptococci

↑CONTENTS

Streptococcus pneumoniae: virulence and variation

Tim J. Mitchell

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Streptococcus pneumoniae (the pneumococcus) can cause serious diseases such as pneumonia and meningitis in humans. The pneumococcus produces several virulence factors that are important in the pathogenesis of infection. These include a polysaccharide capsule, a protein toxin (pneumolysin) and a range of surface proteins including the enzymes neuraminidase and hyaluronidase. The contribution of these virulence factors to pathogenesis will be discussed. It is clear from analysis of the increasing number of genome sequences of this organism in conjunction with comparative biology that the contribution of virulence factors to disease varies between strains of the organism. For example, we have examined a panel of serotype 1 strains of the pneumococccus and found them to be remarkably different in their ability to cause disease in murine models of infection. Analysis of these strains by comparative genomic is providing some clues as to why these strains differ in this way. We have also found that pneumococcal isolates from different sites on infection in the same patient can differ by appearance of single nucleotide polymorphisms (SNPs) and these can affect gene expression and virulence of the strains isolated. In this presentation I will give an overview of these findings.

Streptococcus agalactiae adaptation to the human lifestyle

Barbara Spellerberg

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Streptococcus agalactiae (Group B streptococci, GBS) is an important human and veterinary pathogen. Its host range includes fish and cattle as well as humans. During the course of invasive human infections, GBS adapt to different environments, requiring the colonization of epithelial surfaces, the binding to extracellular matrix proteins, penetration of cellular barriers and the evasion of host immunity. Genes responsible for these events are often localized on pathogenicity islands (PAI). So far 14 PAIs have been identified in GBS, encoding the genes for the ß-hemolysin, the CAMP-factor and numerous regulatory systems. Especially involved in adaptation processes is PAI XII, harboring the structure of a composite transposon that contains the genes for the C5a-peptidase and for the adhesin Lmb. Genes in this region are responsible for the binding to fibronectin and laminin, the invasion into eukaryotic cells and the escape of the immune system. The importance of this region for human infections is underlined by the specific induction of the C5a peptidase upon exposure to human serum and the absence of the whole region in many bovine isolates. Detailed understanding of microbial adaptation and interactions with the host will be crucial for the development of novel therapeutic and prophylactic strategies.

Oral streptococci behaving badly

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It is generally accepted that to grow and survive in the oral cavity bacteria must adhere to hard or soft oral tissues, resist clearance, and then become incorporated into biofilm communities. Specific molecules promoting physical interactions of streptococci with oral cavity surfaces include cell-wall-anchored polypeptides, polymeric structures such as pili, and extracellular polysaccharides. Some of these components have been demonstrated also to be actively involved in biofilm formation, although metabolic functions and small-molecule communication systems appear to be highly influential. Oral microbial biofilms provide a source of bacteria for systemic infections. Oral streptococcal adhesins that recognize salivary glycoproteins also bind extracellular matrix proteins. Furthermore, the adhesins are recognized by circulating platelets thus facilitating development of thrombotic vegetations of the cardiovascular system. A picture is emerging of orthologous cell-surface proteins of different streptococci being employed in mediating colonization of mucosal surfaces. In addition, these same adhesins operate in jailbreak interactions with host systemic components, when oral streptococci are behaving badly.

Genome-based approaches to identify virulence factors and vaccine candidates of *Streptococcus suis*

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Streptococcus suis is an important cause of meningitis, arthritis and sepsis in young piglets. Adults pigs can carry *S. suis* asymptomatically on their tonsils. Infant piglets can become infected after early contact with colonized adult pigs. *S. suis* can also cause disease in humans.

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In Western countries human infection with *S. suis* is observed occasionally However, *S. suis* seems to be an important and under-reported human pathogen in South-East Asia. Therefore, control of *S. suis* infections both in humans as well as in pigs seems to be of high importance. So far, however, control of the disease caused by *S. suis* is hampered by the fact that the *S. suis* population is very heterogeneous. 33 serotypes of *S. suis* have been described and several different serotypes can cause disease. Moreover, differences in virulence exist between as well as within serotypes. Genome based approaches were used to get more insight into the genes involved in virulence in the various serotypes. The relationship between gene content, serotype and virulence was studied using comparative genome hybridization studies. In addition, whole genome sequences of various serotypes were analysed. The results of these studies will be discussed.

Streptococcus equi and strangles

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The continued evolution of bacterial pathogens has major implications to both human and animal disease, but the exchange of genetic material between host-restricted pathogens is rarely considered. *Streptococcus equi* subspecies *equi* (*S. equi*) is a host-restricted pathogen of horses that has evolved from the zoonotic pathogen *Streptococcus equi* subspecies *zooepidemicus* (*S. zooepidemicus*). These pathogens share approximately 80% genome sequence identity with the important human pathogen *Streptococcus pyogenes.* We have identified the genetic events that shaped the evolution of the *S. equi*, and led to its emergence as a host-restricted pathogen. Our analysis provides evidence of functional loss due to mutation and deletion, coupled with pathogenic specialization through the acquisition of bacteriophage encoding a phospholipase A_2 toxin, and four superantigens, and an integrative conjugative element carrying a novel iron acquisition system with similarity to the high pathogenicity island of *Yersinia pestis*. *S. equi*, *S. zooepidemicus* and *S. pyogenes* share a common phage pool that enhances cross-species pathogen evolution. Emerging sequencing data highlights that the complex interplay of functional loss, pathogenic specialization and genetic exchange between *S. equi*, *S. zooepidemicus* and *S. pyogenes* continues to influence the evolution of these important streptococci.

Streptococcus uberis – a relentless, opportunistic pathogen of dairy cattle

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Intramammary infection of dairy cattle by *Streptococcus uberis* is a common cause of bovine mastitis worldwide and the leading cause of mastitis in England and Wales. This bacterium can also asymptomatically colonize a number of sites in cattle and persists within environmental reservoirs such as pasture, soil and bedding material. Procedures aimed at preventing spread of infection from cow to cow during milking, although successful in the control of other mammary gland pathogens, have not controlled infection with this organism. *S. uberis* enters the bovine mammary gland via the teat canal and once inside the bovine mammary gland replicates within the secretion, induces a massive influx of neutrophils and a severe and painful inflammatory response; characteristic of the disease. The infection is highly resistant to clearance by neutrophils, a phenomenon that, despite much evidence from studies conducted *in vitro*, is not mediated by the hyaluronic acid capsule. The strategic use of a disease model in the target host species coupled to the availability of a complete genome sequence and the ability to isolate strains genotypically from a bank of random insertional mutants has enabled identification genes from *S. uberis* that do, and do not, have an impact on disease pathogenesis.

Offered paper Bioluminescent imaging of *Streptococcus pyogenes* infection

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Background *S. pyogenes* causes a range of severe diseases such as necrotizing fasciitis, puerperal sepsis and toxic shock, as well as non-suppurative sequelae such as rheumatic heart disease. These diseases are believed to follow from carriage in the nasopharynx. Little is known of the factors that influence carriage and dissemination from this site. Bioluminescence imaging (BLI) can determine the spatial location and abundance of bioluminescent bacteria within a host. This technique was used to examine carriage of *S. pyogenes* within the nasopharynx. *Results* Bioluminescence genes were integrated and expressed in *emm75* strain of *S. pyogenes*. Following intranasal administration of bioluminescent *S. pyogenes* to mice, carriage and the dissemination of bacteria from the respiratory tract to other tissues were

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monitored using BLI. In addition BLI was used to examine the role of virulence genes in carriage in an isogenic *cov* mutant of the bioluminescent strain.

Conclusions These findings demonstrate the applicability of bioluminescence imaging to monitor infections caused by clinical isolates of *S. pyogenes*. The development of BLI should greatly enhance our understanding of streptococcal pathogenesis. It is envisaged that such models will provide a much-needed and widely used system for evaluation of novel vaccines and treatments for streptococcal carriage.

Offered paper **Screening of *Streptococcus suis* mutants during infection of a pig respiratory tract *in vitro* organ culture**

ROY R. CHAUDHURI[1], Jinhong Wang[1], Dhaarini Raghunathan[1], Sarah E. Peters[1], Sabine E. Eckert[2], Gemma C. Langridge[2], Stephen D. Bentley[2], A.W. (Dan) Tucker[1] & Duncan J. Maskell[1]

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Streptococcus suis is a common commensal of the upper respiratory tract of pigs which can cause severe systemic disease in infant animals, and zoonotic meningitis, pneumonia and arthritis in humans. We are investigating the genetic requirements for survival in an *in vitro* organ culture (IVOC) model of the pig respiratory tract. IVOC allows explants of respiratory tract epithelial tissue to be maintained with a physiologically realistic air-liquid interface. We have generated a complex pool of *S. suis* mutants using a novel *mariner* transposon mutagenesis strategy. This pool, together with a pre-existing library of ~2000 Tn*917* mutants, has been screened for survival after growth on the IVOC model system. The relative fitness of each mutant is being quantitatively assessed using TraDIS (Transposon Directed Insertion-site Sequencing), an Illumina-sequencing based form of signature-tagged mutagenesis (STM). Mutants that are attenuated for growth on the IVOC model represent candidates for the development of novel live-attenuated *S. suis* vaccines.

Offered paper **Investigating intracellular survival mechanisms of *Streptococcus agalactiae* within macrophages**

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The opportunistic human pathogen *Streptococcus agalactaie*, or group B strep (GBS), is a leading cause of neonatal septicaemia and meningitis. The organism is found as a commensal of the gastrointestinal and genitourinary tract in approximately 30–50% of the population and the vast majority of individuals exhibit no symptoms of infection.

Phagocytic cells are the first line of the innate immune system and act to engulf and destroy a wide range of microorganisms. We have been investigating the interaction of GBS with the mouse derived macrophage like cell line J774. We now show that GBS can survive for several hours within the phagosome, significantly longer than the non-pathogenic species *E.coli* and *Lactococcus lactis.* We have been using antibody markers to confirm that the GBS phagosome matures normally and indeed phagosome acidification is required for the organism to survive intracellularly, since pharmacological inhibition of maturation leads to GBS death. In addition, we demonstrate that intracellular survival requires the activity of the global regulator CovR/S and are currently examining the role of other well characterized GBS virulence factors.

Offered paper **Molecular basis for a new quorum sensing mechanism in streptococci**

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Rgg-like proteins have been primarily identified in streptococci, where they play the role of transcriptional regulators. Recently, we have discovered, in nearly all streptococci genomes, a genetic context encoding an Rgg-like protein and a small hydrophobic peptide (SHP). We have studied one of these *shp/rgg* loci in *Streptococcus thermophilus* and found a target gene which encodes a secreted peptide. We have shown that the expression of this target is drastically decreased in mutants deleted for the gene *rgg*, *shp* or *ami*, which encodes an oligopeptide transporter. These results led us to hypothesize that Rgg in association with a pheromone (SHP) is involved in a new quorum sensing regulatory mechanism.

To confirm this, we validated these steps: (i) secretion, (ii) maturation, (iii) detection and (iv) reimportation of the pheromone inside the cell and (v) interaction of the transcriptional regulator Rgg with SHP and DNA.

With these experiments, we have confirmed a new quorum sensing mechanism involving for the first time a transcriptional regulator belonging to the Rgg family and a small hydrophobic peptide SHP. Currently, studies on two other *shp/rgg* loci of pathogenic streptococci are making it possible for us to extend this mechanism within this genus.

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Offered paper **Dissecting virulence of the pneumococcus**

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Streptococcus pneumoniae is generally considered to be a strict human pathogen however it has been reported to infect and cause respiratory disease in horses. Molecular characterization of *S. pneumoniae* isolates from horses led to the discovery of a conserved *lytA-ply* (autolysin-pneumolysin) genetic fusion as the result of a recombination-deletion event. This event not only deleted the intervening ~7kb of sequence, including 10 predicted open reading frames (ORFs), between the two genes, but also truncated the *lytA* and *ply* coding sequences abolishing their characterized function.

To investigate the implications of this naturally occurring mutation on the virulence of *S. pneumoniae*, we amplified the *lytA-ply* with upstream and downstream flanking sequence from equine pneumococcal strain A45 and transformed into TIGR4 to introduce the deletion. Gene replacement was confirmed by PCR, bile solubility tests and haemolytic activity assay. We will discuss the impact on virulence of this isogenic mutant compared to that of the wild-type in a murine model using both intranasal and intraperitoneal challenge.

As the deletion is conserved in all equine pneumococcal isolates tested as yet, we will also discuss how the contribution of pneumococcal virulence factors to pathogenesis may be host-restricted.

Offered paper **Role of pneumococcal virulence factors and host protection in long-term nasopharyngeal carriage and subsequent re-infection**

LUKE RICHARDS[1], Daniela Ferreira[2], Peter W Andrew[1] & Aras Kadioglu[1]
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We have developed a model of pneumococcal nasopharyngeal carriage, in which we have examined the role of the pneumococcal virulence factors pneumolysin, neuraminidase-A and B and pneumococcal surface protein-A. We have used this model to determine the immune response over an extended period and also evaluate the immunogenicity of a cleared carriage event in subsequent exposure to pneumococci, either during re-colonization or invasive pneumococcal disease.

MF1 mice infected with WTserotype-2 D39 had detectable colonies in the nasopharynx up to 28 days post infection whereas isogenic mutants were cleared from the nasopharynx by day 14. Strong anti-capsular IgM and anti-Psp-A IgG antibodies were detected by ELISA over both carriage of the WT and mutant pneumococci, and were correlated to numbers of recovered bacteria.

Mice which had cleared their nasopharynx of carriage were subsequently re-infected 14 days later with WT pneumococci. The protection offered by the clearance of Ply-deficient colonization was sufficient to prevent a lethal outcome in an acute lower respiratory challenge with parent D39 pneumococci. This protection correlated to anti-PspA antibody but was not dependent on capsular serotype, however, protection against a subsequent re-colonization was. The contribution of each immunogen to overall protection is under further study.

Offered paper **Proteomic investigations of the Group B *Streptococcus***

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The Group B Streptococcus (GBS) is a leading cause of neonatal invasive disease globally. During the progression of GBS neonatal disease, GBS must adapt to and survive in a range of distinct environmental conditions, notably those associated with vaginal carriage in pregnant women (pH typically <4.5; O_2 & nutrient limited) and those associated with amniotic fluid, the neonatal lung and blood (pH typically neutral; aerobic; rapid growth). We have used *in vitro* culture to explore GBS growth and long-term survival under conditions of low pH and nutrient limitation. Perhaps surprisingly, growth and long-term survival in stationary phase were diminished at low pH compared to neutral pH, which has implications for our understanding of colonization. Subsequently, we have used proteomic methods to investigate GBS strain A909 protein expression under *in vitro* conditions modeling these different environmental conditions. Furthermore, to model the influence of host derived nutrients, an *in vitro* model of GBS colonization was devised by culturing GBS on Todd Hewitt agar plates with and without human serum. We observed significant differences in protein expression associated with conditions modeling 'invasion' compared to 'colonization'. Notably this included induction of the Bac protein, a putative virulence factor linked to immune evasion, in the presence of serum and in growth conditions associated with invasion of the neonatal host. The

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data add to our understanding of the mechanisms that may underlie the adaptation of GBS as it transits from maternal colonization to neonatal disease.

Comparative genomics workshop – introduction

Matthew T.G. Holden[1] & Andrew Waller[2]

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Since the first bacterial genome was published in 1995, the complete genomes of nearly 50 streptococci have been deposited in the public sequence databases. Comparative genomic analysis using these data has provided new insights into the genetic basis of host specificity, pathogenesis and evolution of this genus. As a result of recent technological advances, the scale and scope of sequencing that can be undertaken has dramatically increased. In this workshop we will review the current state of the art, and discuss the application of comparative genomics to investigate bacterial diversity at the population level.

Offered paper SeM variation within outbreaks. Is there an antigenic or functional consequence?

Katy Webb

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SeM is an immunodominant virulence factor of *S. equi* which is variable at its N-terminus with a dN/dS ratio of 3.054. There are 70 different SeM sequences identified as of 26/03/10 on the online SeM database (http://pubmlst.org/szooepidemicus/sem/) and sequence variability of SeM can be seen during the course of strangles outbreaks with up to 9 different SeM alleles identified in one outbreak. We hypothesized that sequence variability of SeM may provide a selective advantage to *S. equi* in evading protective immunity in the guttural pouch, whilst maintaining its fibrinogen binding function enabling the development of a carrier state, a major factor in the persistence of strangles.

Functional analysis of SeM variants has revealed that there are significant differences in the relative amount fibrinogen binding to 24 recombinant SeM alleles. In a minority of SeM alleles, fibrinogen binding function is lost or severely reduced for several reasons including a truncation of the protein due to a premature stop codon; a deletion/insertion in the variable region or an amino acid substitution in the variable region resulting in a charge and structural change. To test if there was an antigenic consequence of SeM variation, recombinant SeM variable region (aa37–184) was generated for 25 SeM alleles and the relative binding of sera from horses naturally infected with *S. equi* was determined using an ELISA. It has been determined that there is no significant affect with sera from naturally infected horses.

51 genomes of *S. equi* have been analysed using Illumina genome sequencing technology. Single Locus Polymorphisms (SNP's) in the genomes have been identified and analysed using Artemis. SNP's accumulating in the genomes of isolates during the course of an outbreak can be seen. The most variable LPxTG anchored protein of *S. equi* is SeM. FneE; SclF; SzPSe and Cne also contain non-synonymous SNP's compared to Se_4047. The frequency of carrier horses is likely to be higher than previously thought as many isolates which would have initially been thought of as the same or likely to have evolved from an earlier isolate in the same outbreak are different by genome analysis. Gene deletions have been observed in some isolates including phiSeq1 and *hasA* and *hasB*. Interestingly, isolates from the guttural pouch of one horse have lost several genes including the equibactin locus on ICESe2; the citrate utilization locus; and phiSeq1. It is likely that this isolate was in the guttural pouch for a long period of time and has accumulated a number of changes in its genome to enable it to persist and has lost genes not required for survival in chondroids. It would be interesting to see if this isolate was still able to cause strangles in a naïve horse.

Offered paper Diversity of the emerging pneumococcal serotype 6C in the UK

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6C is a recently recognized serotype of *Streptococcus pneumoniae*. This serotype is now responsible for significant serotype replacement in UK carriage and disease. Importantly, it is not included in any of the licensed pneumococcal conjugate vaccines. We sought to describe the genetic diversity of serotype 6C isolates from carriage and disease in the UK.

Serotype 6C isolates from carriage (n=14) and from invasive disease (n=4) were analysed to determine genetic diversity. Nine different

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STs were observed in serotype 6C carriage over the winters 2006/7 to 2008/9. Sequence type (ST) 1692 was the predominant ST amongst carried serotype 6C during winter 2008/9. Prevalence of this ST increased significantly between winter 2006/7 and 2008/9. Importantly a 6C invasive disease isolate from 2008 was also determined to be ST 1692.

The pneumococcal serotype 6C population in the UK is both diverse and dynamic. Traditionally, serotype 6C has not been associated with invasive disease. The occurrence of serotype 6C invasive disease has substantial clinical repercussions, especially in light of the recent introduction of pneumococcal conjugate vaccines. The observed diversity of the serotype 6C population demonstrates that clonal expansion and resultant serotype replacement is occurring in the UK. This has implications for future conjugate vaccine policy.

Physiology & communities workshop – introduction

Nick Jakubovics[1] & Angela Nobbs[2]

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In natural environments most streptococci, like the majority of bacteria, exist in mixed-species communities such as biofilms. Interactions with neighbouring bacteria play a fundamental role in community development and in shaping the physiology of streptococcal cells. Some interactions are competitive, whilst others are clearly beneficial to one or more of the interacting organisms. The consequences of interbacterial interactions are extremely complex, yet they cannot be ignored if we wish to understand the success of streptococci in colonizing their natural habitats. This workshop will focus on the mechanisms utilized by streptococci for colonization of host organisms and for integration into mixed-species communities. There will be an opportunity to discuss our current understanding of streptococcal interactions with bacterial and eukaryotic cells, and to explore the best approaches to advance these areas in the future.

Offered paper Characterization of the FeoAB iron transport system of *Streptococcus thermophilus* and link with oxidative stress response

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Streptococcus thermophilus – a non pathogenic *Streptococcus* – is widely used as starter in dairy product manufacture, in combination with *Lactobacillus bulgaricus* in yoghurt. *S. thermophilus/L. bulgaricus* co-culture in milk leads to the decrease of transcription of most of the genes potentially involved in iron transport, whereas ferritin-like and ferric up-take regulator gene expression increases (1) which probably result in reduction of intracellular [iron]. We explored here the role of *S. thermophilus* FeoAB system – a system poorly characterized in non-pathogen Gram$^+$ bacteria – and its potential involvement in oxidative stress.

Characterization of growth of the wild-type and the Δ*feoAB* negative mutant indicated that i) *feoA* and *B* genes are dispensable for growth, ii) FeoAB is the major transporter of iron, and iii) FeoAB is dedicated to ferrous (Fe^{2+}) iron transport. Furthermore, we showed that *feoB* gene expression was not regulated by extracellular iron. In addition, *feoA* gene expression is repressed in presence of H_2O_2 (1). Δ*feoAB* growth is less affected by H_2O_2 than wild-type growth, probably because of a quasi absence of iron transport in Δ*feoAB*. Altogether these results suggest that FeoAB system is regulated by H_2O_2 in order to limit the intracellular Fenton reaction ($H_2O_2 + Fe^{2+}$ => ROS + Fe^{3+}) that is highly deleterious for the bacterium.

Reference 1.Herve-Jimenez, L., Guillouard, I., Guedon, E., Boudebbouze, S., Hols, P., Monnet, V., Maguin, E. & Rul, F. 2009. Postgenomic analysis of *Streptococcus thermophilus* cocultivated in milk with *Lactobacillus delbrueckii* subsp. *bulgaricus*: involvement of nitrogen, purine, and iron metabolism. *Appl Environ Microbiol* 75:2062–2073.

Offered paper *Streptococcus pyogenes* AspA (antigen I/II) polypeptide interacts differentially with salivary agglutinin (gp-340)

SARAH E. MADDOCKS[1], Christopher J. Wright[1], Angela H. Nobbs[1], Linda J. Franklin[1], Nicklas Stromberg[2], Aras Kadioglu[3], Mark A. Jepson[1] & Howard F. Jenkinson[1]

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Streptococcus pyogenes is the causative agent of a number of infectious diseases ranging from tonsillitis to necrotizing fasciitis with 500,000 associated deaths per annum. It belongs to a heterogeneous group that readily colonizes humans, relying upon the primary interactions of streptococcal cell surface adhesins with host proteins. Antigen I/II (AgI/II) polypeptides are cell wall anchored surface adhesins expressed by most indigenous oral streptococci and have been recently identified on the surface of *S. pyogenes* (AspA). They

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interact with multiple host tissue proteins and cellular receptors, specifically recognizing innate immunity glycoprotein gp-340, found at mucosal surfaces. AgI/II proteins of oral streptococci are known to contribute to biofilm formation, but no such role has been previously described for AspA.

AspA shares significant amino acid sequence similarity (30–40%) with other AgI/II family members, including the common NAVPC structure with conserved alanine (A) and proline (P) repeats supporting the more divergent N and V regions, and a conserved C region. The V region of AspA contains a cluster of histidine residues characteristic of structural or metalloprotease metal ion-binding regions and is a feature that is absent from viridans AgI/II proteins. Regional amino acid differences between AspA and other AgI/II protein family members have the potential to confer a differential binding function. This study identified the differential interaction of AspA with gp-340 and clarified a role for AspA in mediating biofilm development.

Functional and comparative assays using a surrogate *Lactococcus lactis* expression system confirmed that AspA interaction with immobilized gp-340 is comparable to that of other members of the AgI/II family, but AspA is not involved in inter-cellular aggregation mediated by fluid phase gp-340 which is a unique characteristic compared to AgI/II proteins of the oral streptococci. Recombinant protein fragments and Far Western blotting identified a unique gp-340 binding profile for AspA, strongly suggesting that the three dimensional protein structure is necessary for interaction with gp-340, possibly reliant on the metal ion binding site, which was found to be associated with Zn^{2+}.

NT04 Acid stress

↑CONTENTS

My gut feelings on acid tolerance of food-borne pathogens: a view from fresh produce processing and storage practices

Arvind Bhagwat

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Ready-to-eat, minimally processed fruits and vegetables ideally suit health conscientious busy societies. However ready-to-eat produce is not subjected to any 'kill-steps' such as surface pasteurization or cooking and it heavily relies on refrigeration temperatures and modified atmosphere packaging to restrict the microbial load. Thus minimally processed fresh-cut produce, which is often eaten raw, represents a new challenge to food safety. Many of the foodborne pathogens themselves are evolving, and new pathogens and strains are emerging that are adapted to new environmental niches. The presentation will focus on understanding and addressing acid tolerance of food borne pathogens with special emphasis on outbreak associated strains. Some of the important topics covered are gene regulation for acid tolerance and selective pressures exerted by fresh-cut preparation methods, and relation between acid-tolerance and infective dose.

Glutamate-based acid resistance in *Escherichia coli*: biochemical and regulatory aspects

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Escherichia coli has the ability to resist severe acid stress, as that encountered during transit through the host stomach, and this is instrumental to host gut colonization. The glutamate-based acid resistance (AR) system plays a major role in the protection of the cell from the deleterious effects of a high-proton-concentration environment. Structural genes of this system are *gadA*, *gadB* and *gadC*, which encode two glutamate decarboxylase isoforms and a glutamate/γ-aminobutyrate (GABA) antiporter, respectively. Glutamate decarboxylation leads to both proton consumption and production of GABA, a neutral compound exported via the GadC antiporter. Even though the *gadA* and *gadBC* genes are 2,1 Mb apart, their transcription is under the control of the same regulators: GadE, GadX, GadW, H-NS and RpoS. These regulators also affect the expression of 12 genes located in the acid fitness island (AFI). We have identified the GadX (GadW) binding site, a 42 bp sequence, in the regulatory regions of *gadA*, *gadBC*, *slp*, *hdeAB*, *gadE* and *gadY*. All are AFI genes, but *gadBC*. In my talk I will show the most recent results from *in vivo* and *in vitro* analyses aimed at fully characterizing the GadX regulon. In addition I will show biochemical data on the decarboxylase's intracellular activation/inactivation process and on additional effectors involved in GABA export.

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Offered paper **Protection of *Escherichia coli* by putative alarmones against acid stress**

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We have investigated the activity of putative alarmones which have been hypothesized to confer bacterial protection against inimical treatments. Filtrates, prepared from *Escherichia coli* C600 after growth at pH 4.0 and pH 7.0, were adjusted to pH 7.0 and incubated for 30 min (1:1 ratio) with mid-log phase *E. coli* C600. Aliquots (20µl) were then dispersed in pH 3.0 nutrient broth (100 ml) and 60 minutes kill-curves generated. Supernatants from acid-equilibrated cultures conferred statistically significant protection ($P<0.01$) on recipient cultures. Alarmones were inactivated by heat (60°C) and by proteinase K, but not at 4°C or 20°C. Microarray analyses of transduced cultures indicated that 671 open reading frames (ORFs) were significantly differentially expressed between alarmone-protected and control populations (FDR<0.05). ORFs (508) were up-regulated in the induced cells including different multidrug efflux system proteins (6) and an acid-resistance membrane protein whereas 163 ORFs were down-regulated. This work confirms previous observations that *E. coli* releases diffusible signalling compounds which mediate adaptation to stress of recipient/neighbouring cells (Rowbury & Goodson, 1998, 1999).

References Rowbury, R.J. & Goodson, M. (1998). Induction of acid tolerance at neutral pH in log-phase *Escherichia coli* by medium filtrates from organisms grown at acidic pH. *Lett Appl Microbiol,* 26, 447–451; Rowbury, R.J. & Goodson, M. (1999). An extracellular acid stress-sensing protein needed for acid tolerance induction in *Escherichia coli*. *FEMS Microbiol Lett,* 174, 49–55.

Offered paper **A systems biology approach identifies OmpR as a key regulator of acid adaptation in *Escherichia coli* BW25113**

ANNA STINCONE, Nazish Daudi, Ayesha S. Rahman, Phillip Antczak, Wazeer Varsally, Nil Turan, Peter Lund & Francesco Falciani

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The ability to survive to strong acidic conditions is an important pathogenicity factor in *E. coli*. So far four main acid response systems have been identified but our understanding of the regulatory networks underlying adaptation to an acid environment is still poor. The aim of our study was to characterize the transcriptional response of the *E. coli* strain BW25113 to exposure to mild acid conditions, to identify the molecular components required for survival in strong acid conditions and to infer the molecular networks regulating such a response.

We have discovered that mutant strains deficient in the regulation of a number of energy metabolism and transport pathways have more severe phenotypes than mutants in genes belonging to canonical stress response systems, suggesting that a tight integration between proton detoxification mechanisms and bacterial physiology is required for efficient survival. In addition, the application of network inference techniques has lead to the hypothesis that OmpR may be a key regulator of the complex transcriptional program involved in the regulation of acid adaptation. Experimental validation of our model, based on the analysis of a ompR strain has shown that this regulator is associated to the stronger phenotype we have observed. consistent with this observation, the transcriptional response to acid exposure in this mutant is severely impaired.

EvgS/EvgA signal transduction: transcriptional cascades and acid resistance

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Acid resistance in *Escherichia coli* is regulated by various factors. Among these, the EvgS/EvgA system is the major two-component signal transduction system (TCS) that confers acid resistance. We focus on the sensor EvgS and investigate how this system regulates such resistance. An initial transcriptome analysis of a constitutively active EvgS mutant revealed the induction of acid resistance genes, the PhoP regulon, and RpoS-regulated genes initiated by EvgS/EvgA activation. Activation of EvgS in fact initiated a transcriptional cascade of regulators, EvgA YdeO GadE, and conferred acid resistance to log phase cells. The EvgS/EvgA system also activated the PhoQ/PhoP TCS through a connector, SafA (encoded by b1500 and constituting an operon with ydeO), which is a small membrane protein that directly activates the sensor PhoQ. PhoQ/PhoP then induced another connector, IraM, which binds to a response regulator RssB and then accumulates RpoS in log phase cells. Studies on the acid resistance of mutants showed that SafA, PhoQ/PhoP, and IraM all participated in the EvgS/EvgA-induced acid resistance. We propose a model showing how the signal transduction cascade from EvgS/EvgA to PhoQ/PhoP and RssB contributes to the acid-resistant phenotype. Results of ongoing studies on the environmental signal to which EvgS responds are also introduced.

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Offered paper **The *Campylobacter jejuni* acid-induced *cj1501* gene is required for formate dehydrogenase expression and activity**

F.L. SHAW, I Porcelli, B.M. Pearson, F. Mulholland & A.H.M. van Vliet

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Campylobacter is one of the major causes of foodborne bacterial gastroenteritis worldwide, but its pathogenicity is still poorly understood. Recent advances in functional genomics now allow for the identification of genes which are up-regulated during biologically relevant stress conditions. For *Campylobacter*, low pH is a relevant stress, since the pathogen has to survive passage through the acidic stomach to reach its intestinal niche. Here, we have used transcriptomic approaches to investigate the response of *C. jejuni* to *in vitro* shocks at acidic conditions (pH 3.6 and 5.0). Within the ~100 genes up-regulated at acidic pH, we further characterized the *cj1501* gene which encodes a small protein with a SirA-like domain. A *cj1501* knock-out mutant was constructed by insertional inactivation, and comparison of protein profiles of the wild-type strain and *cj1501* mutant by proteomic approaches revealed that the formate dehydrogenase (FDH) enzyme was absent only in the *cj1501* mutant. The *cj1501* mutant lacked FDH enzyme activity when assessed by a benzyl viologen dye-coupled reaction, thus confirming the proteomic investigations. We are further characterizing the function of Cj1501 to allow for improved understanding of the adaptations used by *C. jejuni* during gastrointestinal infection and transmission via the food chain.

Offered paper **Investigating physiological markers for onset of growth of *Bacillus cereus* during acid-induced lag phases**

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Physiological parameters of *Bacillus cereus* were investigated during acid stress induced lag times to indentify markers for the transition between lag-phase and growth on both population and single cell level. During lag times induced by exposure to pH 4.9, pH 5.3 with lactic acid and pH 7.0, the pH of the medium, ATP levels and cell counts remained stable. In contrast, an increase in optical density was observed. Flowcytometry in combination with fluorescence probes, such as PI (membrane permeability), $DiOC_{2(3)}$ (membrane potential), cFDA (esterase activity), and C12-resazurin (electron transfer), were used to assess performance at single cell level. Although different time frames were observed in the control versus acid-stressed conditions, electron transfer chain activity, membrane integrity, and esterase activity, based on induction of cFDA fluorescence, increased upon the onset of growth. Assessment of the membrane potential showed high signals in early lag phases, followed by decreased signals during the lag phase and a complete depolarization after growth resumed.

This study shows that similar physiological trends were observed in lag-phases of non-stressed and acid-stressed cells. Based on the probes used, electron transfer and membrane potential can be used as markers for transition to the growth phase at single cell level.

Keywords: Flowcytometry, pH, lactic acid, growth boundary

What's a tRNA modification enzyme doing in my regulatory soup?

John Foster

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Glutamate-dependent acid resistance (GDAR) is the most effective AR system in *Escherichia coli*. Expression of GadE, the central transcriptional activator of GDAR, is activated by several regulatory systems that converge on an upstream 798 bp sensory integration region (SIR) composed of three promoters. The main *gadE* induction pathways are EvgAS-YdeO (minimal media pH<7), RpoS-GadY-GadX (stationary phase in LB) and an ill-defined pathway induced by growth in LB glucose. We now show numerous carbohydrates added to LB will repress the RpoS-dependent pathway and induce the alternate pathway. Activation of the alternate pathway requires the tRNA modification enzymes MnmE and GidA. MnmE activates, probably indirectly, the *gadE* P2 promoter. In LB, added carbohydrate must be metabolized to inhibit *rpoS* transcription. This inhibition diminishes RpoS-specific *gadE* activation and makes *gadE* expression reliant on MnmE. Surprisingly, carbohydrate metabolism is NOT required to activate the MnmE-dependent pathway. However, knocking out sugar-specific transcriptional activators will prevent induction by the cognate sugar but not by other carbohydrates (e.g. an *araC* mutation prevents induction by arabinose but not glucose). Screening the ASKA library of *E. coli* ORFs identified several genes able to suppress the effect of a *mnmE* mutation on *gadE* expression.

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pH stress in individual cells of *Escherichia coli* and *Bacillus subtilis*

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Neutralophilic bacteria such as *Escherichia coli* and *Bacillus subtilis* maintain pH homeostasis during growth at external pH 5–9, and express systems enabling survival at more extreme pH. The role of pH homeostasis in growth and survival remains poorly understood. Cell-to-cell variation under pH stress is unknown: Do certain subsets of cells maintain pH more strongly than others? Do septating cells become more proton-permeable, and hence more vulnerable to pH stress? Does filamentation protect cells from extreme pH? Cytoplasmic pH was measured for individual cells of *E. coli* and *B. subtilis*, using GFP-pHluorin fluorescence microscopy. Bacteria were sampled on slides coated with agarose or poly-L-lysine and perfused with buffered medium. Ratiometric imaging (425nm/465nm) with LED light sources yielded pH values. Standard curves were obtained by collapsing the transmembrane ΔpH with benzoate and methylamine. Cytoplasmic pH of slide-trapped *E. coli* appeared comparable to that of suspended cells. In sporulating *B. subtilis* AG174 ($pGFP_{ratio}$), the forespore pH was measured distinctly from that of the mothercell. The bacteria sporulated most effectively at high pH; and, contrary to previous reports, the forespore pH appeared to be higher than that of the mothercell, by approximately 0.5–1.0 unit.

Heterogeneity in the response of *Bacillus cereus* to acid stress: the origin of secondary oxidative response and radical accumulation

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Bacillus cereus is a notorious food borne pathogen and spoilage organism. Low pH and organic acids are often used to preserve foods. Therefore, we dedicated our research to elucidate the phenotypic and transcriptional responses of *B. cereus* to acid stress. By investigating physiological, transcriptional, and single cell responses upon exposure to mild, bacteriostatic, and lethal conditions common and specific responses were revealed. The well-known general stress response, including alternative sigma factors, chaperones, and proteases, was induced in *B. cereus*. More interestingly, a common oxidative response was revealed as shown by the induction of oxidative stress-associated genes encoding catalases, superoxide dismutases, and thioredoxins. Furthermore, single cell analysis employing flow cytometry in combination with fluorescent probes, revealed excess formation of reactive oxygen species, such as superoxide and hydroxyl, to be associated with exposure to lethal stress conditions, with no evidence for excess radicals formed in non-lethal conditions. Since stress-induced secondary oxidative stress and radical formation appeared to be absent in anaerobically stressed cells, it is proposed that this secondary oxidative response is induced in actively respiring cells by superoxide originating at the electron transport chain. Subsequently, highly reactive oxygen species can be formed that damage vital cellular components and macromolecules.

Listeria monocytogenes: overcoming acid stress in food and *in vivo*

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Listeria monocytogenes encounters many different stresses at different points in its infectious cycle, which may involve prolonged periods in the environment and in food before encountering the biological stresses associated with infection. Acid stress is one of the principal challenges in all environments (organic acids in food, inorganic acids in the stomach and even bile acids in the gut environment. It has been known for some time that *Listeria* can mount an acid tolerance response (ATR) which can assist its survival under pH stress, and we are now beginning to understand the molecular mechanisms responsible for the ATR. Could a better understanding of the ability of *Listeria* to overcome acid stress help us to predict the outcome of infection? In addition, the role of the food vehicle in foodborne disease and the impact of food constituents on the physiology of both organism and host may have been underestimated. Does the immediate 'history' of the food in terms of processing or storage conditions impact on microbial physiology? Could the presence of certain food ingredients or micronutrients help to 'program' a pathogen in a way which may make it more or less likely to lead to an infection? This concept will be explored using *Listeria monocytogenes* as a model organism.

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Acid stress response and survival in *Listeria monocytogenes*

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Survival of the foodborne pathogen *Listeria monocytogenes* in acidic environments (e.g. during gastric passage) is vital to its transmission. *L. monocytogenes* can acquire enhanced resistance to lethal acid conditions through multiple mechanisms. Our laboratory has focused on elucidating the contributions of the stress responsive alternative sigma factor, σ^B, which is encoded by *sigB*, to *L. monocytogenes* acid stress response and survival. An *L. monocytogenes* Δ*sigB* mutant is severely reduced in its ability to survive acidic conditions (pH 2.5) compared to its parent strain. σ^B also regulates transcription of genes contributing to acid resistance, including genes encoding components of the glutamate decarboxylase (GAD) system. Growth temperature (i.e., 7°C, 30°C, or 37°C) also affects subsequent *L. monocytogenes* survival in artificial gastric fluid (AGF) or acidified BHI (ABHI) at 37°C. *L. monocytogenes* cells grown at 7°C were less resistant to AGF or ABHI than bacteria that had been grown at higher temperatures (i.e. 30°C or 37°C). For *L. monocytogenes* grown at 7°C, stationary phase cells were more resistant to ABHI than log phase cells, indicating both temperature and growth phase effects on acid survival. Temperatures commonly encountered during food storage and distribution affect the ability of *L. monocytogenes* to survive acid challenge.

Acid tolerance and dental caries: the story of *Streptococcus mutans*

Dennis Cvitkovitch

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Streptococcus mutans resides in the biofilm of dental plaque where it produces acid from dietary carbohydrate to cause dental caries. It utilizes a number of physiological traits that allow it to persist in the oral cavity and increase its numbers in the complex plaque community. These properties include its abilities to: produce and tolerate acid end-products, primarily lactate; synthesize insoluble extracellular glucan; make intracellular glycogen and rapidly metabolize sugars. *S. mutans* uses a quorum-sensing signaling system in biofilms to activate multiple phenotypes including acid tolerance. This presentation addresses mechanisms by which cell-cell and environmental signals activate this acid resistant 'biofilm phenotype'. DNA microarrays and proteomic analyses have identified genes and proteins activated by acidic pH and CSP. Mutants defective in various components of the CSP and ATR systems have shown that *S. mutans* senses a number of environmental signals and activates specific regulons via a number of TCSTS. Virulence properties including extracellular glucan production and detection of acidic environments are modulated through the vicRKS and liaRS systems. A number of cross interactions between various TCSTS are also proposed to exist. This presentation will discuss some of these interactions and strategies to dissect and exploit them to modulate the virulence of *S. mutans*. These studies were supported by NIH NIDCR grant DE013230 and CIHR grant MT-15431.

Acid tolerance strategies of commensal and pathogenic oral streptococci

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The microbial biofilms coating the surfaces of the oral cavity are diverse communities that remain stable over time unless subjected to stress. pH has an especially profound effect on the composition and pathogenic potential of the biofilms on teeth. While commensal oral streptococci and the caries pathogen *Streptococcus mutans* share some common acid tolerance systems, fundamental differences in the way these organisms utilize arginine or its derivative agmatine in a manner that affects acid tolerance are evident. Many commensals associated with dental health harbor the arginine deiminase (AD) pathway, which is absent in *S. mutans*. Conversely, a subset of oral streptococci, including *S. mutans*, harbors the analogous agmatine deiminase (AgD) pathway. Induction and repression of the AD and AgD genes is orchestrated by pathway-specific transcriptional regulators, various two-component systems and global regulators in response to many factors, including substrate, low pH, oxygen and nutrient source. Despite similarities in the biochemical reactions of the A/AgD systems, substantial variation in the contributions of the systems to acid tolerance and in the control of induction, pH-responsiveness and catabolite repression of the A/AgD genes is evident. Collectively, these studies highlight some important differences in acid adaptive strategies of commensal and pathogenic oral streptococci.

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Resistance of yeasts to weak organic acid food preservatives

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The plant materials where fungi grow as saprophytes often contain high, potentially toxic concentrations of weak organic acids. Yeasts are endowed with protective mechanisms that ensure that the acid does not accumulate at a high level within their cells. Though these systems probably evolved to facilitate growth at low pH in the presence of organic acids, they pose problems for the food industry as they enhance the resistance of spoilage organisms to the major organic acid food preservatives.

In *S. cerevisiae* resistance to moderately lipophilic propionate, benzoate and sorbate depends upon the ability to induce a plasma membrane ATP binding cassette (ABC) transporter (Pdr12p) [1, 2]. This confers resistance by catalyzing the active efflux of the acid from the cell. In contrast, resistance to acetate is by destabilizing Fps1p, the plasma membrane aquaglyceroporin through which undissociated acetic acid enters the cell. Acetate stress activates Hog1p MAP kinase which then phosphorylates Fps1p, this being the signal for Fps1p to be ubiquitinated, endocytosed, and then degraded in the vacuole [3].

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Offered paper The decarboxylation of the weak-acid preservative, sorbic acid, is encoded by linked genes in *Aspergillus* spp.

MICHAELA NOVODVORSKA, Andrew Plumridge, Petter Melin, Malcolm Stratford, Lee Shunburne, Paul S. Dyer, Jacques Stark, Hein Stam & David B. Archer

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The ability to resist anti-microbial compounds is of key evolutionary benefit to micro-organisms. Certain fungi exploit such resistance mechanisms to overcome food preservatives and cause spoilage in foods and beverages. *Aspergillus niger* has previously been shown to require the activity of a phenylacrylic acid decarboxylase (encoded by *padA1*) for the decarboxylation of the weak-acid preservative sorbic acid (2,4-hexadienoic acid) to 1,3-pentadiene. It is now shown that this decarboxylation process also requires the activity of a putative 4-hydroxybenzoic acid (3-octaprenyl-4-hydroxybenzoic acid) decarboxylase, encoded by a gene termed *ohbA1*, and a putative transcription factor, sorbic acid decarboxylase regulator, encoded by *sdrA*. The *padA1, ohbA1* and *sdrA* genes are in close proximity to each other on chromosome 6 in the *A. niger* genome. Further bioinformatic analysis revealed conserved synteny at this locus in several *Aspergillus* species and other ascomycete fungi (including the yeast *Saccharomyces cerevisiae*) indicating clustering of metabolic function. This cluster is absent from the genomes of *A. fumigatus* and *A. clavatus* and, as a consequence, neither species is capable of decarboxylating sorbic acid, and show sensitivity to this preservative.

Offered paper Utilization of a high-throughput screening process to characterize the sensitivity of ethanologenic yeast to inhibitors released by the breakdown of lignocellulosic material

D. GREETHAM, T. Wimalasena, S. Lawrence,, R. Linforth, M.E. Marvin, F. Dafhnis-Calas, S. Brindley, G. Liti, E. Louis & K.A. Smart

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Fermentation of sugars released from lignocellulosic biomass (LCMs) is potentially a sustainable option for the production of bioethanol. LCMs release both hexose sugars and pentose sugars but five-carbon sugars are not efficiently fermented by *Saccharomyces* yeasts; ethanol yield from lignocellulosic residues is dependent on the efficient conversion of all available sugars to ethanol. One of the challenges facing the commercial application for the conversion of lignocellulosic material to ethanol is the presence of inhibitors released by the physicochemical breakdown of plant cell walls. Using Phenotypic Microarray PM (Biolog) analysis a screen has been developed to determine the sensitivity of yeast strains to inhibitors released by the breakdown of straw under anaerobic and aerobic conditions. Inhibitor sensitivity showed that pentose utilizing yeasts (*Candida* and *Pichia*) are sensitive to the inhibitors released by the breakdown of the plant cell wall but *Saccharomyces cerevisiae* strains are more robust. This study focused on the effect of acetic acid, formic acid and furfural as they were present in the highest concentrations from the breakdown of hemicelluloses to pentose sugars. Other inhibitors were present, but at concentrations which did not affect yeast viability. Presence of acetic acid or formic acid at mM concentrations in the fermentation media lead to a loss of viability, a decrease in sugar consumption and impaired ethanol production

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in *S. cerevisiae.* There appeared to be a synergistic effect when acetic and formic acid were present in the same assay. The results also revealed that the inhibitory effect of acetic acid could be reduced by increasing media pH but that the presence of oxygen had little effect on inhibitor toxicity.

The genetic regulation of pH sensing and adaptation in *Aspergillus*

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Aspergilli are tolerant to a broad range of pH perturbations. In part this is due to a dedicated regulatory pathway comprising the PacC transcription factor, and several upstream signalling molecules. PacC regulates the expression of genes required for pH tolerance and this conserved regulatory system is widely implicated in fungal virulence of animals, plants and insects.

Recently we undertook a detailed analysis of alkaline adaptation by the pathogenic mould *Aspergillus fumigatus* by profiling global gene expression, in a temporal fashion, following alkaline shift. Studies of the wild type isolate identified a heavy reliance upon metabolic adaptations during the early phase of alkaline stress. This was accompanied by altered ion transportation and a later decline in respiration, thereby placing the activity of the fully functional PacC signalling pathway within the context of cellular homeostatic control. Relative to other model fungi we identified significant regulatory divergence in *A. fumigatus* whereby alkaline adaptation occurs in a calcium non-dependent manner. Transcriptional studies of a *pacC* null mutant during murine infection identify stage-specific deficits, predominantly in transporter expression. Collectively these analyses point to a heavy dependence upon the vacuole during fungal alkaline growth and virulence.

NT05 Bioremediation of metals ↑CONTENTS

Nano-scale bioengineering; harnessing subsurface bacteria for bioremediation

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The microbial reduction of Fe(III) minerals plays a critical role in controlling the mobility of both inorganic and organic species in the subsurface and offers the basis for flexible and robust bioremediation processes. The nature of the Fe(II)-bearing biomineral phase formed is especially important in mediating 'indirect' reductive transformations of xenobiotic organics and redox active toxic metals and radionuclides during contaminant clean up. We have used a range of approaches to optimize bioproduction of the nano-scale Fe(II)-bearing mineral phase for reductive transformations of organic and inorganic substrates. These include physiological manipulations of bacterial cultures, the selection of the optimal Fe(III)-mineral phase for bioreduction to highly reactive nano-scale biomagnetite and the incorporation of highly reactive transition metals into or onto the post-reduction biomineral to enhance performance. The physiological processes underpinning biomagnetite production will be described, in addition to the molecular-scale characterization of the resulting functional bionanominerals and their use in the detoxification of model organic contaminants, metals and radionuclides. Experiments conducted in batch contactors and sediment columns confirm that optimized nano-scale biomineral phases can be used effectively for both *in situ* and *ex situ* remediation of a broad-range of contaminants.

Bioremediation of subsurface aquifers

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Biostimulation experiments in a shallow aquifer at the U.S. Department of Energy's Integrated Field Research Challenge site in Rifle, Colorado are providing key information on subsurface biogeochemical processes controlling the transformation of aqueous U(VI) to immobile sediment-associated U(IV). These studies exhibit a general consistency in microbially-mediated changes in uranium redox status that supports the potential deployment of engineered bioremediation of metals in groundwater systems. In the initial ~30 days of acetate amendment, U(VI) concentrations decrease below the regulatory limit concomitant with elevated Fe(II) concentrations indicative of active iron-reducing bacteria. This is followed by dominantly sulfate reducing conditions accompanied by a partial rebound in U(VI) concentrations. Continued Fe-reduction during sulfate reduction likely explains the sustained removal of U(VI) even during maximum rates of sulfate reduction. Interestingly, post-biostimulation removal of influent U(VI) continues for several months depending on the duration of biostimulated sulfate reduction. Microbial community composition, activity, and metabolic status are tracked via

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proteogenomics in order to constrain reactive transport models that employ either Monod kinetics or *in silico* models of microbial metabolism. The coupled-process simulations are used to mechanistically explore controls on *in situ* bioremediation with the prospect of optimal design over a wide range of subsurface hydrologic and biogeochemical conditions.

In situ bioremediation of metals: case studies and metal sulfide stability issues

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Heavy metal contamination of soil due to anthropogenic sources e.g., non ferrous industrial sectors, is a worldwide problem. Further, the leaching of metals by infiltrating rainwater from the polluted soil eventually contaminates the groundwater. In the last decades, a lot of research has been accomplished to develop efficient technologies for the treatment of metal-polluted effluents and groundwater. However, most of these methods adapted are mostly pump and treat, which become particularly impracticable for huge contaminated zones where large volumes of groundwater need to be pumped and treated. Biotechnology, in terms of *in situ* bioprecipitation (ISBP), offers an interesting possibility of metal removal from groundwater without the requirement of pumping up the large volumes of contaminated groundwater. ISBP involves amendment of a carbon source (electron donor) for metal bioprecipitation *via* the formation of metal sulfides, a process mediated by sulfate reducing bacteria (SRB). The SRBs, when provided with a suitable electron donor, reduce the available sulfate to sulfides, which then concomitantly precipitate the heavy metals and immobilize them. Despite the effectiveness as has been demonstrated in the field at several sites, the acceptance of *in situ* immobilization as a competent reclamation method therefore depends upon its ability to predict the long term stability of the metal precipitates, formed after ISBP process and proving the economic benefit of the technology.

Cold war biogeochemistry: microbial heroes in a former uranium mining site

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Groundwater near Ronneburg, Germany, is impacted by acid mine drainage, due to legacy uranium mining. Previous work in other uranium-contaminated environments has emphasized the role of biostimulated Fe(III)-reducing microorganisms on mediating uranium reduction. However, long term stability is a concern due to the potential for remobilization after stopping carbon addition. Therefore, natural attenuation processes might be an alternative. We aimed to achieve a detailed understanding of the microorganisms involved in the formation or reductive dissolution of minerals in the presence of heavy metals under slightly acidic conditions. Our studies demonstrate that heavy metal tolerant Fe(II) oxidizers play an important role via coprecipitation of metals with Fe(III)-oxides. In contrast, Fe(III) reduction was associated with an increase in Ni, Zn, and surprisingly, U concentrations suggesting a release of sorbed metals. However, downstream migration is expected to be low due to the low *in situ* activity of Fe(III) reducers which are affected by even small amounts of heavy metals. Sulfate reducers were more tolerant and formed metal sulfides. The experimental formation of amorphous and nanocrystalline nickel sulfides was confirmed via high resolution TEM and EDX analysis. Bacterial cell surfaces were highly encrusted by about 5 nm small precipitates. Altogether the results indicate that biomineralization is an important natural attenuation process.

Use of microbial biosensors in metal remediation

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Mitigation of metal contamination may either be achieved through the imobilsation of metals into a less toxic form or through enhanced leaching by forming labile ligands. Both are valid approaches and are being activley engaged at the field scale for sustainable solutions. Microbial biosensors are key to this process because they enable the practitioner to better understand the form and speciation of metals and this allows a solution to be matched to an individual problem. The sensors are able to interrogate complex matrices when applied appropriately.

In this study, a software system was developed to assess the most effective process for remediating metal impacted soils. In laboratory studies there was a close fit between an environmnetal scenario and the most suitable remedial strategy. The biosensor technology helped in the validation of the strategy implemented.

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The clean-up targets are derived from risk-based corrective actions. This means that they are protective of named receptors. However, on site metal solution analysis is time consuming and problematic to make chemical assessments, so once again the respose was calibrated against the biosensor.
Risk-based corrective targets validated by constitutive and elemental-specific sensors represents a new and sustainable approach for the real-time confirmation of remedial management.

Geomycology: metals, actinides and biominerals

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'Geomycology' can be considered a subset of 'geomicrobiology' and simply defined as the impact of fungi on geological processes, including the alteration and weathering of rocks and minerals, the accumulation of metals, and their roles in element and nutrient cycling. Many geomicrobial processes are of relevance to pollutant fate in the environment and our research seeks to understand the mechanisms of metal and mineral biotransformations, and their environmental and applied significance in bioremediation and also biodeterioration. This presentation will include examples of our research on fungi inhabiting certain rock types, soil, including the mycorrhizosphere, particularly regarding mineral dissolution and transformation, and the formation of secondary mycogenic minerals. An important mechanism of metal mobilization from minerals is a combination of acidification and ligand-promoted dissolution: if oxalic acid is produced, the production of metal oxalate biominerals may result. In other cases, mobilized metal species may interact with phosphate. Examples outlined in this presentation will include fungal communities and roles in degradation and transformations of rocks and metal-containing minerals, depleted uranium and uranium oxides, and fungal biodeterioration of concrete, the latter being of general biodeteriorative significance regarding built concrete structures as well as radionuclide containment and storage.

NT06 Workshop: prokaryotic taxonomy

Bacterial taxonomy in university life science education

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There is an expectation by students that science degrees should be based on the most modern material reflecting recent developments in the subject. Students expect to acquire a wide range of modern theoretical, practical and transferable skills commensurate with the degree title. In microbiology, there has been a shift in emphasis from the fundamental and traditional aspects of the science towards fashionable new areas. This is a pity because some of the essential and elementary skills are virtually ignored. All too frequently taxonomy is viewed by students as boring, old fashioned and unnecessary. Research funding has become difficult to obtain when the emphasis is taxonomic. With the spotlight on biodiversity, there has been a re-kindling of interest in taxonomy. The influence of the environment and climate change on biodiversity is of concern. Thus, there is a clear need for good taxonomy to identify changes in natural populations caused by external influences. 16S rRNA sequencing is a modern technique, which students are keen to learn, and which has led to increased confidence in taxonomic processes, including the recognition of new taxa. There is hope that students may be motivated by bacterial taxonomy by emphasizing it wide applications to real life situations.

The use of whole-genome sequence data for bacterial typing

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Whole genome sequencing has been used to investigate genomic diversity and evolution. Comparative genome analysis can pinpoint diversity across the whole genome, and thus provide high-resolution genotyping. Using second-generation sequencing technology platforms it is now possible to undertake high-resolution genotyping for large numbers of strains, and therefore investigate bacterial population diversity. With the ongoing decrease in sequencing costs, the possibilities for using genome sequencing as a routine genotyping tool are growing. Using Illumina high-throughput genome sequencing we have been able to produce a high-resolution view of the epidemiology and micro-evolution of meticillin-resistant *Staphylococcus aureus* (MRSA). We have investigated globally lineages of MRSA and have been able to identify global geographic structure within lineages, and intercontinental transmission through four decades. Our work has also revealed the potential for detection of person-to-person transmission within a hospital environment.

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Multilocus Sequence Analysis (MLSA) as tool for classification and identification of bacteria

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Bacterial classification and identification form together with bacterial nomenclature, the three cornerstones of bacterial systematics. Whole bacterial genome sequences are becoming available to the public at an exponential speed. It is more and more broadly accepted that about 15% of the genome content is common throughout the bacterial world.

Modern bacterial systematics is based on the natural genomic evolutions and therefore in theory the bacterial genome should form the basis of bacterial taxonomy. Bacterial species delineation is far from straightforward and to some extent artificial. Where can bacterial genomes help to delineate species and do we have a pragmatic approach? In this context, a well underpinned MLSA approach seems to be very useful for building a taxonomic framework and to obtain reliable identifications. Therefore, the loci need to be selected very carefully and the obtained sequences must be compared with care and criticism. Before MLSA schemes can be used they need to be validated to delineate the borderlines of the species. DNA-DNA hybridizations and ANI (average nucleotide identities) are parameters for this validation process.

Introducing students to bacterial taxonomy through a hands-on laboratory experience

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Many students see bacterial taxonomy and systematics as boring topics with no place in modern biological science. A hands on experience in the classroom greatly enhances any educational activity and helps maintain the student's interest in the subject matter. For a number of years I have taught a lecture class entitled 'Prokaryotic Diversity'. Recently I started a laboratory class (BIOL 4126) to complement the lecture class. The students work with isolates from a specific bacterial genus/family group. They use a selective enrichment technique to isolate their own strains from environmental samples. The students apply a polyphasic approach and for those techniques not available at LSU they obtain the data from collaborators or commercial labs. Using the data collected the students prepare a draft *IJSEM* manuscript which after review they correct it to a final submission standard paper, on which they are graded. In addition, they are graded on a database they prepare, on a presentation they give, as well as a blog that they write each week. I find that the majority of the students see the class as a true research experience and make a connection to the importance of bacterial taxonomy in the real world.

Modern prokaryote taxonomy: back to basics

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Taxonomy comprises three major elements, characterization, classification and nomenclature. Of these three aspects nomenclature is the most prominent aspect, particularly when confronted with changing names. However, characterization and classification of an organism are the most important aspects and the name used is a simple consequence of these two processes. Changing names simply flag new knowledge gained at either of the previous levels.

Contrary to popular belief there is no one 'correct' taxonomy or nomenclature, because both depend on aspects of theory and philosophy, as well as opinion/interpretation. A classification system based on risk assessment alone or on cellular morphology may be fully adequate if that is all one is trying to achieve. One reason why such a system may be inadequate is if the addition of more data does not correlate with the existing classification. Past taxonomies (and nomenclature) relied on a limited data set that coloured the process of classification. However, with an ever increasing data set one goal is to cater for the needs of a wide spectrum of end users who will find the resulting classification (taxonomy and nomenclature) useful. In the ideal situation all parameters examined would fit nicely into a simple classification that would remain inherently stable. Unfortunately, we know both from the past and more recent experience that biology does not lend itself well to such wishful thinking and one of the key issues that arises is which data sets are more reliable and provide us with useful classifications?

Confronted with the possibility of gathering all information on an organism (both total genome sequences together with the interpretation of the expression of the genome – at all levels) some of the key issues that arise centre on principles such as 'information content', 'predictability' and the 'purpose' of the classification. Only when one has tackled these issues will one be able to formulate suitable solutions.

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NT07 New insights into secondary metabolism

The regulation of secondary metabolism and mutualism in the insect-pathogenic bacterium *Photorhabdus luminescens*

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Photorhabdus luminescens is a species of Gram-negative bacteria that is pathogenic to insects whilst also maintaining a mutualistic association with nematodes from the family Heterorhabditis. *P. luminescens* elaborates an extensive secondary metabolism during the post-exponential phase of growth that includes the production of an antibiotic called 3-5-dihydroxy-4-isopropylstilbene (ST), an anthraquinone pigment (AQ) and bioluminescence. For a number of years we have been studying the regulation of secondary metabolism in *Photorhabdus* and, during this time, we have identified several mutants that are affected in their ability to express particular aspects of secondary metabolism. Of particular interest is a mutant that we have recently characterized that was unable to produce ST, AQ and light. This mutation was found to be in the *mdh* gene, encoding malate dehydrogenase, a key enzyme in the TCA cycle. Interestingly the *mdh* mutant was unaffected in virulence but was unable to support nematode growth and development *in vivo* or *in vitro*. This clearly establishes that secondary metabolism in *P. luminescens* is required for the mutualistic interaction with the nematode. Furthermore the construction of mutations in key genes in other central metabolic pathways confirmed the critical role for the TCA cycle in both secondary metabolism and mutualism, but not in virulence. Therefore the TCA cycle is required for the transition of *P. luminescens* from pathogen to mutualist implicating the involvement of a metabolic switch in the regulation of life-style decisions in this bacterium.

The VeA protein complex and its role in secondary metabolism

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The fungal *velvet* gene, or *veA*, is necessary for both normal sexual and asexual development as well as for secondary metabolism in *Aspergillus* spp. Deletion *veA* strains of the model fungus and sterigmatocystin-producer *Aspergillus nidulans* and of the aflatoxin-producers *Aspergillus parasiticus* and *Aspergillus flavus* present a dramatically different secondary metabolic profile compared with the wild-type control strains, indicating that *veA* is necessary for the synthesis of numerous natural products, including mycotoxins and antibiotics. The *veA* gene product, VeA, is transported to the nucleus by the KapA -importin. Once in the nucleus VeA forms a complex with light sensing proteins, chromatin-modifying proteins and transcription factors. Interestingly, we found that there are *veA* orthologs not only present in *Aspergillus* spp., but also across fungal genera. In *Fusarium verticillioides* the *veA* homolog, *FvVE1* is necessary for the synthesis of toxins denominated fumonisins and fusarins. Furthermore, our recent studies have shown that *veA* is also necessary for normal *Aspergillus* and *Fusarium* pathogenicity in plants, where virulence was significantly reduced in the absence of VeA.

Assessing the relevance of light for fungi – implications and insights into the network of signal transmission

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Light is of major importance as a signal for most organisms including fungi. They react to illumination in various ways and adjust gene transcription to the altered conditions within a few seconds after a light pulse. Among the numerous pathways which show a response to illumination are also those for regulation of carbohydrate metabolism and secondary metabolism.

In order to get insights into the underlying mechanisms we studied the *Hypocrea jecorina* (anamorph *Trichoderma reesei*) photoreceptors BLR1 and BLR2 as well as the light regulatory protein ENVOY. Since we also had detected a light dependent influence of heterotrimeric G-protein signalling on carbohydrate metabolism, we were interested how the interconnection between these two pathways is established. Our data show that ENVOY plays a major role in this mechanism by establishing a link between light input, nutrient signaling and adjustment of cAMP levels. A genome wide view on microarray data provides insights into how the interplay between the photoreceptors and ENVOY on the one hand and the G-protein β and γ subunits and the phosducin like protein PhlP1 on the other hand works to regulate their output pathways.

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Offered paper **Functional multi-pathway mini networks in *Streptomyces* secondary metabolite production**

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Sensitivity analysis of the range of pathway fluxes (within a genome scale metabolic network) required to achieve a given yield of antibiotic (clavulanic acid and actinorhodin) revealed antibiotic-generating mini-networks, whose reactions are recruited from disparate parts of primary metabolic functions. Fluxes in these critical reactions responded to mutations that affected antibiotic yield (*zwf*) as well as chemostat-induced changes in growth rate that had an affect on specific antibiotic production rate.

The mini-networks could not have been predicted by visual analysis of antibiotic biosynthetic pathways.

Simple metabolic pathway flux analysis, with either maximum growth or maximum antibiotic production as the simulation target (objective function) was not effective in translating these ideas into pragmatic process improvement strategies. We hypothesize that this is because secondary metabolism is not required for cell growth, and antibiotic production is never the prime objective of the *in vivo* network. Evolution, therefore, has not optimized the network to maximize secondary metabolite production.

By comparing candidate process feed nutrients, selected by examining an antibiotic-generating metabolic mini-network we found that maximizing the assimilation rate of primary nutrients by addition of appropriate process feeds, provided more effective objective functions.

Offered paper **The role of nucleoid structure in actinomycete secondary metabolism**

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Actinomycetes have many secondary metabolic functions silently encoded in 'cryptic' pathways which may be an underexploited source of natural product diversity. However these are challenging to activate. Chromatin structure is well known to influence transcriptional activity in eukaryotes and there is increasing evidence for similar processes in bacteria, albeit without nucleosomes. In both kingdoms highly-expressed genes are more accessible to non-specific nucleases such as DNaseI, showing that physical state correlates with transcriptional activity. Small, highly abundant nucleoid-associated proteins (NAPs) contribute to both nucleoid morphology and global patterns of gene expression but little is known about the higher-order chromatin-like structures they may form.

We have begun to generate a high-resolution genome-wide map of nucleoid structure including factors such as physical accessibility, topoisomerase activity, occupancy by NAPs and transcriptional activity. We will also look at how the removal or manipulation with small molecules of NAPs affects nucleoid structure and function. We are currently carrying out a proteomic survey of the *Streptomyces coelicolor* nucleoid to determine which proteins are present and what post-translational modifications they carry.

We hope to use this knowledge to design new ways of activating cryptic clusters and to improve our understanding of how the bacterial nucleoid functions.

Use of antisense RNA and peptide nucleic acid for gene silencing in *Streptomyces coelicolor*

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Small RNAs are known to regulate a number of cellular processes in *Streptomyces*, with antisense RNA (asRNA) predicted to be involved in regulation of gene expression through mRNA silencing. In this study we used a paired-termini (PT) design for expressed asRNA (PTasRNA) and peptide nucleic acid (PNA), to silence the expression of the *act*I-ORF1 gene (β-ketoacyl synthase, α subunit) that is essential for synthesis of the polyketide backbone in actinorhodin biosynthesis. Expression of PTasRNAs from pSH19 (hyper-inducible, high copy number) or pIJ8600 (integrative) vectors resulted in complete silencing of actinorhodin biosynthesis in solid and liquid culture. In *S. coelicolor*, expression of PTasRNA had no affect on growth or the production of other antibiotics (undecylprodigiosin and calcium-dependent antibiotic). RT-PCR analysis of the actinorhodin biosynthetic gene cluster showed that *act*I-ORF1 was specifically silenced. A second gene silencing approach using peptide nucleic acid (PNA)-based silencing of the same gene also prevented actinorhodin biosynthesis, and thus circumvented the need for cloning in order to achieve gene silencing. This work demonstrates that potential for gene silencing approaches is *Streptomyces* and offers alternative and complementary methods for controlling secondary metabolism in *Streptomyces*.

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New insights into biosynthetic mechanisms for polyketides from pathogenic/endophytic fungi and the biocontrol agent *Lysobacter*

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The discovery of new antibiotics and anticancer agents is a pressing and continual need for human health due to the constant emergence of drug resistant pathogens and cancers. Bioactive natural products are a major source for drugs and drug leads. Traditionally, soil bacteria, especially the Actinomycetes, have been the primary source for bioactive natural products. However, there remain many sources that are not well exploited. In the talk, we will discuss our efforts to explore new bioactive natural products from pathogenic/endophytic fungi as well as environmental bacteria such as Lysobacter. The studies have led to natural products with novel scaffolds, new modes of action and insights into the biosynthetic mechanisms.

Chemical epigenetic tools for empowering the natural product-based drug discovery process

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The exploration of microbial natural products for new therapeutic leads is fraught with many challenges. In response to these difficulties, our group has developed new tools for enhancing the role that fungal-derived secondary metabolites play in the drug discovery process. We have employed a multifaceted approach that combines the investigation of fungi from both well-known and relatively unexplored habitats with novel chemical epigenetic tools in order to routinely access new microbial secondary metabolites. A key component of our strategy has been the utilization of small-molecule DNA methyltransferase and histone deacetylase inhibitors, which have enabled us to substantially expand the secondary metabolite profiles of fungi under laboratory culture conditions. Evidence from both genomic and chemically-driven studies employing chemical epigenetic manipulation suggest that this simple tool, along with other related techniques, hold tremendous promise for reinvigorating the role of natural products as a viable source of novel drug leads. The evolution and application of chemical epigenetic techniques as part of the rapidly expanding repertoire of methodologies for accessing silent biosynthetic pathways in microorganisms will be considered and pertinent examples involving the discovery of unique secondary metabolites from a diverse selection of fungal strains provided.

Waking up bacterial sleeping genes – a new insight into secondary metabolism

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The genome sequencing projects have revealed many biosynthetic gene clusters for additional unknown secondary metabolites. *Streptomyces coelicolor*, *Streptomyces avermitilis*, *Streptomyces griseus* and *Saccharopolyspora erythraea* produce 3 to 5 secondary metabolites, but actually possess more than 20 clusters each that encode known or predicted secondary metabolites. Exploitation of the genetic potential in actinomycetes would therefore lead to the isolation of many new biologically active secondary metabolites. We have developed a pragmatical method that activates silent or poorly expressed bacterial genes by generating specific drug-resistant mutants developed spontaneously. For example, the synthesis of antibacterial compounds was markedly activated in soil-isolated *Strepomyces* sp. 631689, which normally does not produce a detectable amount of antibiotics, by generating a rifampicin resistance mutation in the *rpoB* gene, which encodes the RNA polymerase β-submit. Acquisition of a streptomycin resistance *rpsL* mutation resulted in a further increase in antibacterial compounds by the *rpoB* mutant. Mass spectrometry and NMR spectroscopy analyses revealed that the antibacterial compounds are novel cyclic petide antibiotics, named piperidamycins. Similarly, a considerable fraction of actinomycetes (43% in Streptomyces and 6% in non-*Streptomyces*) was activated to produce the antibacterial compounds. In addition, expression of the silent gene clusters present in *S. coelicolor*, *S. griseus*, *S. avermitilis* and *S. erythraea* were markedly activated by generating the *rpoB* mutations. These findings demonstrate that modulation of transcriptional and/or translational apparatus (i.e. RNA polymerase and ribosome) effectively wakes up 'silent genes', leading to the isolation of new antibiotics from bacterial sources. The efficacy of rare earth elements (scandium and lanthanum) in silent gene activation is also discussed.

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Regulation of antibiotic production: from signalling molecules to antisense RNA

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One of the important characteristics of *Streptomyces* is its ability to produce a large variety of secondary metabolites, diverse in both chemical structure and bioactivity. The production pathways of antibiotics have been under intense study; however, there is little knowledge on how antibiotic production is controlled. We have tackled this question by analysis of this control at several levels in the model *Streptomyces* species, *Streptomyces coelicolor* A3(2). In this species the γ-butyrolactones are known to be the signalling molecules (or bacterial hormones) that regulate antibiotic production[1]. The two major players of the butanolide signalling system, γ-butyrolactone synthase and the γ-butyrolactone receptor, exert a concerted effort. Microarray analysis of a γ-butyrolactone synthase-deficient mutant has revealed possible regulatory effects of the synthase itself, while the receptor controls not only the synthesis of the butanolide but also that of the CPK antibiotic biosynthesis pathway. Antibiotic biosynthesis is also regulated at the translational level involving ncRNA. One such example is the antisense ncRNA of glutamine synthetase, which we recently described[2]. These recent advances in our knowledge of the regulation of antibiotic biosynthesis can now be used to awaken the multitude of cryptic antibiotic clusters, 20–50 of which are typically found in each genome[3], as we have demonstrated by awakening the cryptic/orphan CPK gene cluster[4].

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Analysing the switch to secondary metabolism by metabolic modelling

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During the transition from exponential to stationary phase, *Streptomyces coelicolor* undergoes a major metabolic switch, which results in a strong activation of secondary metabolism. We have explored the underlying reorganization of the metabolome by combining detailed transcriptomics time course observations collected within the SysMO-STREAM consortium with computational predictions based on constraint-based modeling. We reconstructed the stoichiometric matrix of *S. coelicolor*, including the major antibiotic biosynthesis pathways, and performed dynamic flux balance analysis to predict flux changes that occur when the cell switches from biomass to antibiotic production. The predicted fluxes of many genes show highly significant correlation to the time series of the corresponding gene expression data. Individual mispredictions identify novel links between antibiotic production and primary metabolism.

Transcriptional regulation of the secondary metabolism network during morphological differentiation in *Streptomyces*

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A complex network of regulation governs gene expression during development of the morphologically and biochemically complex eubacterial genus *Streptomyces*. Transition from primary to secondary metabolism in *Streptomyces* generally is believed to be associated with onset of morphological differentiation. We studied gene expression of secondary metabolite gene clusters during morphological differentiation under ordinary laboratory culture conditions, and find that this association is not as tight as has been supposed. Our results show that genes controlling the synthesis of secondary metabolites were turned on during various stages of the growth cycle: some before, some during and some after the major event of aerial hyphae formation. These findings suggest that certain secondary metabolites may have specialized functions that contribute dynamically to *S. coelicolor* development. Mutational analysis and adventitious overexpression of known key regulators of secondary metabolism has revealed novel regulons that include previously unknown genes for secondary metabolism control. Our genetic and bioinformatic analyses extend knowledge of the complex global network of differentially expressed genes that regulate secondary metabolism in *S. coelicolor*.

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An energetic deficit might be the real trigger of antibiotic biosynthesis in *Streptomyces* species

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In *Streptomyces* species, antibiotic production usually takes place in the periods of slow or no growth (weak anabolism) and is triggered by a nutritional limitation in Pi, condition that correlates with a weak energetic charge. The interruption of the *ppk* gene leads to an huge increase in the production of the polyketide antibiotic, actinorhoddin, in the weak producer, *S. lividans* (3). *In vitro*, Ppk acts either as a polyphosphate kinase (PPK) or as an adenosine di phosphate kinase (ADPK) depending on the ATP/ADP ratio in the reaction mix (3). *In vivo*, *ppk* is mainly expressed in condition of Pi limitation (1). In these conditions, the analysis of the cellular content in polyphosphate, ATP and ADP suggested that Ppk was acting as an ATP regenerating enzyme (an ADPK). Ppk thus likely plays a central role in the energetic metabolism of the cell and its absence is predicted to result in a severe energetic deficit. Indeed, electron microscopic observations as well as proteomic studies demonstrated that a strong activation of the -oxydation of storage fatty acids (mainly TriAcylGlycerol) was taking place in that strain. This process known to generate FADH2 and thus energy generates as well acetylCoA, a precursor entering in the biosynthesis of the polyketide antibiotic, actinorhoddin. In order to test this hypothesis, an artificial ATP deficit was generated by the cloning of an ATPase under the control of a strong constitutive promoter.

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Team building for bacterial impact: management of secondary metabolite production in *Erwinia* and *Serratia*

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We have investigated secondary metabolite production in strains of the enterobacteria, *Erwinia* (*Pectobacterium*) and *Serratia,* including production of the carbapenem antibiotic (a β-lactam), 1-carbapen-2-em-3-carboxylic acid (Car) in both genera and the red, tripyrrole antibiotic, prodigiosin (Pig) in *Serratia*. Small molecules of the carbapenem and prodigiosin classes have utility as antibiotics, immunosuppressants and anti-cancer agents.

The production of the carbapenem is controlled by quorum sensing (QS) via *N*-acyl homoserine lactones but is mediated by structurally different molecules operating on homologous biosynthetic gene clusters delivering the same chemistry, but via different regulatory mechanisms. Both Car and Pig are under QS control but regulatory inputs to Pig biosynthesis are more complex than for carbapenem and involve diverse regulators, reflecting the responsiveness of Pig to more environmental cues. Crypticity occurs among *Erwinia* (*Pectobacterium*) strains for carbapenem production; some are 'activated' by provision of a functional LuxR-type regulator. We have moved chromosomal QS regulator loci and Pig biosynthetic loci between strains and generated recombinants with altered metabolic behavior. Subtle functionality and evolution of the LuxR-type transcriptional regulators plays a key role in physiological control of the 'target' genes.

NT08 Respiratory & septic infections

↑CONTENTS

Spectrum of disease caused by aspergilli

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The genus *Aspergillus*, and its associated diseases, is extraordinary and unparalleled by any other microorganism. The spectrum of aspergillosis extends from allergy in the nose and sinuses (allergic *Aspergillus* rhinosinusitis) to the lungs (manifesting in subtly different ways) (allergic bronchopulmonary aspergillosis and severe asthma with fungal sensitization), to slowly progressive pulmonary or sinus destruction in apparently normal individuals (and other mammals and birds) (chronic pulmonary aspergillosis and granulomatous *Aspergillus* rhinosinusitis) and to immediately life-threatening invasive infection in immunocompromised patients (invasive aspergillosis).

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The basic genomic structure and many facets of biology in the *Aspergillus* genus are also remarkable. The apparently close relatives *Aspergillus fumigatus* and *Aspergillus nidulans* are as distantly related as fish and man. This represents huge evolutionary changes in the space of about 200 million years in an organism with 10,000–14,000 genes. This adaptability may account for the wide environmental comfort zone for the genus in many hostile locales, namely compost, high salinity environments (Dead Sea) and the human lung. Improvement in disease management will come from better diagnosis of invasive and allergic disease. We don't know why *Aspergillus* can disseminate from the lung. We don't know whether to use one or more drugs for treatment. New patient populations with serious infection (especially those with chronic lung disease) or allergy are being defined. We need new and better anti-*Aspergillus* drugs to cope with azole resistance (increasing since 2003), treatment failure and intolerance problems, especially new oral drugs.

The immunopathology of childhood respiratory infection

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Respiratory pathogens are responsible for a huge worldwide disease burden; they rapidly mutate, cross species barriers, threaten patients with pre-existing lung diseases, evolve resistance and respond rapidly to demographic and economic change. Some (e.g. tuberculosis) are on the rise, while others (e.g. influenza) are emerging threats to health and prosperity.

Host inflammatory responses are major contributors to disease pathophysiology, but there is little understanding of the mechanisms of childhood respiratory infection, despite an extensive literature on animal and *in vitro* models. At St Mary's Hospital, London, we have collected clinical data for children presenting with severe respiratory failure, as part of the *Immunopathology of Respiratory Infection Study*, a prospective, observational study which aims to identify pathogen-specific signatures of gene and protein expression, and to identify pathways implicated in patients with severe disease. The talk will describe our research approach to childhood respiratory infection.

Offered paper Development of a novel human *in vitro* model of the blood-brain barrier to study *Acanthamoeba* pathogenesis

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Acanthamoeba granulomatous encephalitis is a serious human infection that almost always leads to death, however the pathogenesis and pathophysiology of this disease remains unclear. Several lines of evidence suggest that haematogenous spread is a prerequisite for *Acanthamoeba* encephalitis, however the mechanism by which parasites breach the blood-brain barrier to gain entry into the central nervous system remains incompletely characterized. Using primary human brain microvascular endothelial cells, here we describe a novel *in vitro* model of the blood-brain barrier under flow conditions. Using this model, we determined the effects of *Acanthamoeba* and their cell-free supernatants on brain endothelial cells under static and flow conditions. The findings revealed that *Acanthamoeba* exhibited significantly lower binding to brain endothelial cells under flow but surprisingly showed increased monolayer disruption. *Acanthamoeba*-free supernatants disrupted the monolayer more rapidly under flow compared with static conditons, indicating increase in blood-brain barrier permeability. In conclusion we have, for the first time, shown that *Acanthamoeba* modulates the integrity of the blood-brain barrier at a faster rate under physiologically-relevant flow conditions. Further understanding of the mechanisms associated with *Acanthamoeba*-mediated blood-brain barrier perturbations using this novel system will allow us to develop strategies to prevent this serious infection.

Offered paper The brain lysates of locusts and cockroaches exhibit potent broad spectrum antibacterial activity

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New antimicrobial agents are urgently needed to meet the challenges posed by the re-emergence of infectious diseases. The search for new antibacterial compounds from novel natural sources is a vital research area. Insects represent 80% of all fauna and the most widespread group within the animal Kingdom. The aim of this study was to investigate the potential antibacterial activity in various tissues of the desert locust; *Schistocerca gregaria* and cockroach; *Periplaneta americana*. Both insects were dissected to obtain muscle, fat body, ganglia and haemolymph. Crude lysates were prepared from pooled insect tissue samples and investigated for their antibacterial activity against Gram-positive (meticillin-resistant *S. aureus)* and Gram-negative bacteria (neuropathogenic *Escherichia coli* K1). Insect

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muscle, fat body and haemolymph produced no bactericidal effects. In contrast, lysates of locust and cockroach ganglia exhibited >90% bactericidal effects against bacteria tested. The bactericidal effect was abrogated by heating lysates to 100°C for 20 min suggesting the activity is proteinaceous in nature. Antibacterial activity was retained in the eluate after passing lysates through 10 kDa size-exclusion spin columns.Brain lysates had no cytotoxic effects on human brain microvascular endothelial cells suggesting that the putative target/s is not present in eukaryotic cells. Work is currently underway to purify and further characterize the antibacterial properties of insect brain lysates.

Roles of Gram-positive pili in adherence to epithelial cells and biofilm formation

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Streptococcus agalactiae (Group B *Streptococcus*) is a leading cause of sepsis (blood infection) and meningitis (brain infection) in newborns. Lung is thought to be the portal of entry. Bacterial pili have recently been recognized in several Gram-positive bacteria and are assembled through a distinct and conserved mechanism involving a transpeptidase called sortase. Our previous functional characterization of the pilus locus in *S. agalactiae* showed that it encodes a major pilin (PilB) and two minor pilins subunits (PilA and PilC) that are covalently polymerized by the action of two enzymes belonging to the sortase C family. One of the accessory pilins PilA is responsible for the adhesive property of the pilus. We recently showed that the pilus is essential for optimal display of the pilus-associated adhesin and overcomes the masking effect of the capsule. Pilus integrity was shown to be critical in adherence assays under flow conditions. We also report that GBS can form biofilms and that pili play an important role in this process.

Offered paper The human fungal pathogen *Cryptococcus neoformans* escapes macrophages by a phagosome emptying mechanism that is Inhibited by Arp2/3 complex-mediated actin polymerization

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The lysis of infected cells by disease-causing microorganisms is an efficient but risky strategy for disseminated infection as it exposes the pathogen to the full repertoire of the host's immune system. *Cryptococcus neoformans* is a widespread fungal pathogen that causes a fatal meningitis in HIV and other immunocompromised patients. Following intracellular growth, cryptococci are able to escape their host cells by a non-lytic expulsive mechanism that may contribute to the invasion of the central nervous system. Here we show that phagosomes containing intracellular cryptococci undergo repeated cycles of actin polymerization. Using fluorescent dextran as a phagosome membrane integrity probe, we find that the non-lytic expulsion of *Cryptococcus* occurs through fusion of the phagosome and plasma membranes and that, prior to expulsion, 95% of phagosomes become permeabilized, an event that is immediately followed by an actin flash. By using pharmacological agents to modulate both actin dynamics and upstream signalling events, we show that flash occurrence is inversely related to cryptococcal expulsion. In conclusion, our data reveal the existence of a novel actin-dependent process on phagosomes containing cryptococci that acts as a potential block to expulsion of *Cryptococcus* and may have significant implications for the dissemination of, and CNS invasion by, this organism.

Offered paper A comparison of methods for the identification of respiratory pathogens from cystic fibrosis patients

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Rapid and reliable identification of bacteria colonizing the lungs of cystic fibrosis sufferers is crucial to specific and effective treatment and control of the infection. Recent studies have shown the inadequacy of current non-molecular techniques in routine diagnostics at identifying particularly clinically important and problematic organisms. *Pseudomonas aeruginosa* is one such organism that is known to colonize a vast number of adult patients and cause rapid deterioration in lung function.

Using a duplex real time polymerase chain reaction (PCR) assay, we were able to use specific primers and probes to detect the presence of the *ecfX* gene (extracytoplasmic function sigma factor) and the *gyrB* gene (gyrase B subunit); two regions which are unique to this *Pseudomonas* species. DNA was extracted and analysed from 99 clinical isolates and directly from 40 sputasol treated sputum samples, which were all collected from the diagnostic laboratory of the Royal Infirmary of Edinburgh. The results were compared with the current routine biochemical techniques; with maldi-tof biotyper analysis; and with sequencing of the 16S rRNA region.

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The real time PCR assay was both accurate and successful at detecting *P. aeruginosa*, with a sensitivity and specificity of 100%. The technique was also found to be more cost effective and rapid than existing detection methods. The study also revealed that routine biochemical methods may misidentify up to 20% of isolates from CF lung.

This investigation highlights the need for improved microbial diagnostics for cystic fibrosis patients. Molecular techniques have the potential to enhance diagnosis by detecting organism directly from the primary specimen therefore accelerating the identification process.

Early life-time of *Aspergillus fumigatus* in the lung

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Establishment of aspergillosis is depending first, upon the survival of the conidia of *Aspergillus fumigatus* in the lung following up their recognition and phagocytosis by the cells of the host innate immunity and second, the exit from dormancy and germination of these conidia in the lung environment. In addition to major transcriptional changes controlling the exit of dormancy, it was shown that the cell wall plays a major role during these morphogenetic events. First, the lack of recognition of the dormant conidia is due to the surface rodlet layer of the conidium as shown by the use of morphotypes in which the rodlet layer was removed either biologically, chemically or genetically. Early germination of conidia was associated with a cell wall surface remodelling and the synthesis of an extracellular matrix that resulted in the modifications of the adhesive and immunological properties of the fungus. Two polysaccharides (α1-3glucan and galactomannan) are specifically associated in these surface modifications. Their role of will be discussed in my talk.

The lipid arsenal of the tubercle bacillus

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Mycobacterium tuberculosis, the causative agent of tuberculosis has a lipid rich cell wall that acts as a permeability barrier and also helps the bacterium tolerate inhospitable conditions inside infected macrophages. Major components of this distinct cell wall are mycolic acids, long chain fatty acids that are either bound covalently to the cell wall, or present as components of cell envelope-associated glycolipids. The significance of mycolic acids in the biology of *M. tuberculosis* is underlined by findings that core mycolic acid biosynthesis is essential for survival, and that structural variations in mycolate subclasses play an important role in pathogenesis. Using a highly efficient phage-based knockout strategy, we have generated knockouts (or conditional knockouts) of potential candidates to study mycolic acid biosynthesis and processing in mycobacteria. Mutant strains were subsequently tested in the mouse model of infection to assess effects on virulence. Surrogate organisms like *Mycobacterium smegmatis* and *Mycobacterium marinum* were also used; the latter allowed for testing altered virulence in zebrafish embryos.

Offered paper *Staphylococcus aureus* protein A binds to osteoblasts and inhibits proliferation

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Osteomyelitis is an inflammatory bone cell disease accompanied by progressive bone weakness and bone loss. *Staphylococcus aureus* is found in more than 80% of osteomyelitis patients. Treatment is often less than optimal due in part to a lack of understanding of the molecular mechanisms leading colonization of osteoblasts. This study investigated how *S. aureus* interacts with osteoblasts. Osteoblasts bound to several wildtype *S. aureus* strains. Clumping factor A (ClfA) and Protein A (Spa) are among the most abundant proteins expressed on *S. aureus*. Deletion of Spa but not ClfA from wildtype *S. aureus* significantly inhibited binding to osteoblasts (ΔSpa 60±9% inhibition, $P<0.0001$ and ΔClfA 15 ±4% inhibition, P=NS). Moreover, osteoblasts failed to adhere to immobilized purified ClfA but they did bind to purified Spa. Osteoblasts failed to proliferate in the presence of *S. aureus* but they proliferated normally in the presence of the Spa mutant. Consistent with the wild-type strain, complementing the Spa deletion mutant with wild-type Spa prevented proliferation. These results suggest that Spa may be important in binding of bacteria to osteoblasts and thus might be of relevance in the pathogenicity of osteomyelitis. A better understanding of *S. aureus*-osteoblast interactions will aid development of more targeted treatments.

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Catching a cold: gene expression signatures to predict and classify respiratory viral infection

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Acute respiratory viral infections result in high utilization of health care services by affected individuals. Current diagnostic tests are hampered by lack of sensitivity. Early and accurate detection of infection could have profound implications on patient management and prognosis by allowing prompt initiation of appropriate therapy. Compelling evidence now exists that the HOST RESPONSE to pathogens, in the form of pathogen-specific host gene expression signatures, can serve as a potential early and rapid diagnostic strategy. Gene expression patterns, captured by microarrays, offer a robust means of classifying infectious pathogens and of early and specific diagnosis in advance of standard methods. Using a model of experimental inoculation of healthy volunteers with rhinovirus, respiratory syncytial virus or influenza A (H3N2/Brisbane or H1N1/Wisconsin), we developed peripheral blood gene expression signatures that classify acute respiratory viral infection with a high degree of accuracy and can differentiate from bacterial infection and healthy individuals. This technique has promise for the development of host-based diagnostic tests for acute respiratory viral infection.

A zebrafish embryo model of *Staphylococcus aureus* infection

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Staphylococcus aureus is a major human pathogen of increasing clinical importance due to the spread of antibiotic-resistant strains. In order to understand infection processes, animal models are commonly used. Two novel vertebrate models of staphylococcal infection in zebrafish embryos have been developed, with bacteria injected into two different sites within the embryo. The kinetics of bacterial growth and host mortality were established, and fluorescence microscopy was performed to visualize the interaction of labelled pathogen and host cells.

The circulation model most closely mirrors the infection in the mammalian system. Using this model, both host and pathogen factors important in staphylococcal pathogenesis were identified. Myeloid cell depletion renders embryos more susceptible to *S. aureus*. Additionally, a preliminary screen for *S. aureus* virulence determinants has revealed that mutations in *perR*, *pheP* and *saeR* lead to attenuation. A combinatorial approach involving phagocyte-depleted embryos infected with the *saeR* mutant of *S. aureus* showed full recovery of virulence, as opposed to *perR* and *pheP* mutants, which virulence was not fully restored by myeloid cell depletion. Using bacterial population studies, the importance of the immunological checkpoint in bacterial within-host selection was revealed. It was hypothesized that very limited numbers of bacteria are required to found and initiate successful infection.

Clinical impact of the molecular diagnosis of respiratory viruses

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Acute respiratory disease (ARD) accounts for an estimated 75% of all acute morbidities in developed countries, and most of these infections (approximately 80%) are viral. Acute viral respiratory tract infection is the leading cause of hospitalization for infants and young children in developed countries and is a major cause of death in developing countries. The timely detection of a respiratory virus can help in treatment, prognosis, outbreak management and patient management in the short and long term. Typically influenza A and B viruses, parainfluenza virus (PIV) type 1 (PIV1), PIV2, PIV3, respiratory syncytial virus (RSV), adenovirus are the traditionally accepted major causes. It is now clear that rhinoviruses, human coronaviruses, human metapneumoviruses and hPIV4 can cause LRTI and ARD and can be fatal in some cases. Indeed, all of the viruses mentioned above have overlapping clinical presentations and cause both URTI and LRTI and the causative agent is not identified without a laboratory diagnosis. In Edinburgh extensive molecular diagnosis and epidemiology analysis has been performed to assess impact of these viruses in all hospitalized patients including those admitted to ITU.

Pneumocystis pneumonia

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Different species of the ascomycetous fungus *Pneumocystis* asymptomatically infect a wide range of mammalian hosts and may cause a pneumonia, known as PCP (from PneumoCystis Pneumonia). In humans *Pneumocystis* pneumonia is caused by *Pneumocystis jirovecii* (previously *Pneumocystis carinii*). Detection of *Pneumocystis*-specific DNA using PCR in BAL fluid and induced sputum is superior to

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histochemical staining, but specificity is <100%. The mortality rate for PCP has fallen to <10%. Clinical and laboratory factors that predict a poor outcome include increasing patient's age, a second or subsequent episode of PCP, medical co-morbidity, low haemoglobin, low albumin, poor oxygenation, peripheral blood leucocytosis and a raised serum LDH level, identification of pulmonary KS, neutrophilia or a co-pathogen in BAL fluid and raised serum LDH levels that remain increased despite treatment, need for mechanical ventilation and/or development of a pneumothorax. Co-trimoxazole remains the treatment of choice for PCP, but ≤25% of patients will have treatment-limiting side-effects and ≤10% will fail therapy; prognosis in the latter group is poor. Clindamycin/primaquine has been shown to be superior to iv pentamidine as salvage therapy. All patients with a PaO_2 <9.3kPa should receive adjunctive corticosteroids. The optimal time to start antiretroviral therapy during treatment of PCP remains undetermined.

Cold in the corps: respiratory viral infection among military recruits in the UK and USA

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Military recruits receiving training are historically vulnerable to viral respiratory illness and large epidemics at training centres have, in the past, incapacitated commands and halted training programmes, with repercussions on military readiness. While adenoviruses & influenza viruses have been identified in most cases, a significant proportion of febrile respiratory illness (FRI) is of unknown aetiology. Although previously rarely considered as an infective agent in adults, within the last decade Respiratory Syncytial Virus (RSV) infection has been increasingly recognized as a cause of significant morbidity and mortality in certain adult populations. The question thus arises as to whether RSV contributes to viral respiratory illness in military recruit populations. We determined the proportion of FRI, the clinical presentation and the severity of illness associated with common respiratory viruses among symptomatic Royal Navy and US Army recruits receiving basic training. We employed standard viral culture together with a novel molecular diagnostic technique and serological analysis to measure the prevalence of RSV infection, its contribution to FRI and the clinical presentation and severity of illness associated with RSV among symptomatic recruits.

How can antibodies help protect against the dissemination of *Salmonella*

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The severity of infections caused by the intracellular pathogen *Salmonella* Typhi, or related non-typhoidal serovars such as *Salmonella* Typhimurium, is associated with bacterial dissemination through the host via the blood. Antibody generated against the bacterium during infection can prevent this dissemination but not moderate bacterial colonization, whereas specific antibody present before infection, resulting from previous infection or vaccination, can help to reduce both colonization and bacteraemia.

In this talk I will discuss our data from murine models of *Salmonella* Typhimurium infection that examines the antibody response to this pathogen. I will present our findings on why the origin of the B cell recruited into the response is important. In addition I will show data on the antibody response to OmpD and flagellin and how antibody to different surface-exposed antigens has a different impact on the host's ability to control infection. This work has implications for understanding how the host controls disseminated Salmonella infection and for selecting bacterial targets as vaccine candidates against these devastating infections.

Chemokine–cytokine network in histoplasmosis

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Resolution of infection with the pathogenic fungus, *Histoplasma capsulatum*, requires collaboration among numerous constituents of the host immune response. Although the necessity for particular cytokines has been established, we have examined the contribution of chemokines and their receptors on host defenses to this fungus. Mice lacking the chemokine receptor, CCR2, succumb to an otherwise sublethal infection. The failed immune response is caused by elevated levels of interleukin-4. Absence of CCL7 and CCL2, two ligands for CCR2, is responsible for altered production of interleukin-4. Protective immunity is restored by neutralizing interleukin-4 or by adoptive transfer of antigen-primed dendritic cells plus elimination of $CD4^+$ cells. Hence, $CD4^+$ cells are deleterious to host defenses in this model. In contrast, the absence of the chemokine receptor CCR5 accelerates clearance of infection. The improved outcome is a result of a shift in the balance between regulatory T cells and interleukin-17^+ $CD4^+$ T cells. The lack of CCR5, or one of its ligands, CCL4, prevents migration of regulatory T cells from the thymus and inhibits their expansion in the lungs, but facilitates Th17 cell growth. These findings highlight the complex nature of chemokine signaling and the interaction between chemokines and cytokines in this infectious disease.

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NT09 Extremophiles

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Halo-alkalithermophiles: polyextremophiles living in sun-heated alkaline salt lakes

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Microorganisms living in extreme environments utilize a number of adaptive mechanisms in order to enable them to proliferate, and this is true to an even greater extent for poly-extremophiles, i.e., microorganism able to grow under various combined extreme growth conditions. We have investigated the properties of anaerobic and aerobic microorganisms which grow optimally under the concomitant stressors of temperatures between 46 and 66°C, (thermophilic) , $pH^{55°C}_{opt}$ at or above 9.5 (obligately alkaliphilic) , and between 3.7 and 4.3 M Na^+. (obligately halophilic). Key objectives of this work are to elucidate the diversity of this novel and unusual group of microorganisms and to characterize the major biochemical and physiological mechanisms that enable these poly-extremophiles to grow under the above triple stress conditions. The study includes the isolation and characterization of members of the novel order *Natranaerobiales* (Phylum *Firmicutes*) from the sun-heated salt lakes Wadi An Natrun (Egypt) and Lake Magadi (Kenya) and of a novel archaeal species and in *Natranaerobius* species the characterization of the stress-coping mechanisms such as cytoplasmic acidification, use of proton-sodium/potassium sym- and antiporters, and a sodium ion-dependent ATPase.

The Cyanidiales: unicellular red algae of acidic hot springs

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In the Rhodophytan order, Cyanidiales, three morphologically described genera, *Cyanidium, Galdieria, and Cyanidioschyzon,* are recognized. These few genera probably consist of many additional taxa (some at the genus level), and are characterized by using sequence information from several genes. Most inhabit acid (pH 0.2–4.0) hot springs from ~35°C to 56°C, this upper temperature limit being close to the maximum known for eukaryotes. Members of the Cyanidiales occur in volcanic areas world-wide. The most abundant culture isolates from many sites in Yellowstone National Park (the 'enigma' strains), using the sequences of nuclear 18S rDNA and chloroplast rbcL match those of *Cyanidioschyzon merolae* at the 99–100% level. However, large morphological and reproductive differences challenge this close relationship. Although many physiological differences distinguish many different culture isolates, only Illumina-generated sequences of genes on all 20 chromosomes demonstrate the great differences between these common 'enigma' strains and those of the more rare *C. merolae.* The next step will be to correlate the physiological and genetic characters among the many isolates with the varied and specific chemical and physical characteristics of the acidic habitats in Yellowstone National Park from which they came.

Polar diatoms and their adaptation to the polar environment

Thomas Mock
University of East Anglia, Norwich

Diatoms contribute 25% of global carbon fixation but their contribution to the carbon cycle is even higher in polar systems where they are the major primary producers in the marine system. None of the other phytoplankton groups have been so successful in occupying this extreme environment characterized by overall low temperatures and strong seasonality of solar irradiance in addition to nutrient limitations such as limitation by iron in the Southern Ocean. However, our knowledge about the evolution and adaptation of polar diatoms is still in its infancy despite their significance for the whole polar ecosystem. Thus, genome-enabled technologies have been used to identify functional adaptations in these organisms, which are absent in non-polar diatoms and therefore probably responsible for their successful adaptation. The genome sequence of *Fragilariopsis cylindrus* and the first eukaryotic metatranscriptome from diatom-dominated Southern Ocean sea ice communities has been pivotal for the identification of unique adaptations to the polar ecosystem. Both projects together with accompanied studies on specific genes and proteins revealed remarkable and unique adaptations, which weren't anticipated but which are subject of this talk.

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Life in subglacial polar ecosystems

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The existence of subglacial Antarctic lakes has been recognized for almost two decades though we have yet to penetrate and sample any of these remarkable water bodies. Recent revelations of the vast scale and complexity of the subglacial hydrological system in Antarctica has significantly ramped up interest in establishing what life forms may exist in such extreme environments, what the challenges are for subglacial life and the potential of these ecosystems as analogues for possible life in extraterrestrial environments. Under the auspices of the Subglacial Antarctic Lake Exploration (SALE) programme an international group of researchers has been developing understanding of these environments over the past decade through a variety of remote sensing technologies, use of accretion ice and exploration of a number of complementary polar ecosystems. Despite the technological challenges of clean entry and sampling of these pristine systems through several kilometres of glacial ice, SALE has a series of lake penetration and sampling projects underway to enter three subglacial environments between 2011 and 2013. This talk will outline our current understanding of life in subglacial environments and the environmental challenges that subglacial life forms face.

Aerobiology, extreme life in the atmosphere

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A study of air-borne microbial biodiversity above two contrasting scientific research stations in Antarctica, one continental, one maritime, was undertaken to assess the likely origin, influence of the local environment and potential for human impact. Overall, the continental site had low microbial biodiversity, and many of the sequences obtained were from as yet uncultivated organisms. Those detected, were subjected to extreme environmental conditions for extended periods of time. However, no significant patterns were detected in aerial biodiversity between the austral summer and the austral winter, very few marine sequences were detected irrespective of the distance to open water and around one-third of sequences detected were similar to those identified in human studies. For the maritime Antarctic, microorganisms typical of the local terrestrial environment were detected and microorganisms that would be considered evidence of human activity were not found. The detected aerial microorganisms were therefore markedly different between the continental and maritime Antarctic sites.

Extreme fungi

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Recent studies have revealed the diversity of fungi that can occur in stressful environments that are hostile to most eukaryotes. Such habitats are characterized by high salt concentrations, high and low temperatures, acidic and basic conditions, high hydrostatic pressures, high ionizing radiations and toxic environments, to name but a few extremes. When we reviewed these studies, we repeatedly encountered the same groups of fungi, polyphyletic in origin, that have evolved a plethora of adaptive mechanisms to cope with these different extremes. The same groups also harbour potential novel fungal pathogens. In our research work we focused in particular on life at high salt concentrations and in ice, since there is a surprising similarity between the fungal populations in these two kinds of environments, both of which are characterized by low water activity. We have proposed some mechanisms that would allow for the evolutionary adaptation of eukaryotic microbial life under extreme conditions, with steps of evolution from generalist species towards the development of specialists in extreme habitats. Traits present in some fungal groups, such as asexuality, synthesis of melanin-like pigments and a flexible morphology, are probably preadaptations that facilitate persistence and eventual adaptation to conditions on the ecological edge, as well as biotope switches. These steps, important for understanding the evolution of extremophiles, will be exemplified by studies on three fungal model organisms: ubiquitous, halotolerant *Aureobasidum pullulans*, the extremely halotolerant black yeast *Hortaea werneckii* and the halophilic fungus *Wallemia ichthyophaga.* These fungi differ considerably in their adaptations to hypersaline conditions and in their ability to grow at low a_w due to high concentrations of NaCl. *A. pullulans* can grow up to 3.0 M, *H. werneckii* up to 5.0 M, *W. ichthyophaga* requires at least 1.5 M and thrives up to saturation (5.2 M) NaCl, while *Saccharomyces cerevisiae,* the usual model organism for adaptations in eukaryotes, can grow up only to 1.2 M NaCl. Due to these differences we argue that studies of responses to elevated salt concentrations should be performed on (possibly several) halotolerant/halophilic models rather than on the salt-sensitive *S. cerevisiae*.

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Extreme predators and prey; haloviruses and their hosts

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Haloquadratum walsbyi is a square-shaped, extremely halophilic archaeon, and is a dominant member of the microbial populations in salt lakes and salterns around the world. The genome sequence of the type strain of this genus has been determined and compared to the previously published sequence of a Spanish isolate. The two strains share an extremely high level of similarity, particularly given that were recovered from sites that are 17,000 km apart. This has allowed a detailed analysis of the changes that have occurred between them, providing insights to the mechanisms underlying these changes. The role of viruses in the evolution of *Haloquadratum* and other haloarchaea will also be reviewed in the context of genomic and metagenomic data.

Chaotropicity: implications for the limits of the microbial biosphere

John E. Hallsworth

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The functionality of microbial cells and – by implication – the microbial biosphere is constrained by physicochemical parameters such as temperature, desiccation and solute activities. Cells are aqueous systems so the diverse types of solute-induced water stress represent the most potent sources of microbial-growth inhibition: eg salt-, matric- and osmotic stress. We have shown that chaotropic solutes (such as ethanol, urea and phenol), that can disorder macromolecular structures, induce a genome-wide microbial stress response mostly via the upregulation of proteins involved in stabilization of biological macromolecules and membrane structure. We found that the exceptional chaotropicity of a salt, $MgCl_2$, determines the limit of biosphere function in specific locations on Earth. Unexpectedly we found that some fungi grow optimally under chaotropic conditions, providing evidence for a previously uncharacterized class of extremophilic microbes, *chaophiles*. In these studies it was established that chaotropicity (not water activity) determined the growth windows of xerophilic fungi at high solute concentrations, and obtained mycelial growth at water activities as low as 0.647 (below that recorded for any microbial species) by addition of compounds that reduced the net chaotropicity. Further studies established that chaotropes can expand the windows for microbial survival and growth at subzero temperatures. These data have implications for the possibility of microbial-cell function in extreme environments on Earth as well as extraterrestrial environments. We are currently investigating the impact of chaotrope-induced microbial stress in relation to sea-ice ecosystems, oil seeps and spills, and the environmental tenacity of emerging pathogens.

Offered paper Extremes for extremophiles: thermophilic bacteria in permanently cold sediments

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Many rare taxa in the marine environment include dormant organisms that constitute a natural microbial 'seed bank'. In permanently cold Arctic sediments of Svalbard fjords, dormant thermophiles are estimated to make up 0.01% of the total microbial population. Experimentally heating Arctic sediments to 50°C stimulates these diverse anaerobic heterotrophs that are naturally present as endospores that survive the cold *in situ* temperature (<4°C). Quantitative spore-germination assays and sediment accumulation rate measurements reveal a constant annual influx of 10^8 thermophilic spores per square meter of the seabed. This suggests that Arctic thermophiles must be supplied from a large, warm habitat. Eight phylotypes of these bacteria were detected and 16S rRNA gene sequencing confirms their genetic affiliation with endospore-forming *Firmicutes*. Closely related bacteria are found in warm deep biosphere habitats including petroleum reservoirs, hydrothermal vents and in ocean crust fracture fluids near mid ocean ridges. Geofluid dynamics associated with these extreme environments may therefore be supplying thermophiles to another extreme environment– polar sediments. The biogeography that connects these misplaced members of the rare biosphere to very different extreme habitats suggests geological factors have an important influence on the passive dispersal of microbial cells.

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Offered paper **The role of bacteria-like rhodopsins in marine eukaryotic phytoplankton**

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A rhodopsin gene was identified from the whole genome sequence of the polar diatom *Fragilariopsis cylindrus* and was found highly expressed in metatranscriptome data sets from the Southern Ocean and the Equatorial Pacific Ocean, indicating its ecological importance. Sequence analysis suggests that the protein is similar to *Gloeobacter* rhodopsin (GR), a fast-cycling rhodopsin capable of light-driven proton transport and most probably obtained by lateral gene transfer. The physiological role of a proton-pumping rhodopsin in the presence of the proton gradient-generating chlorophyll-based photosynthetic apparatus remains puzzling. To further analyse its function, the rhodopsin from *F. cylindrus* was cloned from cDNA, fused to GFP and heterologously expressed in the diatom *Phaeodactylum tricornutum*. After expression of the fusion protein in *Phaeodactylum*, GFP accumulates within 'blob'-like structures (BLS) around the chloroplasts, which suggests targeting to the periplastidic or inter-envelope space. The screening for a phenotype is currently in progress, as are experiments to further characterize the protein by heterologous expression in *E. coli* and the diatom *Thalassiosira pseudonana*.

Bioprospecting extreme environments: retrieval of enzymes for nucleic acid processing and potential industrial applications

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Biotechnology & Biomolecules, Matis Ltd, Vínlandsleið 12, 113 Reykjavík, Iceland

In recent decades, bioprospecting of extreme geothermal environments has received a considerable interest as they are a source of stable and robust biocatalysts suitable for industrial applications or exploitation in the recombinant DNA technology. Furthermore, the potential of using thermophiles as cell-factories in biorefining processes is widely being investigated. The biotech company Matís Ltd., previously Prokaria, has been active in the bioprospecting of extreme environments in Iceland since 2000. It's expertise is based on ecological enrichment methods and sequence based techniques for screening and exploitation of natural diversity. The company focuses on the discovery of novel biocatalysts such as glycosyl degrading and modifying enzymes for the carbohydrate industry, and cellulases and hemicellulases for the biofuel processing technology. Also, work involving discovery and production of novel nucleic acid processing enzymes has been substantial. Matís has put an increasing emphasis on the metabolic engineering of production strains, e.g. for the production of ethanol and compatible solutes and process development of bioactive compounds from various marine sources. Different bioprospecting projects will be reviewed; some of those have lead to commercialization, other brought up new problems, requiring further research and development for eventual exploitation.

Evolution of Haloarchaea

Emma White & R. THANE PAPKE

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Phylogenetic analysis of ribosomal proteins and RNA typically indicate that members of the order Halobacteriales (called haloarchaea here) evolved from methanogenic archaea, however very little is known about the relationships among the haloarchaea themselves. To better understand the evolution of haloarchaea, we analysed hundreds of strains representing a broad diversity of officially and unofficially classified genera, species and strains, including many type strains, using PCR amplification of five loci and DNA sequencing (multilocus sequence analysis). Our data demonstrate that haloarchaea are highly recombinogenic in nature, e.g., lateral gene transfer plays a major role in haloarchaeal evolution and speciation, and that each gene has a separate evolutionary history. Furthermore, it appears that LGT does not occur randomly, but is biased; that is, it appears to occur more frequently between closely related individuals than distantly related ones. This suggests that LGT is an active homogenizing force for reinforcing species and generic diversity and may explain why we see evidence for taxonomic groups in the face of high LGT rates.

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Extreme radiation resistance: the magic of *Deinococcus* manganese complexes

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For *Deinococcus radiodurans* and other bacteria which are extremely resistant to ionizing radiation (IR), ultraviolet radiation, and desiccation, a mechanistic link exists between resistance, manganese accumulation, and protein protection. We show that ultrafiltered, protein-free preparations of *D. radiodurans* cell extracts prevent protein oxidation at massive doses of gamma-rays. In contrast, ultrafiltrates from IR-sensitive bacteria were not protective. The *D. radiodurans* ultrafiltrate was enriched in Mn, orthophosphate, nucleosides and bases, and peptides. When reconstituted *in vitro* at concentrations approximating those in *D. radiodurans*, these constituents interacted synergistically and formed complexes which preserved the activity of large, multimeric enzymes exposed to 50,000 Gy, conditions which destroyed DNA. When applied *in vivo*, they protected *Escherichia coli* and human Jurkat T cells from extreme cellular insults caused by IR. By establishing that orthophosphate Mn^{2+}-metabolite complexes of *D. radiodurans* specifically protect proteins against indirect damage caused by gamma-rays delivered in vast doses, our findings provide the basis for a new approach to radioprotection and insight into how surplus Mn budgets in cells combat reactive oxygen species.

Life after catastrophe; microbial ecology, extreme impacts and the late heavy bombardment

Charles Cockell

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Asteroid and comet impacts are known to be able to cause devastation to surface dwelling life, yet their effects on the deep biosphere are unknown. Results from the deep drilling of the ~35 Myr-old Chesapeake Bay impact structure, USA are discussed. Microbial enumerations display a logarithmic downward decline, but the different gradient of decline compared to previously studied subsurface sites and the scatter of the data are consistent with a microbiota influenced by the profound geological disturbances caused by the impact. Coupled with the low hydraulic conductivity, the data suggest that the deep biosphere has not recovered from the impact. Microbial enumerations, molecular analysis of microbial enrichment cultures and geochemical analysis show recolonization of a deep region of impact-fractured rock that was heated to above the upper temperature limit for life at the time of impact, demonstrating that impacts can extend the depth of the biosphere. This phenomenon would have provided deep refugia for life on the more heavily bombarded early Earth. Similar effects are observed in surface rocks of the Haughton impact structure in Canada, which are fractured and become an improved habitat for phototrophs following impact bombardment. These data yield insights into the effects of impacts on microbial ecosystems through time.

Smooth lipopolysaccharide plays a key role in the ability of the deep-sea bacterium *Photobacterium profundum* SS9 to grow optimally at cold temperatures

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Although over 70% of the Earth's biosphere is comprised of oceans our knowledge of pressure- and cold-loving deep-sea bacteria is lacking. The deep-sea bacterium, *Photobacterium profundum* SS9 grows optimally at 28 MPa, 15°C but its ability to grow at atmospheric pressure (0.1 MPa) has made it genetically tractable. My research has focussed on the characterization of two transposon mutants (FL25 & FL26), which are defective in their cold-adapted growth. Interestingly, both mutants only display cold-sensitivity on marine agar. Since the genes disrupted in FL25 and FL26 encode a putative glycosyl transferase and O-antigen ligase, respectively, we hypothesized that these mutants would be affected in their lipopolysaccharide (LPS) biosynthesis. We found that both mutants produced rough LPS but unlike the parent strain, they were defective in the biosynthesis of smooth LPS, which possesses the O-antigen subunit. Since the lack of smooth LPS in *Salmonella* Typhimurium did not affect its ability to grow at cold temperatures, this suggests that the key role of smooth LPS in the cold-adapted surface growth could be a unique feature of cold-loving deep-sea bacteria, such as *P. profundum* SS9.

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High-throughput screening of the Keio Collection identifies novel genes required for growth of *Escherichia coli* at pressure

S. LUCAS BLACK[1,2], F. Bruce Ward[1] & Rosalind J. Allen[2]

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Introduction Screening of deletion/mutation libraries is a simple and powerful method to search for conditionally essential genes. We have used the systematically constructed Keio collection library (for *Escherichia coli*), combined with a novel, quantitative, high-throughput growth method to screen for mutants with a pressure sensitive growth phenotype.

Methods We have developed a quantitative high-throughput method of monitoring growth, fluorescence and luminescence of cultures grown in a standard pressure vessel. Our method, based on standard 96-well plates, uses a combination of clear seals and epoxy resin to seal the plate in a way that is sterile, flexible enough to transmit pressure, sturdy enough not to leak in the pressure vessel and compatible with standard plate readers. This high-throughput method was used to screen the Keio library of single deletion mutants in *E. coli* for the ability to grow at 0.1MPa, 30MPa & 50MPa.

Results Our screen highlighted a set of nine novel genes which are required for growth at pressure. These genes can be grouped into three categories; cell shape, DNA replication & repair and transport genes.

Discussion We discuss these results in the light of known protein functions for these gene products and highlight their connections with previously identified pressure-sensitive cellular processes.

Life on the edge: growth at the thermodynamic limit

MICHAEL J. MCINERNEY[1], Jessica R. Sieber[1], Bryan Crable[1], Housna Mouttaki[1], Neil Q. Wofford[1], Lars Rohlin[3], Rachel R. Ogorzalek Loo[2] & Robert P. Gunsalus[3]

Botany & Microbiology[1], University of Oklahoma, Norman, OK, USA; Dept of Biological Chemistry[2] & Dept of Microbiology & Molecular Genetics[3], University of California, Los Angeles, CA, USA

Methanogenesis is an essential process in global carbon cycle that has long been used to covert wastes into the energy-rich compound, methane. Methane production from natural polymers involves a consortium of interacting microbial species. The Gibbs free energy changes involved in the conversion of key intermediates such as fatty and aromatic acids to methane are very low, close to the minimum free energy change needed to sustain microbial growth. Genomic and metabolic analyses of bacteria capable of syntrophic metabolism, *Syntrophus aciditrophicus* and *Syntrophomonas wolfei*, reveal that they are specialists with few options to generate a proton motive force needed for reverse electron transfer, nutrient uptake, and motility. Proton and sodium gradients can be formed by membrane-bound pyrophosphatases, decarboxylases, and proton- and sodium-dependent ATP syntheses and possibly by the concomitant consumption of protons inside of the cell and production of protons on the outside of the cell by cytoplasmically and externally-oriented hydrogenases or formate dehydrogenases. Their very restricted metabolic potentials probably reflect the outcome of evolutionary selection for energy conservation systems that are highly efficient at using small amounts of free energy. Methanogenic consortia provide excellent models to study interspecies interactions and highly efficient energy economies.

Detection of biomass and metabolic activity in subsurface sediments

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The subsurface biosphere is the one of the largest ecosystems on this planet, still we know very little about its inhabitants and their metabolic activity. Several factors make exploration of subsurface environments so challenging. Sample availability has improved over the last few years, although many areas and environments still have never been sampled, especially in the terrestrial realm.

The concentration of cells per volume of sediment decreases rapidly with depth, making it difficult or even impossible to use the same techniques as in surface sediments. Subsurface metabolic activity is extremely low making detection and quantification extremely difficult. Some data indicate metabolic activities that challenge our concept of the minimum requirements of life. Although major technical improvements have been made over the last few years, e.g. the detection of sulfate reduction through radiotracer has reached its absolute physical limit; other techniques still have room for improvements. But even with the most sensitive methods, some processes proceed much too slow for any direct measurement. In such cases modelling may be the only solution, although such approaches depend on high-quality chemical and physical data, which may not be available in all cases. Only by combining different approaches we will be able to learn more about the subsurface biosphere.

The deep sub-seafloor biosphere: a large but extreme habitat

R John Parkes

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Ocean sediments cover ~ 70% of the Earth's surface and although average depths are ~500 m, they can be up to 10 km. This environment is high-pressure (average seawater pressure alone is 38 MPa), initially cold (~2°C) with temperatures gradually increasing with depth (~30°C/km), and normally, low available energy (low concentrations of recalcitrant organic matter). Remarkably, however it may contain up to 70% of all prokaryotes. This large biomass has been questioned due to energy limitation, 'Most microorganisms in subseafloor sediments are either inactive or adapted for extraordinary low metabolic activity'. Measurement of active cells (CARD-FISH), however, shows that a large fraction of sub-seafloor prokaryotes are alive. This is supported by measurement of intact membrane lipids and prokaryotic community and activity changes at deep interfaces (7 – 11 My old). Currently, the deepest sediments with detected prokaryotes is 1,626 metres in the Newfoundland Margin. These sediments are 111 My old, temperatures are 60 to 100°C, and thermophiles and hyperthermophiles (novel *Pyrococcus* and *Thermococcus*) are present. In addition, the presence of Anaerobic Methane Oxidizing sequences suggested that hydrocarbons of deep thermogenic origin are also being utilized as energy sources.

Dark life in the fracture labyrinth of deep hard rock

Karsten Pedersen

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Since two decades, life has been shown to exist and to be active in groundwater extracted from aquifers in fractured Scandinavian, granitic hard rock to depths below 1000 m. Microscopic counts, ATP determination, cultivation and DNA analyses have revealed a large diversity comprising oxygen-, nitrate-, iron-, manganese- and sulfate-reducing bacteria, homoacetogens and methanogens. New species such as *Desulfovibrio aespoeensis* and *Methanobacterium subterraneum* have been named and described. Geochemical and hydrological investigations suggest that deep groundwater may have been isolated from the surface for thousands of years. This poses a conceptual problem for the microbes detected there. What ultimate energy source are they using? Organic carbon from a sun-driven surface ecosystem would not last for a long time because of the documented *in-situ* activity of the found microbial populations. An energy source at this depth must be renewable. Many of the found microorganisms are chemolithotrophs that may utilize hydrogen as a source of energy. Therefore, a hypothesis of a hydrogen-driven biosphere in deep granitic rock aquifers has been suggested; an extreme ecosystem that survive in total darkness feed by hydrogen from the interior of our planet. However, the experimental testing of this hypothesis is extremely challenging as will be exemplified.

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Supertolerance: stress and inhibitor tolerance in bioethanol yeast strains

Katherine Smart

BBSRC Sustainable Bioenergy Centre, Nottingham University

Abstract not received

Renewable chemicals: opportunities and challenges for industrial biotech

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Even in these years with unprecedented scrutiny of the chemical industry, the drivers for adapting technology to develop chemicals based on renewable feedstocks are recognized by the industry as very important. Independency of oil price volatility combined with the growing demand for decreasing carbon footprint are the key drivers. A variety of different routes from renewable feedstock to chemicals can be envisioned and many of those routes embark on cell factories (microbial strains producing chemicals). Some are already realized and in production scale (e.g. polylactic acid and 1,3-propanediol), while the majority are still waiting to be developed. Systems biology will be a key technology in such development. Furthermore, integration with chemistry will be important as many routes combine both fermentation and additional chemical conversion step to the final product, which has to be cost-competitive to the petro based product. This represents a huge challenge as petrochemical processes have been optimized over many decades whereas

bioprocesses are to in large in their infancy. This presentation will highlight Novozymes activities in the biorefinery area, providing biotechnology for producing the fermentable sugars and engineered microbial strains to produce 3-hydroxypropionic acid, a precursor for acrylic acid. Models for process economics and indifference curves will be presented.

Metabolic engineering of *Geobacillus* spp. for ethanol production – degrees of improvement

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The production of ethanol from lignocellulose requires either the addition of catabolic capabilities to known ethanologens, or the redirection of fermentation pathways in catabolically versatile hosts. Facultative *Geobacillus* spp are excellent hosts for the latter strategy. Working with TMO Renewables Ltd we have demonstrated that it is possible to create a competent ethanol pathway involving overexpressed pyruvate dehydrogenase (Cripps et al 2009, Metabolic Engineering 11,398). The alternative approach, involving pyruvate decarboxylase, is not naturally available in thermophiles due the lack of a thermophilic Pdc. In this talk I will present our strategy and recent progress towards creating such an enzyme.

Micro-organisms as green chemists

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Microorganisms can be used as green chemists, a field usually referred to as Industrial Biotechnology. Even though, the development has been driven by the large attention given to production of biofuels, many other basic chemicals can be produced by a biotech based process as alternative to petroleum based processes. Efficient cell factories based on well established microbial platforms are one critical factor in developing novel processes for Industrial Biotechnology. A strong technology platform encompassing modelling tools (genome scale models), data mining tools, systems biology experimental tools, gene mining tools, a large strain collection, advanced fermentation technology and novel molecular biological tools are necessary to support this development . Microbial production platforms are suitable for production of both simple molecules as well as more complex ones. The prerequisite for the growth of industrial biotechnology; the availability of cheap raw material and enabling technologies, will be discussed in detail. During this presentation the challenges and prospects will be discussed and examples of improvement strategies with regard to the fermentation process and design of the microbial production platforms from our research program which includes production of bulk chemicals (bioethanol and succinate) as well as more advanced molecules (selenometabolites with cancer preventive effects) will be given.

Engineering improved fermentation of ethanol production from xylose and cellobiose in *Sheffersomyces* (*Pichia*) *stipitis*

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The fermentation of D-xylose, L-arabinose, cellobiose and various other components of lignocellulose for the production of renewable fuels has proven to be very challenging. The larger sizes, thicker cell walls, and low nutritional requirements of yeasts all confer advantages for the large-scale fermentation of cellulosic and hemicellulosic sugars. The dominance of *Saccharomyces cerevisiae* in the ethanol fermentation industry has spurred numerous attempts to engineer it with the enzymatic machinery for the efficient fermentation of cellulosic and hemicellulosic sugars. In many cases, these efforts have drawn on enzymes from native xylose fermenting yeasts such as *Sheffersomyces (Pichia) stipitis.*

S. stipitis can ferment xylose, galactose, mannose, glucose, cellobiose and various hemicellulosic oligosaccharides in mixtures of pure sugars and from hemicellulosic hydrolysates. It produces over 60 g/l ethanol from xylose and some strains can ferment cellobiose almost as rapidly as glucose. Much can be learned from this model organism in developing improved yeasts. We have used whole genome expression array technology to identify distinct genes involved in the assimilation and fermentation of cellulosic and hemicellulosic sugars, and we have targeted critical genes for engineered expression. Since *S. stipitis* and related yeasts substitute serine for leucine upon encountering the CUG codon, we have synthesized several selectable markers for drug resistance genes to optimize codon usage, and flanked the markers with LoxP sites to enable highly efficient transformation and subsequent excision of markers with an engineered Cre recombinase.

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With these tools, we have been able to carry out repeated rounds of transformation and marker recovery using up to 15 different autologous *S. stipitis* genes. We have obtained numerous strains of *P. stipitis* engineered with multiple genes that show significantly better ethanol production than the best wild-type strains known. We have also developed mating and selection systems that enable rapid crosses and identification of hybrids with improved performance properties.

This presentation will review gene targets, global expression array results and the performance of genetically engineered strains on mixed sugars, hydrolysates and pretreated cellulosics.

Synthetic biology: building new pathways for chiral chemicals

John Ward
Institute of Structural & Molecular Biology, Darwin Building, University College London WC1E 6BT

It is predicted that the market share of bio-based chemicals by 2025 will be 50% in the specialty and fine chemical areas. Biocatalysis and Synthetic Biology will be the cornerstone of this and we need to increase work using all aspects of the available biological toolkit to make this a reality. The use of single enzymes in biocatalysis is well established and our group has explored enzymes such as transketolase, transaminases and the oxygenases of the Baeyer-Villiger and cytochrome P450 class. To make complex chiral compounds organisms have evolved pathways where multiple enzymic steps are put together to build complexity from simple starting structures. *De novo* pathway construction is now possible in the lab and we are building short pathways using transketolase and transaminases to access chiral aminodiols. Mutants of the *E. coli* transketolase and a wide range of wild-type transaminases from many different bacteria have been combined. *de novo* designed pathways to build complexity can draw on the wealth of enzymes now available from over a thousand fully sequenced genomes of bacteria, archaea, plants, fungi and higher eukaryotes. High throughput DNA sequencing techniques of metagenomic DNA can also yield novel enzymes. The increasingly low cost of synthetic DNA makes the construction and alteration of genes from different organisms such as plants feasible. We are building novel alkaloids using synthetic norcoclaurine synthase genes based on plant enzymes to construct pathways in *E. coli* that synthesize novel benzylisoquinolines.

Novel enzymes and their applications in commercial biocatalysis

Jennifer Littlechild
Exeter Biocatalysis Centre, Biosciences, University of Exeter, Stocker Road, Exeter EX4 4QD

The Exeter Biocatalysis Centre specializes in the isolation and characterization of novel enzymes from extremophilic organisms which are used for the production of new optically pure drugs of interest to pharmaceutical companies. These enzymes are more robust to organic solvents used in the biocatalytic process and immobilization techniques. The biotransformation process can be operated at elevated temperatures where potential substrates are more soluble. Enzymes developed at Exeter that are already used commercially will be discussed including the L-aminoacylase from *Thermococcus litoralis* for the resolution of aminoacids and aminoacid analogues, the γ lactamase from *Sulfolobus solfataricus* for the production of optically pure γ lactam – the building block for anti-viral carbocyclic nucleotides and alcohol dehydrogenase from *Aeropyrum pernix* for the production of optically pure alcohols. Enzymes in development include a transaminase and a dehalogenase from *Sulfolobus* species. The transaminase can be used for the asymmetric synthesis of homochiral amines of high enantioselective purity. The L-2-haloacid dehalogenase has applications both in biocatalysis and in bioremediation. Several of these enzymes have been immobilized into micro-reactors to allow rapid substrate screening and optimization of the biotransformation process. A novel cofactor NADH recycling system has been developed which eliminates the use of another recycling enzyme.

Conversion of grass to fuels, polymers and value-added biochemicals

Maria Tuohy
National University of Ireland, Dept of Biochemistry, University Road, Galway City, Ireland

The competence centre for Biorefining and Bioenergy (CCBB) is an Industry lead initiative which engages in cutting-edge research and development whose outputs to support a sustainable & competitive Irish biomass (bioenergy and bioproducts) industry. This research theme is investigating high-potential, next-generation feedstocks, such as grass and algae that do not compete with food, and can achieve a higher energy balance and a greater potential to reduce greenhouse gas emissions relative to current bioenergy and biorefinery. The CCBB is investigating both primary and secondary conversion technologies to produce multiple products such as energy, fuel, biopolymers, and biochemicals. In particular enzymatic and thermochemical techniques are being investigated to unlock the energy and building blocks from feedstocks with a particular relevance to Ireland. The building blocks are subsequently converted through secondary conversion technologies for value added product synthesis.

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Offered paper **Efficiency of enzyme production technologies**

Mads O. Albaek

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Growing markets and new innovative applications of industrial enzymes leads to increased interest in efficient production of these products. Most industrial enzymes are currently produced in traditional stirred tank reactors in submerged fed batch culture. The limiting parameter in such processes is often oxygen transfer from the gas to the liquid phase. In many systems there is a trade-off between productivity and efficiency. Often high productivity technologies and high productivity processes are preferred. We have studied the efficiency of oxygen transfer versus productivity in fed batch fermentations of filamentous fungi in 550 litre pilot scale stirred tank reactors for a range of process conditions. At low energy inputs there is a significant increase in oxygen transfer efficiency. A number of alternative technologies for enzyme production have been reported in the open literature; some with quite promising results. In order to compare these results, predictions of the large-scale process performance have been made. Further investigations are needed to objectively compare these technologies with the existing ones. Production of enzymes can possibly be carried out in energy efficient operational regimes and by means of alternative technologies, leading to better energy utilization, lower carbon emissions, and new markets for industrial enzymes.

Offered paper **Feasibility of bioutilization of carbon dioxide stored for the geological sequestration: bioconversion of carbon dioxide to methane energy by geo-micro-organisms**

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Accumulation of too much of CO_2 in the atmosphere causes global greenhouse effects. Treatment and utilization of CO_2 have globally been one innovative direction of environment technologies. After many years' studies, it has been generally accepted that storage of CO_2 in deep geological formations is of a feasible solution. Geological storage options of CO_2 include depleted oil and gas reservoirs, discarded coal beds, deep saline formations, and impermeable geological cap structures. According to some of the previous studies, abundant geological micro-organisms have been found in these geological storage sites, which consist of methane-producing bacteria (methanogens). Based on the biogeochemical information, here, we firstly present the proposal: CO_2 stored in deep geological formations could be converted to methane energy by geo-methanogens. As a result of long-term reaction, a large amount of methane will be produced and then capable to be extracted out for combustion energy. The CO_2 emitted from the burning of methane is captured and pressurized, and then again stored in the geological formations. In such recycle will meet carbon 'zero emission' ideal.
In this report our preliminary studies showed that methane-producing geo-micro-organisms seem to prevail over the geological environments. Here, two cultures of methane-producing geo-micro-organisms FJ10 and TP-01 were obtained and proven easily to grow and produce methane well under the 100 atm condition similar to the environment pressure of geological CO_2 storage. By PCR techniques culture TP-01 was analysed with a consortium of methanogens, archaea, and bacteria. A comprehensive survey of the previous research publications and the studies in this report implies that producing methane energy converted from the CO_2 stored in the geological reservoirs by methane-producing geo-micro-organisms seems to be quite feasible.
Keywords Carbon dioxide, geo-microbiology, greenhouse gas, mitigation, storage, treatment, utilization

BBSRC support for industrial biotechnology

Colin Miles

BBSRC

Colin Miles will explain BBSRC's background in supporting research, training and knowledge transfer activities in industrial biotechnology and present a number of approaches to ensure the future support for this area of science which features in the new BBSRC Strategic Plan, *The Age of Bioscience*.

How will industrial biotechnology feature in a low-carbon economy?

Garry Staunton

The Carbon Trust, 6th Floor 5 New Street Square, London EC4A 3BF

The carbon Trust was established in 2001 with the mission to facilitate the move to a low carbon economy. It does this by providing

specialist advice and finance to help organizations cut carbon, setting standards for carbon reduction as they cut their carbon emissions now, In addition we are investing to cut potential future carbon emissions by opening markets for low carbon technologies, leading industry collaborations to commercialize technologies and investing in early stage low carbon companies. Whilst the carbon Trust is, in principle, technology blind we recognize that industrial biotechnology has the potential to play a significant role in the emerging low carbon economy. This paper will set out how we approach the assessment of applications for support, and how we then set about matching the support we can offer to what is required to help move low carbon (IB) prospects on their journey towards commercial scale deployment. This process will be illustrated through case studies based on work we are supporting in second generation biofuels and biopolymer production.

NT11 Bioleaching of metals: new developments in technologies

Acid and hard rock: the odd world of biomining bacteria

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Micro-organisms that thrive in anthropogenic environments created to accelerate the dissolution of sulfide minerals, and thereby facilitate the recovery of precious and base metals, need to survive and be active in extremely acidic liquors that contain elevated concentrations of soluble transition metals and other solutes, and conditions of often fluctuating temperatures and aeration. As a group (and sometime as individual species), biomining micro-organisms (chiefly bacteria and archaea) are extremophiles that exhibit considerable physiological diversity in terms of the varieties of electron donors and acceptors they use, the temperature and pH ranges in which they are active, and how they respond to other environmental variables, such as osmotic stress. Recent isolation and characterization species from biomining operations and mine-impacted environments of novel bacterial genera and that are capable of biomineralization as well as mineral dissolution, has expanded the biotechnological potential of acidophiles beyond mineral processing. Selective precipitation of transition metals and metalloids from polluted streams and groundwaters, and industrial process waters, has been demonstrated in the laboratory and (in one case) in a pilot-scale plant using currently unclassified species of acidophilic sulfate-reducing and iron-oxidizing bacteria.

Arsenic resistance in acidophiles and its significance to bioprocessing of gold-bearing ores

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Microbial consortia used in continuous-flow, stirred tank processes to treat gold-bearing arsenopyrite concentrates have became adapted to very high concentrations of arsenic. The dominant microorganisms, *Acidithiobacillus caldus* and *Leptospirillum ferriphilum*, were found to contain two sets of arsenic resistance genes. One set of *ars* genes located on the chromosome and present in all isolates of a species irrespective of whether they were highly arsenic resistant or not. A second set of *ars* genes was present on Tn*21*-like transposons and was found in all strains tested that had been adapted to high concentrations of arsenic. The arsenic resistance transposons present in *At. caldus* and *L. ferriphilum* were closely related, but sufficiently different to indicate that they had been acquired independently rather than having been passed from one bacterium to the other. The transposons were transpositionally active in *Escherchia coli* and were shown to confer higher levels of arsenic resistance than the chromosomally-located *ars* genes where it was possible to test this. Transposons containing arsenic resistance genes that were identical or closely related to the transposon from *L. ferriphilum*, originally found in South Africa, were also found in both *L. ferrooxidans* and *L. ferriphilum* isolates from South America and Europe.

Insights into the metabolic potential of bioleaching micro-organisms from genome analyses

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Bioleaching (the use of microorganisms to solubilize metals from ores) is carried out by consortia of microorganisms. Novel insights into the metabolic potential of these consortia is beginning to emerge from analyses of 48 complete or draft genomes of bioleaching microorganisms or their close acidophilic relatives (29 bacterial and 19 archaeal genomes) and 4 metagenomics projects. Pathways or processes have been predicted for chemotrophic energy transduction, aerobic and anaerobic respiration, autotrophy including CO_2 and

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N_2 fixation, central carbon metabolism, aspects of acid tolerance and metal resistance, iron and sulfur assimilation and biofilm formation and chemotaxis. Information is also emerging regarding the role of lateral gene transfer, mobile elements and phage in genome evolution and genome flux. The composition and activity of these consortia are known to change over space and time as bioleaching proceeds and a knowledge of their composition and function are prerequisites for a more complete understanding of the biology of bioleaching. Predictions of metabolic potential from genomic data allow preliminary models of the ecophysiology of such consortia to be built that begin to address questions such as who is capable of doing what, to whom, where, when and under what circumstances. It is hoped that the initial results presented here will serve as starting points for several interesting lines of further inquiry.

Acknowledgments Fondecyt 1090451 and Conicyt Basal CCTE PFB16.

Mechanisms of iron and sulfur oxidation in acidophilic mineral-leaching prokaryotes

Violaine Bonnefoy

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The most important role played by microorganisms in the solubilization of metals from metal sulfide ores is their ability to oxidize ferrous iron (Fe(II)) and reduced inorganic sulfur compounds (RISC). The products of these oxidations, that is ferric iron and sulfuric acid respectively, then chemically attack the mineral releasing the metal, Fe(II) and RISC. Therefore, the 'biomining' prokaryotes allow the recycling of these chemical agents. Understanding the molecular mechanisms involved in Fe(II) and RISC oxidation is difficult because these microorganisms are not easy to handle experimentally (slow growth, poor cell yield, etc.) and genetic manipulation is a real challenge and, even when developed, is fastidious. Most progress has been made in the mesophilic acidophilic bacterium *Acidithiobacillus ferrooxidans* using biochemistry, molecular genetics and functional genomic tools. In the other known 'biomining' microorganisms, knowledge of these processes remains rudimentary. Nevertheless, a number of metabolic pathways have been reconstructed *in silico*, sometimes supported by proteomics and/or transcriptomics, based on the data obtained from *At. ferrooxidans*. The most obvious conclusion that can be drawn from the data obtained so far is that different Fe(II) and RISC oxidation pathways exist, even between closely related prokaryotes.

Biomining: old technologies, new organisms?

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The development of mineral sulfide bioprocessing in tanks and heaps has seen more control of conditions (such as temperature) that can define which microorganisms might be most profitably used, and this has coincided with more awareness of the diversity of organisms that could find application. The draft genomes for two recently described 'novel' organisms that could fill certain biomining niches are being examined with reference to metabolism that underlies their biogeochemical activity and potential application. These organisms are a thermo-, halo-tolerant proteobacterium related to *'Thiobacillus prosperus'* and a mineral sulfide-oxidizing actinobacterium, *'Acidithiomicrobium* sp'. Exceptionally for actinobacteria, *'Acidithiomicrobium'* species grow autotrophically on sulfur as efficiently and extensively as proteobacterial acidithiobacilli. Its major cluster of Calvin cycle genes appears to have been obtained by lateral transfer from an *Acidithiobacillus* species or its immediate ancestor. In contrast, RuBisCO-encoding genes of the thermotolerant *'T. prosperus'*-like strain are related to those of its alkaliphilic phylogenetic neighbours. It does, however, have a cluster of genes reported to encode proteins involved in electron transfer from ferrous iron to oxygen in the more distantly related *Acidithiobacillus ferrooxidans*, although two key genes of the rusticyanin operon found in the acidithiobacilli are missing.

From Rio Tinto to Mars: the terrestrial and extraterrestrial ecology of acidophiles

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Centro de Biología Molecular Severo Ochoa, UAM; Centro de Astrobiología, Madrid, Spain

The discovery of mineral on Mars similar to those produced in acidic environments associated to metal mining activities (jarosite, goethite, hematite) has put these habitats in the list of terrestrial analogues of Mars. Between them, Rio Tinto (Iberian Pyritic Belt) stands up do to its size (92 km) and the extreme pH and metal concentrations detected. The use of conventional and molecular ecology techniques allowed to determine the microbial diversity associated to the water column of the river, which is dominated by microorganisms of the iron cycle (*Leptospirillum ferrooxidans*, *Acidithiobacillus ferrooxidans* and *Acidiphilium* spp.). Recently we have initiated the characterization of the subsurface geomicrobiology of the Iberian Pyritic Belt to prove the hypothesis that the river is the

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exhausting pipe of an underground reactor operating in the subsurface of the IPB. Expected microbial activities like iron and sulfur oxidizers have detected together with unexpected activities like sulfate reducers and methanogens. The possibility that this type of geomicrobiology could be operating on Mars will be discussed on the light of the current knowledge of the red planet.

NT12 Protein folding & misfolding

↑CONTENTS

Mycobacterial chaperonins: evidence for nucleotide-dependent oligomerization

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Molecular chaperones act in various ways to reduce aggregation and promote folding of many proteins. The chaperonins are probably the best understood molecular chaperones. They are found in nearly all organisms and are generally essential for viability. They have a remarkable structure consisting of two rings, of seven or eight sub-units, each with an internal cavity where protein folding takes place. Most Mycobacteria have two chaperonins, Cpn60.1 and Cpn60.2. The Cpn60.1 proteins from *M. tuberculosis* and *M. smegmatis* are implicated in granuloma formation and biofilm formation respectively. Uniquely, Mycobacterial chaperonins have been reported not to form the higher order oligomers which are essential for chaperonin function. We have confirmed that these chaperonins are monomeric under standard conditions. Nonetheless, we have shown that Mycobacterial *cpn60.2* can completely replace the essential *E. coli groEL* gene, although *cpn60.1* cannot. Analysis by analytical ultracentrifugation of the Cpn60.2 proteins shows that they form large complexes in the presence both of high salt and nucleotide. We have used nanoelectrospray QTOF mass spectroscopy to confirm that the *M. tuberculosis* Cpn60.2 assembles into large complexes, including tetradecamers, in a nucleotide and high salt dependent fashion, a behaviour which is unique among chaperonins studied to date.

Genetic selection designed to stabilize proteins uncovers a chaperone called Spy

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Proteins are often frustratingly unstable. To explore the *in vivo* folding environment and to optimize *in vivo* folding we have developed a way to impose a fold or die dilemma on bacteria. We link the folding of an unstable protein to two different selectable markers, each of which independently monitors its stability. Variants that simultaneously enhance resistance to both selectable markers overproduce a host protein called Spy. Spy overproduction was found to be necessary and sufficient to increase the soluble level of unstable Im7 mutants up to nearly 700 fold. Spy was purified shown to be a very effective ATP-independent chaperone, which suppresses protein aggregation and aids in protein refolding. The ability to tailor the cellular folding environment opens up new routes toward understanding and controlling protein stability.

Functions and mechanisms of AAA+ proteins in protein disaggregation and protein secretion

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AAA+ proteins are involved in a multiplicity of cellular functions by coupling ATP hydrolysis to the remodelling of specific substrates. We are studying the functions of mechanisms of AAA+ family members involved in protein quality control and protein secretion. In bacteria the AAA+ chaperone ClpB mediates the reactivation of aggregated proteins in cooperation with an Hsp70 chaperone system (KJE), an activity that is crucial for cell survival during severe stress. We demonstrate that KJE has an essential function during the initial phase of the disaggregation process by recruiting ClpB to protein aggregates. In contrast to ClpB, the AAA+ protein ClpV operates without a partner protein. ClpV is an essential component of type VI secretion systems (T6SS), which are present in a multiplicity of pathogenic proteobacteria. Secretion systems are frequently energized by hexameric ATPase components that directly act on secretory proteins. Surprisingly, ClpV does not process the exoproteins of T6SS, but disassembles tubular VipA/VipB complexes, illuminating an unexpected role of an ATPase component in protein secretion. The physiological relevance of the ClpV severing activity will be discussed.

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Offered paper **Insertion of β-barrel proteins into the outer membrane of Gram-negative bacteria**

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The outer membranes of Gram-negative bacteria function as a barrier to protect cells from toxic compounds such as antibiotics and detergents. They are composed of phospholipids, lipopolysaccharide and two major classes of proteins, lipoproteins and β-barrel containing integral outer-membrane proteins (OMPs). Insertion of OMPs into the outer membrane of *E. coli* is achieved by the multi-protein BAM complex. An important component of this complex is the BamA protein, which is an essential protein that binds to unfolded β-barrel precursors via the polypeptide transport-associated (POTRA) domains at its N-terminus. The C-terminus of BamA contains a β-barrel domain, which tethers BamA to the outer membrane and is thought to be involved in OMP insertion. As BamA orthologues are found in all Gram-negative bacteria, we examined whether orthologues from a wide range of bacteria could functionally replace *E. coli* BamA. We demonstrate that only the closely related *Salmonella enterica* serovar Typhimurium BamA orthologue could function in *E. coli*. Experiments with chimeric protein fusions demonstrate that the β-barrel domains of many of the BamA orthologues can functionally replace that of *E. coli* but that their POTRA domains cannot.

Structure–function analyses of outer-membrane protein biogenesis

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The outer membrane of Gram-negative bacteria is composed of an asymmetric bilayer of phospholipid and lipopolysaccharide, peripheral lipoproteins and integral outer-membrane proteins which predominantly adopt a β-barrel structure. Recent investigations have demonstrated that correct folding and insertion of folded β-barrel proteins into the outer membrane is mediated by the essential β-barrel assembly machine (BAM). The BAM machinery is composed of an integral outer-membrane proteins (BamA) and four accessory lipoproteins (BamB-E). Comprehensive mutagenesis and interaction studies have defined the complex role of BamE within BAM, and mapped key determinants for complex targeting, phosphatidylglycerol binding and preserving outer-membrane integrity and cell viability.

The periplasmic chaperone Skp and its role in delivering transmembrane proteins for folding into the outer membrane

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In bacteria, periplasmic chaperones facilitate the transport of membrane proteins (OMPs) from the cytoplasmic to the outer membrane. Urea-unfolded outer-membrane protein A (OmpA) from *Escherichia coli* inserts and folds spontaneously into preformed lipid bilayers upon dilution of the denaturant urea. We have isolated the periplasmic chaperone Skp from *E. coli* and examined its impact on insertion and folding of OmpA into lipid bilayers. Skp facilitated folding of OmpA only when the bilayers were negatively charged, containing phosphatidylglycerol, which is also a component of the outer membrane of *E. coli.* When phosphatidylglycerol was absent, Skp inhibited insertion and folding of OmpA. Analyses of the pH-dependence of the fluorescence spectra of unfolded OmpA in buffer after urea-dilution revealed that two different aqueous conformations of OmpA coexist at pH 7. The folding kinetics of OmpA indicated that these forms fold in parallel with rates that differ by one order of magnitude. The analysis further showed that Skp increases the contribution of the faster rate to the overall kinetics of OmpA folding.

Yeast [*PSI*$^+$] prions: protein misfolding in yeast cells and machines for disaggregation

HELEN SAIBIL[1], Petra Wendler[1], Jens Tyedmers[2], Anja Seybert[3], Mikhail Eltsov[4], Daniel Castano[4], Uta Haselmann[4], Susan Lindquist[5], James Shorter[6] & Achilleas Frangakis[3]

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When cellular protein quality control fails, in ageing organisms or genetic disease, misfolded proteins escape the surveillance of molecular chaperones and accumulate as non functional aggregates in cells and tissues. During the course of aggregation, toxic species are generated, causing damage or cell death. In budding yeast cells, the translation termination factor Sup35, a normally soluble protein, can form amyloid-like fibrillar aggregates, and the misfolded form converts the normal protein into the amyloid state, resulting in the infectious, [*PSI*$^+$] prion. Prion propagation requires coordinated activity of the AAA$^+$ protein remodelling factor Hsp104 and the Hsp70 system. We have used electron tomography of freeze-substituted and vitreous sections of prion containing yeast cells to examine these prion aggregates *in situ*, and single particle analysis to study the action of Hsp104.

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Functions and mechanisms of cytosolic chaperones in protein homeostasis

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The yeast Hsp70/40 system Ssb/RAC binds to ribosomes and contacts nascent polypeptides to assist cotranslational folding. Here, we demonstrate that NAC, another ribosome-tethered system, is functionally connected to Ssb/RAC and the cytosolic Hsp70 network. Simultaneous deletions of genes encoding NAC and Ssb caused conditional loss of cell viability under protein folding stress conditions. Furthermore, NAC mutations revealed genetic interaction with a deletion of Sse1, a nucleotide exchange factor regulating the cytosolic Hsp70 network. Cells lacking Ssb or Sse1 showed protein aggregation which is enhanced by additional loss of NAC, however, these mutants differ in their potential client repertoire. Aggregation of ribosomal proteins and biogenesis factors accompanied by a pronounced deficiency in ribosomal particles and translating ribosomes only occurs in *ssb*Δ and *nac*Δ*ssb*Δ cells suggesting that Ssb and NAC control ribosome biogenesis. Thus, Ssb/RAC and NAC assist protein folding and likewise have important functions for regulation of ribosome levels. These findings emphasize the concept that ribosome production is coordinated with the protein folding capacity of ribosome-associated chaperones.

Protein folding and associated stress in the endoplasmic reticulum of fungi

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Yeasts (e.g. *Saccharomyces cerevisiae*, *Pichia pastoris*) and filamentous fungi (e.g. *Aspergillus* spp.) are eukaryotic microorganisms that serve as cell factories for the secreted production of both industrial and therapeutic proteins, including heterologous proteins (encoded by non-native genes). The filamentous fungi, in particular, secrete a variety of hydrolytic enzymes that facilitate their lifestyles and are also exploited in applications, e.g. in production of biofuels. Secretory proteins fold in the lumen of the endoplasmic reticulum and high (or changing) flux through the system, or the expression of proteins that fold slowly, lead to induction of a stress response called the unfolded protein response (UPR). The UPR encompasses a variety of homeostatic responses that mitigate against the stress and the UPR in *S. cerevisiae* serves as the model for all eukaryotes. The yeast responses to stress in the ER are far less versatile than the system in higher eukaryotes but recent information also shows divergence between fungal species. The molecular basis of the UPR will be compared between fungi, as will the consequences for fungal biology and strain improvement in the secreted production of heterologous proteins in biotechnology.

Offered paper Expression of plant Hsp70 and Hsp101 in a yeast-based system reveals both conservation and divergence in chaperone function with respect to heat-shock response and prion propagation

Emma Guinan, Ciara Moran, Harriët M. Loovers & GARY W. JONES

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Hsp104 is a hexameric AAA+ ATPase found in *Saccharomyces cerevisiae* that in conjunction with cytosolic Hsp70 chaperone machinery resolves denatured protein aggregates. As well as providing acquired thermotolerance in yeast, Hsp104 is also an essential factor in maintaining propagation of ordered protein aggregates such as prions. While absent in metazoa, plants are the highest eukaryotes to possess an obvious Hsp104 orthologue. To assess evolutionary conservation of protein dis-aggregase function we have expressed *Arabidopsis thaliana* Hsp70 and Hsp101 in *S. cerevisiae*. Two highly homologous cytosolic plant Hsp70s are able to provide essential chaperone functions in yeast as well as propagate the [PSI^+] prion to varying degrees. Upon introduction of analogous yeast Hsp70 mutations known to disrupt prion propagation into plant Hsp70s, these proteins are destabilized in yeast. An unstable peptide-binding domain mutant can be stabilized by second-site mutations within the ATPase domain. While expression of Hsp101 can provide acquired thermotolerance in an *hsp104* deletion strain, Hsp101 cannot maintain propagation of [PSI^+], even when expressed in conjunction with plant Hsp70s. This suggests that Hsp101's inability to propagate [PSI^+] is due to a defect in recognizing or processing prion aggregates rather than an inability to collaborate with the endogenous yeast Hsp70 chaperone machinery.

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Offered paper **Genetic control of *de novo* formation of the yeast [*PSI*+] prion**

Klement Stojanovski[1], Nadejda Koloteva-Levin[1], Theodora C. Sideri[2], Tobias von der Haar[1], Chris M. Grant[2] & MICK F. TUITE[1]

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The *de novo* formation of native prions in the yeast *Saccharomyces cerevisiae* requires the presence of at least one particular prion, [*PIN*+]. In most stains [*PIN*+] is the prion form of the Rnq1p protein and is the only prion so far reported to exist in natural (wild-type) strains. In [*PIN*+] strains the *de novo* formation of [*PSI*+], the prion form of Sup35p occurs spontaneously at a frequency between 10^{-6}–10^{-7} and is not usually associated with any genetic change either at the *SUP35* locus or at any other unlinked locus. However, we have identified several nuclear gene mutations that elevate the frequency of [*PIN*+]-dependent, *de novo* formation of [*PSI*+]. One strategy has been to identify proteins that physically interact with Sup35p and to determine whether, in their absence, the rate of *de novo* formation increases. This strategy has identified Ppq1p, a putative protein serine/threonine phosphatase. Deletion of *PPQ1* leads to an increase in the frequency of spontaneous *de novo* formation of [*PSI*+] and when induced by overexpression of Sup35p. We have also identified two other genes whose products suppress *de novo* prion formation, namely the *TSA1* and *TSA2* genes. Tsa1p and Tsa2p are sequence-related antioxidants that protect cells against endogenous reactive oxygen species (ROS). A small fraction of the more abundant Tsa1 protein and majority of Tsa2p localize to ribosomes. The deletion of *TSA1* and *TSA2* leads to a significant oxidative damage-induced aggregation of Sup35p which leads to the formation of the transmissible [*PSI*+] prion. These data support a model where peroxiredoxins function to protect the ribosomal machinery against oxidative damage, but when these systems become overwhelmed, [*PSI*+] prion formation provides a mechanism for uncovering genetic traits which aid survival during oxidative stress conditions.

Protein misfolding done right: the biogenesis of bacterial amyloid fibres

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Functional amyloids are produced by organisms that span nearly every facet of cellular life. Microbial functional amyloids are especially interesting because they can fulfill essential physiological roles for the microbe, but also because these organisms can offer sophisticated genetic and biochemical tools for understanding how cells coordinate and control amyloid formation. Curli are functional amyloids produced by many Gram-negative bacteria, including *E. coli*. Curli fibers are associated with biofilm formation, host cell adhesion and invasion, and immune system activation. The major curli subunit protein, CsgA, polymerizes into amyloid after interacting the CsgB nucleator protein. CsgB presents an amyloid-like template to CsgA on the cell surface that initiates fiber formation. We have identified anti-aggregation/chaperone-like activities in *E. coli* that are induced during curli assembly. Characterization of this protein-mediated activity will be presented. Furthermore, we have also found that the CsgA and CsgB proteins have evolved such that their aggregation propensity inside the cell is tempered, but promoted on the cell surface. CsgA has five imperfect repeating units (R1-R5) that are each predicted to form strand-loop-strand structures. Asn and Gln residues in R1 and R5 were found to be required for efficient amyloid formation and for interaction with the CsgB nucleator protein. Conversely, CsgA polymerization is slowed by the presence of conserved aspartic residues in R2, R3 and R4. When these aspartic acid residues were changed to alanine (CsgA*), polymerization was significantly faster *in vitro*. Even more remarkable was the observation that CsgA* assembled into an amyloid fiber *in vivo* in the absence of CsgB. The ability of CsgA* to polymerize into amyloid more efficiently, and in the absence of CsgB, was not without consequences. Cells expressing CsgA* grew more slowly when compared to cells expressing wild type CsgA. This analysis suggests that aspartic acid residues can potently inhibit functional amyloid formation. CsgA has apparently evolved to efficiently assemble into an amyloid *in vivo* only in the presence of CsgB. This suggests an elegant mechanism to control amyloid formation by regulating the temporal and spatial interactions between CsgA and CsgB.

Redirecting amyloidogenic trajectories

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Safely eradicating prions, amyloids and preamyloid oligomers may ameliorate several fatal neurodegenerative disorders. Yet, whether small-molecule drugs can directly antagonize the entire spectrum of distinct amyloid structures or 'strains' that underlie distinct disease states is unclear. Here, I present our efforts to illuminate this issue using the yeast prion protein Sup35. We have established how various small molecules block synthetic Sup35 prionogenesis, eliminate preformed Sup35 prions, and disrupt inter- and intramolecular prion contacts. Unexpectedly, these direct activities can select for drug-resistant prions. To combat drug-resistant strains we have isolated minimal drug cocktails (small molecule combinations) that directly eradicate a broader spectrum of prion strains.

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NT13 Learning from the evidence: improving microbiology teaching through educational research

↑CONTENTS

Pedagogic research – the why and the how

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There is increasing emphasis on pedagogic research at university level, including its performance by staff, its application to improving courses and its significance in contributing to promotion prospects. This is particularly so when, as currently, teaching methods, tools, styles and expectations are undergoing great change. University staff who are usually trained and experienced in researching the science of their discipline, are sometimes less confident in tackling pedagogic issues and the purpose of this presentation is to provide some pointers for those wishing to get started in pedagogic research. The presentation addresses a number of issues such as : how to get started in pedagogic research (PedR); does participation in PedR matter to staff; how to fund PedR; providing time to conduct PedR; choosing a PedR area in which to work; differences between PedR and science research; publishing the results from PedR; and coping with the background literature for PedR. A case study of an individual's involvement in both science research and PedR will be presented. Following the presentation university staff should be in a better position to participate in PedR which should improve the student experience and enhance promotion prospects for staff.

Collecting the evidence – an introduction to research methods and methodologies

Mike Joy

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Scientists who are undertaking educational research in their discipline need to use research methodologies grounded in the social sciences, and these can be very different from the approaches used in scientific disciplines. This talk aims to help with 'getting started' in educational research, and to de-mystify how social science research takes place. Some types of methodologies commonly used in Education are introduced, and these are contextualized from a scientist's standpoint.

Teaching the skills of experimental design: a research project to compare the effectiveness of face to face group work and online approaches

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Rigorous evaluation through research is important for providing evidence for the effective future development of approaches. We have developed resources to help students design experimental strategies. Students work independently from the tutor, who takes the role of facilitator for several groups at the same time. Students are given a 'real' problem to solve; relating to situations encountered in industry, medicine or academic science.

For example: How would you identify targets for drugs that may potentially be used to treat infections of the pathogenic fungus Candida albicans? Students are presented with an extensive set of options. Right and wrong decisions can be taken and there is more than one solution to the problem. The programme also gives students the opportunity to develop their skills in group interaction, decision-making and oral communication. For group work the sets of researched options are presented to the students as packs of decision-making cards. We have also developed online versions that enable the students to work individually through the exercises. We have carried out research to compare the effectiveness of the two approaches in improving the students' ability to answer factual questions and to write a short summary of their final experimental strategy.

Getting published: writing papers for pedagogic research journals

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University scientists often put considerable thought and creativity into the development of novel teaching resources and interventions without necessarily thinking of this as publishable material. This session will consider some of the benefits arising from writing up and sharing educational innovations, offer tips on some relatively minor tweaks to the work you are already doing to bolster the likelihood that it will be accepted for publication and some suggestions regarding suitable journals to which pedagogic research can be submitted.

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Education research projects for final-year students – the supervisor's perspective

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Education projects are undertaken by a number of final year students as an alternative to laboratory research projects, and have gained approval from external examiners as providing an adequate training in scientific methods (experimental design, data evaluation and science communication). The projects appeal to students interested in postgraduate teaching qualifications or with general interests in science communication. Students develop a 'product' or resource of value for teaching and learning. A major part of the project is to evaluate the effectiveness of their educational intervention, relate their results to educational literature, and present these findings in a report. At The University of Manchester, Faculty of Life Sciences, we place students in primary, high schools & colleges, in the Faculty and in museums. Students have developed laboratory classes; novel board & card games and run 'after-school' workshops; have inspired their pupils to make models of neurones using sponges; justify pharmaceutical spending in song and make 3-D posters to explain meiosis. The distribution of marks awarded to Education projects is similar to those of laboratory-based projects. Anecdotal evidence from former students has shown they have found Education projects to be enjoyable and provide valuable transferable skills. Feedback from schools is invariably excellent, as the projects allow difficult or recent scientific advances to be communicated to their students in novel and interesting ways. The school is then left with a tried and tested educational resource that can be used in future years.

Developing a learning resource for teenagers: a student's perspective of educational research

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A computer-based sexual health learning resource for pupils aged 15–16 years has been developed. Sexually transmissible infections are rising yearly in the UK, and disproportionately affect young people. The need for effective sexual health education is clear. The resource provides an introduction to microbiology using sexually transmitted infections as a framework. Through understanding the causative agents, pupils can appreciate the disease courses and treatment options. Contact was made with a local school where a baseline questionnaire was conducted to ascertain the existing knowledge of the students and to establish the key areas of focus. Additionally, opinions were gathered regarding current sexual health lessons. A detailed literature review was performed to allow medically correct and relevant authoring of the resource. Using the information derived from the baseline questionnaire and literature review, a computer resource was designed using *Questionmark Perception*. Following construction, a pilot was conducted at the pilot school. Students were asked to complete a pre-tutorial and post-tutorial questionnaire, and a final evaluation. Results suggested that the resource successfully increased the students' knowledge. Use of larger pupil cohorts, inclusion of a greater diversity in pupil ability and assessment at various time points following resource use are potential improvements for this project.

Crossing the divide: experiences of a 'quantitative' microbiologist going 'qualitative'

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Educational research is by nature often qualitative, whereas microbiologists are trained and experienced in quantitative research. Both terminology and methodology of the new subject are not easily accessible. Therefore getting started and actually wanting to conduct research outside one's comfort zone, using recently acquired skills, is challenging. Anticipation of standard and levels of competition can easily discourage: one doubts whether one's contribution would be accepted by traditional educational scholars and within one's own disciplinary culture. The opinion of the former cannot easily be gauged and 'quantitative' scientists culturally do not readily accept qualitative research results. Understanding that one has to consciously step away from one's quantitative mindset is crucial. Persistence to gain experience in the new field will then enable to apply necessary scrutiny and will eventually lead to the confidence one would usually get from a large sample size and dataset. Being aware that in qualitative research one does not test hypotheses but analyses beliefs, combined with a 'quantitative' scientist's passion, scientific rigour and other skills will allow for educational research of acceptable standard. Findings of a qualitative research project regarding the 'divide' as well as personal experiences of the author will be subject of the session.

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Using research to inform and enhance microbiology teaching

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A second year research skills module was developed, which acted as an intermediate module between the prescriptive practicals at levels one and two and the final year research project. The module was evaluate pre and post course and the findings suggested that understanding of the research environment and research process which was poor pre course, improved after completion of the module. This increase in student understanding and confidence was also observed in their understanding of research literature, their ability to write scientifically, and present scientific material. The research projects carried out by the students as part of the module pedagogy were successful, but during the process it was found that those projects which were less defined at the start and offered the opportunity to design research questions were more successful in the development of higher cognitive skills than projects which were highly defined at the start. The research project allowed the students to become emotionally attached to their work and this substantially increased motivation to succeed. This enquiry based learning module was an ideal way to provide a platform for the meeting of teaching and research. One of the research projects developed during the module, investigating the microbial content of commercially available probiotic products was then adapted for a level 2 microbiology practical for approximately one hundred students. This practical enabled the students to work in small 'research' teams, where they used molecular techniques for identification and traditional culture techniques to study the survival of bacteria in artificial gastric juice. The students had to think about experimental design and the appropriate use of statistics as well as making their own growth media and solutions for their investigations.

In a separate project, also as a spin-off from the research skills module, a second year microbiology student had a summer placement funded by the SGM to take still images and video clips of fungi, to try and enhance the teaching of mycology at undergraduate level. This has resulted in a website where people can download images and movies to illustrate their lectures free of charge.

References www.bioscience.heacademy.ac.uk/journal/vol11/beej-11-5.pdf
www.bioscience.heacademy.ac.uk/ftp/TeachingGuides/studentresearch/yeoman.pdf;
www.uea.ac.uk/bio/joyoffungi

NT14 Microbiology in the indoor environment

↑CONTENTS

Legionella pneumophila survival in aquatic environments

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The human pathogen *Legionella pneumophila* is the causative agent of both a potential fatal atypic pneumonia (Legionnaires' disease) and a less severe self-limiting flu (pontiac fever). In order to grow as a pure culture in the lab, *L. pneumophila* requires specific conditions like an incubation temperature of 37°C and nutrients including iron salts and a number of amino acids such as *L*-cysteine, which are used as carbon, nitrogen and energy sources. These specific growth requirements contradict with the widespread distribution of *Legionella* bacteria in freshwater environments, where they occur under non-stringent conditions, namely temperatures of 5.0–63.0°C and a pH range of 2.7–9.2. Based on recent experimental data, it has been hypothesized that under environmental conditions *L. pneumophila* obtains its necessary nutrient supply from the microbial consortium located in biofilms. Biofilms are defined as spatially structured, highly dynamic polymicrobial communities that commonly occur in freshwater environments. Their physical and chemical habitat heterogeneity and the multitude of biotic interactions result in the creation of distinct niches on a microscale, which might allow survival of bacteria with unusual nutritional requirements, such as *L. pneumophila*, under a broad range of environmental conditions.

For ten years, Dr. Declerck performs research on the ecology of *L. pneumophila* in both anthropogenic and natural aquatic environments. An overview of this research will be presented.

Sink drain biofilms; recalcitrance and antimicrobial resistance

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Recalcitrant and taxonomically diverse biofilm communities which form within sink drain outlets in the home and elsewhere represent a potential source of microbial contamination and an environment in which sub-lethal exposure to antimicrobials may occur. In this

presentation, the development and validation of a novel continuous culture drain biofilm model will be discussed. A series of studies into effects of long-term exposure to biocide-containing domestic products on microbial composition and antimicrobial resistance indicated that whilst biocide-exposure caused the clonal expansion of less susceptible clones, statistically significant changes in susceptibility to antibiotics were not detected. Triclosan, a commonly used biocide was partially degraded by domestic drain biofilm consortia.

Fungi and sick building syndrome

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We spend an increasing proportion of our time indoors, and micro-organisms are ever-present in indoor domestic, office, healthcare and industrial environments. In most instances our health is not affected by this exposure, but in non-industrial environments, health problems such as sick building syndrome (SBS) and building related illnesses have been attributed to airborne mould exposure. SBS is a generic term used to describe ill health that is attributed to extended periods spent in a particular indoor environment, with a range of symptoms of ill health. Risk factors include physical (temperature, humidity), chemical (presence of volatile chemicals), psychological (personal control over the environment) and biological (exposure to moulds and endotoxin) parameters. Building related illnesses are more directly attributable to fungal exposure that could result in allergic or toxicological response, with evidence from investigations conducted in USA and northern Europe. This presentation will provide an overview of SBS, a background to fungi in the indoor environment, and describe strategies to investigate, to undertake representative sampling and analysis, and to carry out remediation to prevent further exposure.

Buildings, wood and *Serpula lacrymans*

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Serpula lacrymans, cause of dry rot of wood in buildings, is damaging for two biological reasons: its ability to spread across non-nutrient surfaces to colonize fresh wood elements of the building, and its capacity for rapid destruction of wood tensile strength. Using experiments designed to mimic conditions of successive wood exploitation, mycelial spread and the capture of fresh wood resources, we imaged the flow of free amino acid in mycelial cords of *S.lacrymans*, *Coniophora puteana* and other cord-forming wood decay fungi. The speed and direction of amino acid into freshly-colonized woody resources from the rest of the mycelium is rapid, and responsive to changes in wood resource distribution. These observations support our earlier view that non-metabolized amino acid analogues inhibit mycelial spread by competitive inhibition of nutrient amino acid uptake and metabolism. The low-toxicity compound 2-aminoisobutyric acid can reduce the concentration of toxic fungicide required for fungal inhibition by 50%, and offers a way of reducing the toxicity of wood preservative formulations. Genomic comparison of *S. lacrymans* with close relatives which do not pose a threat to buildings is expected to elucidate the biological features that render *S. lacrymans* pernicious, and to indicate other targets for control.

Offered paper Development of a biofilm remover for dental water units

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The quality of dental unit water lines (DUWL) is very important, as patients and dental staff are frequently exposed to water and aerosols generated from the dental unit. DUWL may become heavily contaminated with opportunistic pathogens such as *Pseudomonas aeruginosa* from the supply water. Even if the units are regularly flushed, it is rarely possible to achieve the recommended, and shortly to be enforced, levels of <200cfu/ml and in untreated systems counts from water from the DUWL often exceed 10^5 cfu/ml. We aimed to develop a biofilm remover formulation that was easy to use, non toxic and biodegradable which was compatible with the materials commonly found in dental units. Formulations were initially tested against biofilms of organisms isolated from DUWL in microtitre plates, and iteratively developed on the basis of crystal violet assays. The organisms were also tagged with marker genes to confer bioluminescent and fluorescent phenotypes which were used to rapidly assess biocidal efficacy of the formulations and readily image the removal of biofilm from intermittent rapid flow models. A biofilm remover formulation which met the initial product specifications was developed, has been field-tested and is planned to be launched into the market in 2010.

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Offered paper **Measuring respiratory output of aerosolized *Mycobacterium tuberculosis* by mask sampling**

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Mycobacterium tuberculosis (Mtb) is transmitted in small aerosol droplets expectorated by individuals with active pulmonary tuberculosis (TB). At present, the World Health Organization estimates that TB is spreading at the rate of one person per second. Transmission of TB often occurs in indoor spaces that are poorly ventilated and overcrowded such as slum housing, prisons, nursing homes, correctional facilities, and refugee camps. The medical practice currently relies on the outcomes of smear microscopy to predict the infectiousness of TB patients; this has been refuted by several studies. Little is known about the relationships between respiratory Mtb output and case infectivity, with one reason being lack of an established method for sampling tuberculous aerosols and measuring the bacillary concentration in them. We proposed the use of a novel mask sampling approach to address this question, and this preliminary study reports its development and evaluation. By coupling this sampling method to downstream detection by a modified mycobacteriophage amplification assay, we were able to detect and to some extent quantify the amount of respiratory-borne Mtb produced by TB patients who were positive by smear microscopy. A total of 11 out of 15 patients gave positive masks in which Mtb was detected in 14 out of a total of 25 masks processed. Preliminary data also suggested possible existence of patterns of infectivity among patients; however this necessitates further investigation. With further optimization and improvement, the mask sampling method has great potential in prompt identification of infectious cases which would fuel subsequent efforts in controlling and interrupting disease transmission.

Liquids, lights and gasses – novel approaches to environmental decontamination

Mathew Upton

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Logic tells us that an environment carrying an elevated burden of potentially pathogenic bacteria is not good for our health. However, there are relatively few good studies that show a correlation between increased, or modified cleaning regimens and reduced infection rates. This has not stopped a raft of novel interventions coming to market with broad claims relating to 'elimination of healthcare-associated infections'. These approaches range from surface modification or novel means of delivering conventional cleaning agents, to some really quite abstract inventions.

We have tested some recently developed approaches in laboratory and clinical settings. These studies included evaluation of a novel cleaning agent that led to a 30% reduction in environmental contamination with MRSA during an 11 month, cross-over trial in which the cleaning agent showed parity with chlorine releasing agents. We have used a simulated hospital side-room to demonstrate significant reductions in bacterial loads on surfaces exposed to gaseous ozone and have seen significant reductions in counts of bacteria and fungi on naturally contaminated surfaces exposed to UV light from a portable unit.

It is possible that some of these interventions could have a positive impact on infection rates in health-care settings, though large randomized trials will be needed to demonstrate such efficacy.

Microbial communities associated with household dust

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Household dust is widely used in epidemiological studies for measuring human exposure in indoor environments; it is considered a collection of everything that has been airborne and it is easy to collect in massive studies with thousands of participants. Apart from infectious diseases, exposure to microbes, their structural parts and metabolites can affect our health in many ways. Therefore there is increasing interest for studies measuring the interactions of indoor microbes and human health. Dust is an assemblage of microbes from different sources; outdoor air, occupants, pets, etc. Microbes also live attached to surfaces that are occasionally wet, for example in the kitchen or bathroom and eventually become airborne to end up in settled dust. In addition, moisture damage of the building is a strong source of dust microbes. We have studied the microbial communities in household dust using molecular and culture methods. Household dust is rich in microbes; depending on the collection site, the species diversity of both fungi and bacteria reaches up to 500–1000 species. The microbial communities in indoor dust are more or less unique for the building apart of some core species that are mostly originating from human occupants of the building. The differences between buildings

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are largely due to the differences between the users of the building but also other factors, such as different cleaning habits, life style factors, etc. For example, farming homes have a richer microbial content than urban homes.

Fungi in museums, collections and libraries: biodeterioration of cultural heritage

Katja Sterflinger

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Fungi play a considerable role for the deterioration of cultural heritage. Due to their enormous enzymatic activity and their ability to grow at low a_w values fungi do not only inhabit but decay paintings, textiles, paper, parchment, leather, oil, casein, glue and other materials used for historical and recent art objects. Also the weathering of stone monuments is significantly increased by epi- and endolitic fungi. Case studies of fungi in museum collections are shown in order to demonstrate how contaminations can be detected, monitored and prevented. In museums and their storage rooms, climate control, regular cleaning and microbiological monitoring are essential in order to prevent fungal contamination. Education and close collaboration of mycologists and restorers are needed to develop object specific methods for the conservation and treatment of contaminated objects. A study comparing different methods for the decontamination of 17th century books – including Gamma radiation, ethanol cleaning, chloro-m-cresol and other treatments methods – is presented as an example of close and successful collaboration between material scientist, restorers and mycologists leading to an optimized procedure for cleaning and conservation of objects threatened by fungal deterioration.

Fungal spores, allergy and asthma

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Fungi are a ubiquitous component of our environment. They form an important part of the soil and airborne ecology and impact on human health in a variety of ways. Fungi are important pathogens of man causing thousands of deaths each year, usually in immunocompromised individuals. However a far greater proportion of the population in affected by fungal allergy with serious consequences in asthmatics. Fungi are common in the indoor environment and have been linked with sickness due to volatiles but it is likely that the most serious impact arises from production of allergen proteins and their persistence. Here we will consider the nature, prevalence and persistence of fungal allergens and their actual and proposed impact on human health.

Molecular environmental microbiology of the intensive care unit environment

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The internal environments of two intensive care units and a high-dependency unit at Sheffield hospitals were investigated in order to explore the use of molecular environmental microbiology techniques within hospital wards during non-outbreak situations. Environmental DNA was isolated from a variety of hard surfaces and handwash sink plugholes in wards. PCR and DGGE techniques were used to characterize microbial populations (via 16S rRNA genes) and a number of antibiotic resistance determinants including *mecA* and β-lactamase genes. Culture-based analysis was performed in parallel. It was found that the molecular techniques allowed detection of 16S rRNA genes specific to groups of bacteria that could not be isolated via culture-based methods and revealed the diversity and persistence of microbial communities associated with various surfaces. Reservoirs of environmental and opportunistic pathogenic genera, including *Burkholderia*, *Pseudomonas*, *Stenotrophomonas* and *Klebsiella* and *Candida* were found in sink plughole samples. The effect of routine ward cleaning was also investigated. DGGE revealed that similar 16S rRNA genes representing Gram-negative opportunistic bacterial species were retrieved from sink samples before and after cleaning. Cleaning was effective in removing culturable antibiotic-resistant bacteria, although antibiotic resistance genes could be amplified via PCR from environmental samples even after cleaning.

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The ability of *Salmonella enterica* to form a biofilm on food contact surfaces

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Background and objective Salmonellosis is the second most common cause of bacterial foodborne gastroenteritis. The ability of *S. enterica* to form a biofilm in food processing environments may be a significant factor in the transmission of foodborne illness. It is important to investigate the ability of *S. enterica* to form a biofilm on surfaces associated with food processing.

Method Biofilm growth was established using the CDC biofilm reactor (CBR) system. The reactor facilitates biofilm growth and examination of up to 24 coupons simultaneously. Coupons representing relevant materials (glass, stainless steel, polycarbonate plastic, glazed tile and concrete) were used. The CRB was operated under batch-phase for 24 hours followed by continuous-flow phase for 24 hours. The coupons were subjected to sonication to remove biofilm mass. Viable count method was used to enumerate cell counts.

Results All nine *Salmonella* (5 *S.* Agona, 2 *S.* Typhimurium and 2 *S.* Enteritidis) form a biofilm on all 5 surfaces tested. The highest mean viable count per standard coupon was for glazed tile for all strains tested followed by concrete and polycarbonate. Viable counts from glass and stainless steel were lower suggesting reduced intensity of biofilm formation.

Conclusion The CBR system is a useful method for investigating the differences in multiple surfaces ability to support biofilm formation.

NT16 Bacterial vesicles

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Contributions of outer-membrane vesicles to toxicity, inflammation and community survival

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Outer membrane vesicles (OMVs) are closed spheroid particles of heterogeneous size released from Gram-negative bacteria during all phases of growth. OMVs are produced by a mechanism yet to be determined. They appear to be formed from OM bulges and subsequent fission of vesicles. In general, OM vesicles reflect the OM composition, including lipopolysaccharide (LPS), phospholipids, OM proteins, as well as periplasmic components. Genetic mutations affect the quantity of OMVs produced, although no null mutant has yet been discovered. OMVs have been shown to have a multitude of possible functions, including the capability to transmit virulence factors in pathogenic strains, as well as to bind to or destroy other bacteria. They have also been shown to act as a method for cells to remove misfolded proteins that activate envelope stress. OMVs can transport toxins and endotoxins *in vivo* leading to important virulence-assocated conseuences. We have also recently found that OMVs can play a role in bacterial resistance to antimicrobials. Thus, OMVs are proposed to play several important roles in pathogenesis.

The role of *Campylobacter jejuni* outer-membrane vesicles in host–pathogen interactions

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Campylobacter jejuni is the most prevalent cause of food-borne gastroenteritis in the developed world, however the molecular basis of pathogenesis is unclear. Secretion of virulence factors is a major mechanism by which enteric bacterial pathogens interact with host cells to enhance their survival and/or damage the host. *C. jejuni* lacks the virulence-associated secretion systems possessed by other enteric pathogens. Many bacterial pathogens utilize outer-membrane vesicles (OMVs) as secretory vehicles for the delivery of virulence factors into host cells. We hypothesized that in the absence of prototypical virulence-associated secretion systems, OMVs are an important mechanism for the co-ordinated delivery of *C. jejuni* proteins into host cells. Proteomic analysis of *C. jejuni* 11168H OMVs reproducibly identified 120 proteins, including a large number of periplasmic and membrane-associated proteins but also previously identified virulence factors. Further analysis indicated that *C. jejuni* OMVs possess proteolytic activity and contain many *N*-linked glycoproteins, which indicates how these periplasmic-located glycoproteins may be able to interact with the host immune system. Co-incubation of T84 intestinal epithelial cells with *C. jejuni* OMVs results in a host cell response similar to that induced by live *C. jejuni*. Our data suggests an important role for *C. jejuni* OMVs during infection.

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Protein cargo selection in the outer-membrane vesicles of a bacterial pathogen

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In contrast to the well established multiple cellular roles of membrane vesicles in eukaryotic cell biology, outer-membrane vesicles (OMV) produced via blebbing of prokaryotic membranes have frequently been regarded as cell debris or microscopy artifacts. Increasingly however, bacterial membrane vesicles are thought to play a role in microbial virulence, although it remains to be determined whether OMV result from a directed process or by passive disintegration of the outer membrane. We have established that the human oral pathogen *Porphyromonas gingivalis* has a mechanism to selectively sort proteins into OMV, resulting in the preferential packaging of virulence factors into OMV and the exclusion of abundant outer-membrane proteins from the protein cargo. Furthermore, we determined a critical role for lipopolysaccharide in directing this sorting mechanism.

Outer-membrane vesicles released by respiratory pathogens interact with the human host defence

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The human respiratory pathogen *Moraxella catarrhalis* reside in tonsils adjacent to B cells and induce a T cell independent B cell response by the immunoglobulin (Ig) D-binding superantigen MID. Whole *Moraxella* are endocytosed and killed by human tonsillar B cells. In contrast, outer-membrane vesicles (OMV) have the potential to interact and activate B cells leading to a potential bacterial rescue. The B cell response induced by OMV was initiated with IgD B cell receptor (BCR) clustering and Ca^{2+} mobilization followed by BCR internalization. In addition to IgD BCR, Toll like receptor (TLR) 9 and TLR2 were found to colocalize in lipid raft motifs. Two components of the OMV, *i.e.*, MID and unmethylated CpG-DNA, were demonstrated to be critical for activation. OMV containing MID activated tonsillar $CD19^{+}IgD^{+}$ lymphocytes resulting in IL-6 and IgM production in addition to increased surface marker density, whereas MID-deficient OMV failed to induce B cell activation. Importantly, this concept was verified *in vivo* as OMV equipped with MID and DNA were found in a 9-year old patient suffering from *Moraxella* sinusitis. In conclusion, *Moraxella* avoids direct interaction with host B cells by redirecting the adaptive humoral immune response using its superantigen-bearing OMV as decoys.

Reference Perez Vidakovics MLA, Jendholm J, Morgelin M, Mansson A, Larsson C, Cardell L-O, and Riesbeck K. B Cell Activation by Outer Membrane Vesicles – A Novel Virulence Mechanism. *PLoS Pathogens* 6(1):e1000724 (2010).

Myxobacterial vesicles: a bacterial minefield?

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Myxococcus xanthus is a ubiquitous soil-dwelling myxobacterium, best known for its social behaviours, which include cooperative digestion of prey organisms. Nutritional phosphatases produce phosphate ions from organo-phosphate substrates and are typically periplasmic or secreted. During attempts to identify phosphatases we found that more than 99.9% of the phosphatase activity of an *M. xanthus* culture was extracellular. Concentration and Mass Spectrometry of extracellular proteins led to the identification of four hydrolases, two outer-membrane proteins, and six lipoproteins. Electron Microscope images showed cells covered with uniform structures, potentially budding vesicles. In addition, ultracentrifugation of spent culture medium produced large amounts of vesicular material, and more than 95% of secreted phosphatase activity was found within this material, suggesting that phosphatases are selectively packaged into vesicles. We propose that on a substrate, hydrolases secreted by non-vesicular pathways would be diluted rapidly by diffusion. In contrast, secretion of hydrolases within vesicles would deliver a high-activity package of digestive enzymes to prey organisms. The increase in effective size of the hydrolases would also reduce diffusion, increasing the proximity of killing to the secreting *M. xanthus* cell, enhancing the efficiency of uptake of prey lysis products.

Phase II clinical trial of *Neisseria lactamica* outer-membrane vesicles in the UK

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Immunity to meningococcal disease is associated epidemiologically with carriage of commensal *Neisseria* species, including *Neisseria lactamica.* Outer membrane vesicles (OMVs) from *N. lactamica* provide protection against lethal meningococcal challenge in mice. We evaluated the safety and immunogenicity of an *N. lactamica* OMV vaccine in a phase I placebo-controlled, double-blinded clinical trial.

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Ninety-seven healthy young adult male volunteers were randomized to receive three doses of either an OMV vaccine or an Alhydrogel control. The OMV vaccine was safe and immunogenic, eliciting rises in titers of immunoglobulin G (IgG) against the vaccine OMVs, together with a significant booster response, as determined by an enzyme-linked immunosorbent assay. Additionally, the vaccine induced modest cross-reactive immunity to six diverse strains of serogroup B *Neisseria meningitidis*, including IgG against meningococcal OMVs, serum bactericidal antibodies, and opsonophagocytic activity. The percentages of subjects showing > or =4-fold rises in bactericidal antibody titer obtained were similar to those previously reported for the Norwegian meningococcal OMV vaccine against the same heterologous meningococcal strain panel. When vaccinated volunteers were challenged intranasally with live *N.lactamica*, there was reduced colonization cf controls, but wild meningococcal carriage-acquisition was not prevented. In conclusion, this *N. lactamica* OMV vaccine is safe and induces good *N.lactamica*-specific immunity but a weak broad humoral immune response to *N. meningitidis*, and does not prevent natural colonization by that organism.

NT17 Microbial models of human disease

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Chlamydomonas as a model for cilia-related disease

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The unicellular green alga *Chlamydomonas reinhardtii* is an ideal model for genetic, biochemical, and microscopic analyses of eukaryotic cilia. Motile and non-motile cilia are widely distributed in the mammalian body. Defective ciliary motility results in male infertility, airway infections, and situs anomalies. Mutation in *hydin* cause hydrocephalus in mice and the 540-kD protein hydin is abundant in *C. reinhardtii* flagella. Loss of hydin affects the ciliary central pair apparatus, which controls axonemal dynein activity. In *hydin* mutant mice, impaired ciliary motility diminishes fluid flow in the brain resulting in hydocephalus. The incidence of hydrocephalus is also increased in patients with immotile cilia syndrome. Non-motile cilia play important roles in sensing and signaling. Bardet-Biedl syndrome (BBS) is a ciliopathy characterized by blindness, kidney disease, and obesity. It is caused by defects in the BBSome, a seven-subunit complex which is conserved and present in *C. reinhardtii* flagella. *C. reinhardtii bbs* mutants are non-phototactic and accumulate signaling proteins in the flagellar membrane. BBSomes are transported by a subset of intraflagellar transport (IFT) particles and might function as IFT cargo adaptors for export of certain flagellar proteins. BBS could be a degenerative disease caused by an abnormal accumulation of proteins in the ciliary membrane.

Using *Schizosaccaromyces pombe* to study human genetic disease

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We use the fission yeast *Schizosaccharomces pombe* as a genetically tractable unicellular model organism to study genetic diseases. Deletion of *btn1*, the *S. pombe* homologue of human *CLN3*, which underlies the most common neurodegenerative disorder of children – neuronal ceroid lipofuscinosis (Batten disease) – causes pleiotropic phenotypes that are rescued by ectopic expression of Btn1p or *H.s.* CLN3, indicating that these proteins are functional orthologues. *H.s.* CLN3 is a multispanning membrane protein that was thought to act at the lysosome. We identified functionally important regions by mimicking disease-causing mutations in Btn1p. Mutations that affect the luminal loops and amphipathic helix have most effect on a set of independent marker phenotypes. Cells deleted for *btn1* area affected in many aspects of cell biology including vacuole homeostasis, cytokinesis, the cell wall, polarized growth, endocytosis, sterol-rich domains and trafficking of vacuolar enzymes. GFP-Btn1p traffics slowly to the vacuole, and its steady-state location is predominantly at the Golgi. Changes in the expression level of Btn1p affect the number, intracellular location and morphology of Golgi compartments (viewed by high pressure freezing EM). We propose that the primary function of Btn1p is at the Golgi, and that its loss affects multiple post-Golgi trafficking pathways.

Lessons from yeast: novel insights into the molecular basis of Parkinson's disease

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Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by the loss of neurons in specific brain regions. The cytoplasmic accumulation of misfolded proteins in the form of Lewy bodies is pathognomonic in PD. Alpha-synuclein (a-syn) is the

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major component of Lewy bodies, which are also positive for ubiquitin and several other proteins. The normal function of a-syn, as well as the molecular mechanisms through which it causes cytotoxicity, are still unclear. *S. cerevisiae* has been extensively used to study a-syn biology. Importantly, a-syn expression in yeast inhibits its growth in a concentration-dependent manner, similarly to what is observed in cell and animal models, and in PD patients. A recent chemical genetics approach enabled the identification of several small molecules which alleviate a-syn-induced toxicity and correct mitochondrial dysfunction. We are now exploring yeast genetics to investigate the role of a-syn post-translational modifications and to uncover novel putative targets for therapeutic intervention.

Offered paper **A high-throughput *Caenorhabditis elegans* assay to efficiently examine virulence of large numbers of *Pseudomonas aeruginosa* strains**

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Pseudomonas aeruginosa is a prominent opportunist pathogen that causes a large burden of severe hospital-associated infections worldwide displaying a wide variety of virulence factors, potentially indicative of a broad spectrum of disease-causing potential. The nematode *Caenorhabditis elegans* has been shown to be a useful tool for the study of *P. aeruginosa* virulence. However, previously described assays are both excessively labour intensive and time consuming. We have developed a new *C. elegans* High-Throughput Assay (HTA) to allow for rapid virulence-associated screening of large numbers of *P. aeruginosa* strains. To validate this *C. elegans* HTA, we obtained HTA results for 14 *P. aeruginosa* strains and compared these to those derived using the well-established Slow-Killing Assay (SKA) which generates *C. elegans* survival curves as assay outputs. The observed correlation was particularly strong and was found to be statistically highly significant (correlation coefficient=0.778; P-value<0.001). Subsequently, we tested the association between *P. aeruginosa* virulence as measured by the HTA in 126 clinical isolates and carriage of genes coding for four *P. aeruginosa* type three secretion system effectors: *exoU*, *exoS*, *exoT* and *exoY*. *exoU*-positive and *exoT*-positive strains were more virulent than *exoU*-negatives and *exoT*-negative strains (P-value=0.027 and 0.015, respectively). No statistically significant association was noted with regards to carriage of *exoS* or *exoY* genes. This new HTA is both a simple and time efficient method for rapid virulence screening of large numbers of *P. aeruginosa* strains. Furthermore, it is particularly suited to providing supporting datasets for future large scale genome-wide association studies that are now feasible due to major reductions in cost of high throughput DNA sequencing.

Offered paper **Yeast as a model for investigation of the human interleukin-1α nuclear signaling**

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Interleukin-1α (IL-1α), a master proinflammatory cytokine regulator and a key player in host immune responses, has been extensively studied for its ability to contribute to various human autoimmune and inflammation-linked disorders including rheumatoid arthritis, Alzheimer's disease, systemic sclerosis or even cardivascular disorders (Vicenova *et al.*, *Physiol Res* 2009). Besides the well-accepted role of IL-1α in extracellular signaling, a significant portion of IL-1α remains in the cell nucleus. We and others have previously discovered the transcriptional activation function of nuclearly localized IL-1α and its cooperation with histone acetyltransferase (HAT) complexes using the yeast model (Buryskova *et al.*, *JBC* 2004, Werman *et al.*, *PNAS* 2004). Employing the power of yeast genetics, we further extended our studies on physical interactions of IL-1α with HAT complexes. Obtained data essentially expand our knowledge on the nuclear function of IL-1α and could contribute to the identification of novel therapeutic targets for inflammation-linked diseases. Concurrently, we gained novel unexpected results increasing our knowledge about molecular composition of yeast SAGA and ADA HAT complexes.

This work was supported by the Ministry of Health of the Czech Republic (project No. NS9819) and by the Ministry of Education, Youth and Sports of the Czech Republic (projects No. MSM0021620813 and LC06066).

Protozoa: cellular models of bacterial virulence

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Environmental bacteria adopt different strategies to resist protozoa. *Legionella pneumophila*, the causative agent of a severe pneumonia called Legionnaires´ disease, replicates in free-living amoebae and, by using a conserved mechanism, also in macrophages of the innate immune system. We use the genetically tractable social amoeba *Dictyostelium discoideum* as a model to study the virulence of *L. pneumophila*. *L. pneumophila* replicates within a unique membrane-bound host cell compartment, the '*Legionella*-containing vacuole'

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(LCV). Formation of LCVs is a complex and robust process that requires the bacterial Icm/Dot type IV secretion system (T4SS) and more than 150 different translocated 'effector' proteins, which subvert host cell signal transduction and vesicle trafficking pathways. LCVs acquire markers of the endoplasmic reticulum and are decorated with small GTPases, phosphoinositide (PI) lipids and PI-metabolizing enzymes. A number of *L. pneumophila* effector proteins anchor *via* PI lipids to the LCV membrane, where they target small GTPases or PI-metabolizing enzymes. Recently, we established a simple and fast LCV purification protocol using fluorescently labeled LCVs from infected *D. discoideum*. Intact LCVs were purified by immuno-magnetic separation with a primary antibody against a T4SS-secreted effector protein localizing exclusively to LCV membranes and a secondary antibody coupled to magnetic beads, followed by density gradient centrifugation. The proteome of purified LCVs was analysed by LC-MS/MS and revealed more than 560 host cell proteins, including small GTPases and protein or lipid kinases and phosphatases. Thus, *D. discoideum* is a powerful and versatile model to analyse on a biochemical, genetic and cell biological level LCV formation and intracellular replication of *L. pneumophila*.

The *Dictyostelium* response to *Legionella* infection: from microarray analysis to functional characterization of infection-specific genes

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The social amoeba *Dictyostelium discoideum* is amenable to a number of genetic, cellular and molecular biology techniques and increasingly being used as a simple model for the investigation of genes that are relevant to human health. Examples include the analysis of immune-cell disease, lissencephaly, protein aggregation disease and mechanisms of drug action. In the last ten years it has also been shown that the professional phagocyte can be used for the study of the medically relevant infection of host cells by pathogenic microorganisms, among them *Mycobacterium marinum*, *Salmonella typhimurium*, *Pseudomonas aeroguinosa* and *Legionella pneumophila*. Infection of *D. discoideum* with *L. pneumophila* resulted in a large number of differentially regulated genes among them three core autophagy genes, ATG8, ATG9, and ATG16. Macroautophagy contributes to many physiological and pathological processes and might also constitute an important mechanism in cell-autonomous immunity. For further studies we selected the highly conserved ATG9. In colocalization studies with GFP-tagged ATG9 and different organelle marker proteins we neither observed colocalization with mitochondria, the ER nor lysosomes. However, there was partial colocalization with the Golgi apparatus and many ATG9-GFP containing vesicles localized along microtubules and accumulated around the microtubule organizing center. ATG9-deficient cells had pleiotropic defects. In addition to growth defects they displayed severe developmental defects, consistent with the known role of autophagy in *Dictyostelium* development. Unexpectedly, the ATG9 mutant also had a strong phagocytosis defect that was particularly apparent when infecting the cells with *L. pneumophila*. However, those *Legionellae* that entered the host could multiply better in mutant than in wild-type cells, due to a less efficient clearance in the early and a more efficient replication in the late phase of infection. We conclude that ATG9 and hence macroautophagy has a protective role during pathogen infection.

Sir Howard Dalton Young Microbiologist of the Year Competition

A novel mechanism of coupling quorum sensing to gene regulation in *Rhizobium leguminosarum*

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Rhizobia are important for their ability to form nitrogen-fixing nodules on legume plants. In several rhizobia, this symbiosis has been shown to be influenced by quorum-sensing gene regulation, which allows them to regulate their gene expression in a population dependent way. Typically, quorum-sensing systems consist of two genes. One encodes an enzyme that synthesizes signalling molecules (acyl homoserine lactones or AHLs), and the other encodes a transcriptional regulator that can regulate specific genes in response to the AHLs made. In *Rhizobium leguminosarum* bv. *viciae,* which nodulates peas, the *cin* quorum sensing system is involved in regulating exopolysaccharide synthesis and processing and also acts as a master regulator for the control of the other quorum sensing systems. The *cin* system is unusual because it contains a third gene, encoding a small protein without homology to proteins outside of rhizobia. This protein, CinS, acts independently of AHLs and appears to act by interacting with a predicted repressor Csi, which represses the expression of several genes, including *csi*. CinS functions as an anti-repressor such that expression of Csi-repressed genes is relieved as *cinS* expression increases in late exponential growth.

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Investigating the role of the small RNA binding protein, Hfq, in virulence and secondary metabolite production in *Serratia* sp. ATCC 39006

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Serratia sp. ATCC 39006 (S39006) is a Gram-negative bacterium virulent in plant (potato) and animal (*Caenorhabditis elegans*) models. It produces two secondary metabolite antibiotics, prodigiosin and a carbapenem, and the exoenzymes, pectate lyase and cellulase. A complex regulatory network controls production of prodigiosin, including a quorum sensing (QS) system, and we hypothesize that Hfq-dependent small regulatory RNAs might also play a role. Hfq is an RNA chaperone involved in post-transcriptional regulation that plays a key role in stress response and virulence in other bacterial species. We constructed an S39006 Δ*hfq* mutant and showed that production of prodigiosin and carbapenem was abolished, while production of the QS molecule, butanoyl homoserine lactone (BHL), was unaffected. Using transcriptional fusions, we found that Hfq regulates the QS response regulators, SmaR and CarR. Additionally, exoenzyme production and swimming motility are decreased in Δ*hfq*, and virulence is attenuated in potato and *C. elegans*. We are currently analysing the whole transcriptome of S39006 WT and Δ*hfq*, sequenced by RNA-seq, in order to define the complete regulon of Hfq. This study confirms a role for Hfq in pathogenesis and the regulation of secondary metabolite production in S39006, increasing further the complexity of the currently described regulatory network.

The vaccinia virus E3 protein is an inhibitor of the RNA polymerase III dsDNA sensing pathway of innate immunity

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The vaccinia virus E3 protein is an important intracellular modulator of innate immunity that can be split into two distinct halves. The C terminus contains a well defined dsRNA-binding domain involved in the inhibition of PKR and dsRNA pattern-recognition receptors (PRRs). The N terminus on the other hand contains a Z-DNA binding domain with amino acid similarity to the cellular proteins ADAR1 and DAI, although the function of this region remains unknown. Both domains contribute to virus virulence. Recently, RNA polymerase III was described as a novel PRR for dsDNA by transcribing AT-rich DNA to produce 5'triphosphate poly(A-U) RNA which subsequently induces type I interferon. Using a series of luciferase reporter based assays we have demonstrated a novel function for E3 as an inhibitor of the RNA polymerase III sensing pathway. Rather than binding directly to DNA via the Z-DNA binding domain, inhibition of this pathway is mediated via the dsRNA-binding domain. We propose that inhibition of this pathway involves E3 sequestering transcribed poly(A-U) RNA preventing the induction of an innate immune response. These studies further extend our knowledge on the function of the vaccinia virus E3 protein as an inhibitor of innate immunity.

Beyond the consensus: using next-generation genome sequencing to dissect within-host viral population diversity of foot-and-mouth disease virus

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Populations of RNA viruses contain many genetic variants as a result of their rapid replication and high mutation rate. However, many of these variants may exist at low frequencies and, to date, characterization of the diversity within these populations has been limited by the resolution of cloning techniques. With the use of the Illumina Next-Generation Sequencing platform, we have measured the frequency of sequence variants within three foot-and-mouth disease virus (FMDV) samples collected during an infection experiment. We compared the diversity of the inoculum with that of samples collected after intra-host replication. In total, we identified 2,622, 1,434 and 1,703 polymorphisms in the inoculum and two foot lesions respectively: most of the substitutions occurred only in a small fraction of the population (<0.5%). We hypothesize those substitutions in <0.1% of the population represents the progeny from cellular replication of FMDV prior to the onset of any selective pressures. Using these data, we estimated an upper limit for virus intra-cellular mutation rate to be 9.5×10^{-4} nucleotides/transcription-event, in line with previous estimates. We conclude that this new sequencing methodology is a powerful tool for dissecting complex viral populations and will be valuable in quantifying the micro-evolutionary dynamics of RNA viruses.

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Construction and analysis of a DnaK mutant in *Clostridium difficile*

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In *Clostridium difficile*, the heat shock response is characterized by the elevated expression of molecular chaperone machineries including the classic HrcA-GrpE-DnaK-DnaJ and the GroEL-ES chaperone complexes. These heat shock proteins play a pivotal role in the folding of nascent polypeptide chains during normal growth conditions and in assisting the refolding of proteins after thermal damage. In order to investigate the importance of the DnaK chaperone in *C. difficile* strain 630, we created a *dnaK* knockout mutant using the well-established ClosTron mutagenesis system. The DnaK mutant strain exhibited a pronounced temperature-sensitive phenotype demonstrating that a functional DnaK protein is essential for normal growth in *C. difficile*. Interestingly, the transcript levels of *groEL* were found to be four-fold higher at 37 °C in the DnaK mutant than in the parent strain. The transcript levels of toxin A and toxin B however remained unchanged, indicating that the knockout had no significant effect upon the toxin production ability of the mutant strain. The mutant was also found to be less motile as compared to the parental strain suggesting that DnaK might be involved in deregulation of flagellin synthesis in *C. difficile*. To summarize, the *dnaK* mutant phenotype of *C. difficile* produces similar and equally severe effects to those observed in *Escherichia coli* mutants, which are more severe than those isolated from *Bacillus subtilis*.

Characterization of the response of clinical isolates of *Pseudomonas aeruginosa* to novel silver(I)–coumarin complexes

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Due to significant increases in *Pseudomonas aeruginosa* antibiotic resistance over the past 30 years, the development of novel antimicrobials specifically aimed at preventing the growth of this pathogen is essential. This study examines the impact of four silver(I)-coumarin compounds and their respective ligands on twelve *P. aeruginosa* clinical strains, isolated from cystic fibrosis patients. All silver(I)-coumarin compounds demonstrated potent anti-bacterial activity against both planktonic and biofilm modes of lifestyle, however the most effective complex was 8-hydroxycoumarin-3-carboxylatosilver (I) ([Ag (8-OHCca)], which reduced viability by >90% at concentrations of 50μM. Bacteria grown within biofilms proved more resistant to treatment with silver(I)-coumarin compounds, e.g. concentrations of 350 μM and 50 μM was observed for biofilm and planktonic cultures respectively of the same strain. Comparative analysis of the transcriptional profile induced upon sub-lethal exposure demonstrated significant changes in expression (10 fold or greater) in 65 genes upon exposure to the silver(I)-coumarin compound. These genes include the metal-binding and transmembrane transport gene (PA3920), which demonstrated a 36-fold increase in expression. Other genes, such as PA3523, which encodes a resistance-nodulation cell (RNC) division efflux membrane fusion protein precursor and PA2850, encoding an organic hydroperoxide resistance protein demonstrated 45- and 55-fold increases respectively upon silver(I)-coumarin exposure. These data indicate that some silver(I)-coumarin complexes are effective anti-bacterial agents, with a possible mode of action distinct from conventional anti-microbial agents. However, exposure to sub-lethal concentrations may induce efflux and transport mechanisms, which may contribute to antimicrobial resistance by clinical strains of *P. aeruginosa*.

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NT01 Metals & microbes

NT01/01 *Withdrawn*

NT01/02 *Withdrawn*

NT01/03 *Not presented*

NT01/04 The importance of the [Fe–S] cluster regulator NsrR in *Streptomyces coelicolor*

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Within the environment bacteria are exposed to a number of stresses, which they need to survive against. One type of stress is the highly reactive molecule nitric oxide (NO) which is an intermediate of the denitrification pathway within the soil. Therefore soil dwelling bacteria, including the non-pathogenic actinomycete *Streptomyces coelicolor*, are exposed to NO stress in their environment. Encoded within the *S. coelicolor* genome is the transcriptional regulator NsrR, which directly senses NO via an [Fe–S] cluster. The [Fe–S] cluster within *S. coelicolor* NsrR is nitrosylated by NO resulting in loss of activity and derepression of genes encoding NO detoxifying enzymes, such as Hmp. We have previously purified *S. coelicolor* NsrR aerobically from *Escherichia coli* and identified that it contains a [2Fe–2S] cluster required for DNA binding activity. Exposure to NO nitrosylates the cluster resulting in loss of DNA binding activity. We have extended this work by purifying native *S. coelicolor* NsrR both aerobically and anaerobically in order to explore the [Fe–S] cluster contained within native NsrR. Results are presented here that shows the anaerobically purified NsrR protein contains a [4Fe–4S] cluster while the aerobic protein contains a [2Fe–2S] cluster.

NT01/05 The complex role of iron uptake systems in *Campylobacter jejuni* virulence

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Bacterial iron acquisition systems are widely studied because during infection pathogens face a state of near continual iron deficiency due to competition from the normal microbiota and iron limitation by the host. Four ferric iron uptake systems have been characterized in *Campylobacter jejuni*; these comprise the CfrA/Ceu (enterobactin uptake), Chu (haem uptake), Cj0178 (iron from transferrins) and p19 (rhodotorulic acid uptake) systems. In those strains, including NCTC11168, that possess all four systems, CfrA and Cj0178 are required for intestinal colonization. In other strains, e.g. 81-176, *cfrA* and *cj0178* are absent and an uncharacterized receptor gene, *cj0444*, is present along with the Chu and p19 systems. We have constructed mutants in 81-176 in *tonB2*, *chuA* and *cj0444*, and have complemented these mutants by introduction of the respective wildtype genes into the *cj0046* pseudogene. Mutant phenotypes have been confirmed in iron uptake assays, and biological effects are currently being determined in a chicken model assessing intestinal colonization and spread to extra-intestinal sites. Preliminary data suggests that TonB2 is required for optimal colonization of chickens by 81-176 whilst ChuA is not. These results confirm that the role(s) and importance of individual iron uptake systems in campylobacters is complex and depends upon genomic context.

NT01/06 Mutation analysis of noradrenaline-dependent enhancement of *Campylobacter jejuni* growth and iron uptake

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Background The catecholamine hormone noradrenaline has been shown to enhance growth of *Campylobacter jejuni* in an iron-restricted medium, CMH (chelex-treated Mueller-Hinton) with added serum (Gut 2007; 56:1060). Here we show that adrenaline and dopamine also alleviate this restriction through iron uptake via the CfrA system.

Methods Knockout mutants were constructed in known iron uptake systems in three *C. jejuni* genome strains, NCTC11168, 81-176 and 81116 and in selected protein glycosylation genes in NCTC11168. Growth assays were performed using CHM, with a starting inoculum of 10^4 or 10^7 cfu/ml, with/without noradrenaline, adrenaline or dopamine.

Results C. jejuni showed a strain-dependent growth response to noradrenaline, adrenaline and dopamine, NCTC11168 growing better than 81-176 or 81116. Mutation of *cfrA*, an enterochelin receptor in NCTC11168, almost abolishes the noradrenaline-mediated growth response in that strain. Mutation of *pglB* and *pglK* in NCTC11168 had no effect on the noradrenaline growth response.

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Conclusion The observed growth restriction in CMH is largely mediated by iron limitation and this suggests that noradrenaline (and possibly adrenaline and dopamine) can augment iron uptake in *C. jejuni* through a siderophore-mediated process utilizing CfrA. It can also be concluded that the observed growth response is not dependent upon the process of protein glycosylation.

NT01/07 Ferric reduction appears to be involved in the acquisition of transferrin-bound iron by *Campylobacter jejuni*

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One iron source available to *Campylobacter jejuni* NCTC 11168 during human colonization is that bound to lactoferrin (Lf) or transferrin (Tf). Mutation of *feoB*, which encodes a cytoplasmic membrane ferrous iron transporter, disrupted ferrous iron uptake in 11168. Establishing a role for FeoB in the uptake of iron from Tf would indicate Tf-bound iron may be removed by reduction producing ferrous iron for uptake into the cytoplasm. *feoB* was inactivated by inserting an erythromycin resistance cassette and wild-type and mutant strain growth was tested using MEMα supplemented with holo-Tf (0.27 µM), ferrous iron (40 µM) or haem (25 µM). Growth of both strains in unsupplemented MEMα was poor. Wild-type growth was promoted by all iron sources. The mutant grew comparably to wild-type in the presence of haem but less under all other conditions, confirming involvement of FeoB in ferrous iron uptake and indicating a role in Tf-derived iron transport. Although there are no obvious ferric-reductase candidates in 11168, mutation of a riboflavin biosynthesis gene (*cj0572*) was previously shown to decrease ferric-reduction compared to wild-type. Acquisition of Tf-bound iron in the ferrous form and the role of ferric-reductases in this process require clarification.

NT01/08 Production of anti-CtuA and investigation of the role of a protease in the uptake of lactoferrin-bound iron by *Campylobacter jejuni*

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Iron acquisition is vital for successful colonization and infection by *Campylobacter jejuni* NCTC 11168. Lactoferrin (Lf)-bound iron supports *C. jejuni* growth; the outer-membrane receptor CtuA (Cj0178) has been found to be partly responsible for this process. To determine whether an extracellular or surface associated protease is also involved in iron liberation, Lf was incubated in MEM in the presence and absence of iron-restricted or iron-replete 11168 cell-culture supernatant or whole cells. Samples were analysed using SDS-PAGE and electroblotting; blots were probed with anti-human Lf polyclonal antiserum. No evidence for the contribution of a protease was found as protein degradation was comparable under all conditions tested. To produce CtuA antisera for use in blocking Lf-binding, the *ctuA* coding region (minus signal sequence) was cloned into pLEICS expression vectors (N- or C-terminal HIS_6 tag). Following transformation into *E. coli* Rosetta, cells were cultured, induced (IPTG), lysed and recombinant protein expression was analysed using SDS-PAGE and electroblotting. Protein was detected using anti-HIS antisera. Expression was optimized using a range of IPTG concentrations, post-induction incubation times and temperatures. CtuA appeared to be insoluble under all conditions tested. Alternative approaches of using protein inclusion bodies or peptides to produce antisera are ongoing.

NT01/09 Site-directed mutagenesis of the *Campylobacter jejuni* Fur box

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Iron homeostasis in *Campylobacter jejuni* is controlled by the ferric uptake regulator (Fur), which represses iron-responsive gene expression by binding to the Fur box sequence. A 19 bp Fur box was identified within the promoter region of the outer-membrane haemin receptor gene *chuA*. The putative fumarate hydratase *fumC* Fur box shows three mismatches (at the 10th, 13th and 19th bases) to the consensus sequence. This variation is predicted to be the key determinant for the contrasting iron regulation and Fur-Fur box binding affinity between *chuA* and *fumC*. Site-directed mutagenesis of the 10th, 13th and 19th positions was carried out for the *chuA* and *fumC* Fur box and their interaction with Fur was determined by electrophoretic mobility shift assay, *lacZ* reporter assay and DNase footprinting assay. A decrease in Fur binding affinity was observed in *chuA* mutants, whereas mutations within the *fumC* Fur box resulted in increased Fur binding. Partial de-repression of the *chuA* promoter was observed for mutants containing 13th and/or 19th base changes in the Fur box and DNase footprints for these *chuA* mutants were also abolished. The 13th and 19th bases in the consensus sequence play crucial roles in Fur-Fur box interactions in *C. jejuni*.

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NT01/10 Influence of the combination and phase variation status of the haemoglobin receptors on meningococcal virulence

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Objectives Neisseria meningitidis can utilize haemoglobin and haemoglobin–haptoglobin complexes as sources of iron via two phase variable haemoglobin receptors, HmbR and HpuAB. The study was conducted to assess whether the distribution, ON-OFF status and poly-C tract length of both receptors varies between disease and carriage isolates and examined the *hpuAB* deletion mechanism.

Results No significant difference was observed in the frequency of both genes relative to *hmb*R only in disease isolates but *hpuAB* only strains were under-represented. Variation in repeat tract length was evident with a slight over-representation of tract lengths longer than the modal numbers. The receptor was in the ON state in >90% disease and ~70% carriage isolates with a single receptor. Isolates negative for *hpuAB* exhibited either complete deletion or replacement by IS1106A3 and events were ST, and/or serogroup associated.

Conclusion The distribution of both receptors varies among isolates with evidence for selection against an Hpu receptor in invasive strains possibly due to a high level of immunogenicity. Invasive isolates have at least one receptor in an ON state indicating that acquisition of haemoglobin is important during invasion and systemic spread. Deletion of *hpuAB* is mediated by recombination and mainly occurred in serogroup B isolates.

NT01/11 *Escherichia coli* monothiol glutaredoxin: complex formation, Fe–S cluster binding and transfer

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Iron–sulfur (Fe–S) clusters are essential cofactors of proteins involved in a variety of functions. Fe–S cluster biosynthetic pathways are remarkably conserved throughout evolution; defects can lead to sickness or inviability in prokaryotes and genetic disorders such as microcytic anemia and Friedreich ataxia in higher organisms. Recent genome-wide screens for genetic interactions of known Fe–S cluster biosynthesis factors in the model prokaryote *E. coli* have linked GrxD, an Fe–S-containing monothiol glutaredoxin (mono-Grx), to a role in Fe–S cluster biosynthesis, possibly as a scaffold protein and novel member of the Suf system. Work in higher organisms have demonstrated that mono-Grxs can transfer their Fe–S clusters to apo-proteins *in vitro* and are further linked to Fe regulation in yeast via an Fe–S-containing complex with Fra2p, a homolog of *E. coli* BolA. Using affinity purification, genetics and biochemistry, we have confirmed that the only *E. coli* mono-Grx, GrxD, can form Fe–S-containing complexes as a homodimer and as a heterodimer with BolA. We have characterized the capacity of these complexes to act as Fe–S scaffold proteins and their spectroscopic properties. This work will aid our understanding of novel factors that play a role in Fe–S cluster biosynthesis and/or iron regulation throughout evolution.

NT01/12 *Not presented*

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NT01/13 Arsenite oxidation in a model thermophile

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Arsenic is prevalent in hot environments and a number of thermophilic arsenite oxidizers have been isolated. To date, two bacterial thermophilic species, *Thermus thermophilus* str. HB8 and *Thermus sp.* str HR13, have been demonstrated to oxidize arsenite. Currently, little is known about the nature of the enzyme in any thermophilic species. The enzyme has been characterized in its mesophilic counterparts and is known to consist of two heterologous subunits; a large catalytic subunit harbouring a molybdenum cofactor and 3Fe–4S cluster and a small Rieske-type subunit. The mesophilic enzyme from NT-26 has been used as a novel environmental biosensor for arsenite and has recently been expressed in *Escherichia coli,* providing the ideal framework for engineering the protein, with an aim of improving the products shelf-life. We aim to achieve this by understanding the basis of thermostability, using the arsenite oxidase from *Thermus thermophilus* as a model. Initial studies of the arsenite oxidase from *Thermus thermophilus* str. HB8 are described. Preliminary biochemical analysis indicates a membrane attached periplasmic enzyme. Sequence analyses show that the enzyme is transported across the cytoplasmic membrane via the Tat (Twin arginine translocation) system and most probably remains membrane attached by an N-terminal transmembrane helix of the Rieske subunit. This work describes how the enzyme was isolated as a soluble complex, allowing for the purification of enzyme for initial characterization studies.

NT01/14 *Escherichia coli* biofilms: gene expression and elemental heterogeneity

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Global analyses examining the differences between bacteria growing in biofilms and their planktonic counterparts have revealed that stress responses are significant in biofilm existence. However, some features of the comparative expression profiles may be unavoidable consequences of growth at high cell densities and not a characteristic feature of the biofilm mode of growth *per se*. Since many biofilm cells are probably anoxic or oxygen-limited, compared to planktonic cells, the gene expression profiles of biofilm and planktonic populations of a facultatively anaerobic bacterium *Escherichia coli* were compared under strictly anaerobic growth conditions. Contrary to some published data, *E. coli* readily forms biofilms under anaerobic conditions and genome-wide transcriptional profiling demonstrated up-regulation in the biofilm of only a small number of genes. A chemostat system was developed with which to expose aerobically-growing biofilms to anaerobic conditions and transcriptomic changes were examined during this transition. As well as predictable changes in transcripts encoding respiratory proteins, biofilms also exhibited changes in the expression of flagella, ribosomal proteins, drug exporters and bacteriophage genes. Biofilms have also been shown to exhibit considerable heterogeneity. To examine elemental heterogeneity in bacterial communities, an emerging analytical technology, laser ablation-inductively coupled plasma-mass spectrometry (LA-ICP-MS) was used. Distinct variations in the distributions of metal ions were detected in colony and biofilm models. Elementally-distinct sub-populations were isolated and their global transcript levels were compared. When compared to the biofilm interior, cells in the biofilm perimeter exhibited higher levels of transcripts related to motility, aerobic respiration and transport.

NT01/15 Copper homeostasis and *Salmonella* pathogenicity

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Salmonella enterica serovar Typhimurium (STy) is an important food-borne pathogen causing serious medical and veterinary problems worldwide. The ability to survive in macrophages is critical for *S.* Typhimurium during systemic disease. Within this compartment the pathogen must sense and respond rapidly to a variety of fluctuating conditions including reactive species of oxygen and nitrogen and varying metal levels. Elevated copper levels are detected by a reporter construct driven by a copper-responsive promoter (P_{copA}) in *S.* Typhimurium during infection of macrophages, which coincides with a requirement for *S.* Typhimurium copper resistance. Our recent progress in further characterizing the role of *Salmonella* copper homeostasis during infection will be presented, with current studies focusing on identifying the source(s) of copper-toxicity within macrophage phagosomes and the contribution of the copper homeostatic systems to reactive oxygen and nitrogen species resistance.

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NT01/16 Investigating the mechanism of inhibition of *Staphylococcus aureus* growth by the human protein calprotectin

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Previous investigations have demonstrated that the protein complex calprotectin, produced in large quantities by mammalian neutrophils and secreted into abscesses, inhibits the growth of *Staphylococcus aureus* (Corbin *et al.*, 2008). Growth inhibition was observed even when calprotectin was separated from the cells by a dialysis membrane, suggesting that the mechanism of inhibition involves nutrient sequestration. Medium treated with calprotectin is depleted for manganese and zinc. Increased sensitivity of Δ*mntA*/Δ*mntB* mutant strains, defective in high-affinity manganese uptake, to growth in the presence of calprotectin in conjunction with transcriptome analysis lead to the hypothesis that calprotectin chelates Mn^{2+} and inhibits growth through manganese starvation. The mechanism by which Mn-starvation inhibits proliferation of *S. aureus* is not yet known. In order to investigate this, here we present analyses of the abundance of metal in the major manganese and zinc pools of the *S. aureus* cytosol. The major proteinaceous manganese pool is shown to be associated with the two superoxide dismutase enzymes, SodA and SodM, and this pool is reduced in Δ*sodA* and Δ*sodM* mutant strains and eliminated in a Δ*sodA*Δ*sodM* double mutant. A second, large pool of manganese is present as low molecular weight, anionic complexes. In contrast, multiple abundant zinc-proteins are observed in addition to a low molecular weight zinc pool. Data will be presented demonstrating the effect on the abundance of manganese and zinc within these cytosolic pools in *S. aureus* cells cultured in the presence of recombinant human calprotectin.

NT01/17 *Not presented*

NT01/18 Role of iron and manganese homeostasis in the response to superoxide stress in *Bacillus anthracis*

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One of the major transcriptional responses of *Bacillus anthracis* to superoxide stress involves the up-regulation of genes of iron homeostasis and uptake. Consistent with this, the total cellular iron content of superoxide-stressed cells increases 2.5-fold after 1 h exposure. Using native two-dimensional liquid chromatography (2D-LC) of soluble cytosolic extracts under rigorously anaerobic conditions in combination with inductively coupled plasma mass spectrometry (ICP-MS), we identified metalloproteins that constitute the abundant intracellular iron and manganese soluble pools in *B. anthracis*, including the superoxide dismutases (SODs) SodA1 and SodA2. We found that SodA1 constitutes the majority of SOD activity *in vivo* and is active with either iron or manganese. SodA2 was found to be specific for Fe^{2+}. The specific activity of SodA1 reconstituted with Mn^{2+} is 30 times higher than the Fe^{2+}-loaded form *in vitro*, and 3-fold higher than that of Fe^{2+}-loaded SodA2. Even though superoxide dismutases are important for the detoxification of superoxide radicals, neither the *sodA1* or *sodA2* genes nor their respective products were induced in response to superoxide stress. We have investigated metal occupancy of cytosolic metalloproteins, including SodA1 and SodA2, in response to superoxide stress *in vivo*, and find that the additional iron accumulated by superoxide-stressed cells is associated with the membrane fraction. We propose that superoxide stress acts as an environmental sensor to initiate the sequestration of iron relevant for the obligatory lifestyle of *B. anthracis*, while maintaining the intracellular redox state and ensuring optimal incorporation of metal cofactors by enzymes.

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NT01/19 A comparative transcriptomic and proteomic analysis of oxidative stress in *Bacillus anthracis*

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The superoxide and the peroxide stress stimulons of *B. anthracis* UM23C1-2 were characterized by a combined transcriptomic and proteomic approach, as a preliminary to studying macrophage-related stresses encountered during infection. For the transcriptome analysis we carried out Northern blots to determine the quality of the RNA preparations and to determine the behaviour of key marker genes shown to be induced by oxidative stress in *B. subtilis*. These experiments indicated that some of the marker genes (*e.g. sodA* encoding superoxide dismutase and *trxA* encoding thioredoxin) were induced by both types of oxidative stress. However, in contrast to the situation in *B. subtilis,* the gene encoding the main catalase (*katB*) was only induced by hydrogen peroxide. Among the most prominent responses to both stresses was the induction of a large number of iron transporter proteins and their cognate substrate binding proteins.

2D-PAGE analysis of extracted cytoplasmic and extracellular proteins showed distinct and rather different patterns of expression in the presence of paraquat or H_2O_2. Significant responses were observed after the addition of H_2O_2, leading to an increased abundance in a small number of proteins. Four of these proteins were identified by peptide mass fingerprinting as alkyl-hydroperoxide reductase, glyceraldehyde-3-phosphate dehydrogenase, RecA and catalase B. In contrast only two proteins were found to be prominently induced in the presence of paraquat, namely, an acyl carrier protein phosphodiesterase and a protein from the glyoxylase family.

NT01/20 Quorum sensing: social benefits of a high cell density behaviour

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As social organisms, bacteria are capable of initiating and sustaining cooperative behaviours. This can take the form of chemical signalling or 'Quorum Sensing' (QS). QS is a regulatory mechanism by which the cell density of a bacterial population can regulate gene expression via extracellular signalling molecules (autoinducers), produced by individuals within the population. Signalling molecules produced by the population are low molecular weight compounds (<1 kDa) which have the ability to activate or repress target genes, as this process is controlled in a cell density-dependent manner, a bacterial population must reach a threshold in order for induction to occur. Despite being defined as a high cell density behaviour in the majority of QS literature, the benefits of achieving a 'quorum' and its role as a social behaviour have never been tested in an experimental model. Here we demonstrate for the first time, an experimental system for testing one of the main assumptions of QS. A minimal medium – Quorum Sensing Media (QSM) was used to culture strains of *Pseudomonas aeruginosa* in varying concentrations of casamino acids (CAA) and bovine serum albumin (BSA) in order to investigate the benefits of QS at a high cell density. Using a set of growth curve experiments it has been demonstrated that a signal negative QS mutant (Δ*lasI*) receives a greater benefit from QS induction when grown at a high cell density when compared to a low cell density culture. A significant difference can be calculated of the benefit of adding signal between these two environments, indicating a benefit for *P. aeruginosa* populations at a high cell density and that the concentration and availability of public goods are vital to this.

NT01/21 Iron uptake systems in *Clostridium difficile*

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Clostridium difficile, a Gram-positive, anaerobic, spore-former is the major cause of antibiotic associated diarrhoea and is also associated with more severe, sometimes life threatening disease.

To date little research has been carried out on iron uptake mechanisms and their regulation in *C.difficile*. In common with other pathogens, iron is likely to be an essential growth factor necessary for the survival of the organism and analysis of available genome sequences reveals the presence of several potential iron uptake systems and regulators. Initially we have focussed studies on homologues of the *E. coli* ferrous iron uptake system (FeoB) and the ferric uptake regulator Fur. The ClosTron mutagenesis system developed in Nottingham has been used to generate knockout mutants in two of the 3 annotated *feoB* homologues and in the single *fur* homologue in *C.difficile*630Δerm.

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Comparisons of the growth characteristics suggest FeoB1 but not FeoB3 is involved in ferrous iron uptake. Co-culture experiments showed that wild type out-competed the FeoB1 mutant in the presence of 2,2,-dipyridyl. As has previously been reported with another anaerobe – *Shewanella oneidensis*, *fur* mutant showed a growth defect under iron replete conditions and enhanced growth under iron limitation compared to wild type and was hypersensitive to hydrogen peroxide.

NT01/22 Inactivation of *fecD* and *ceuE* affects nickel acquisition and urease activity in *Helicobacter mustelae*

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The genome sequences of *Helicobacter* species colonizing the mammalian gastric mucosa contain a large number of genes annotated as putative iron-uptake genes, but only few nickel-uptake genes, which contrasts with the central position of nickel in urease-mediated acid resistance of these pathogens. In this study we have investigated the transcription and roles of the putative iron ABC transporter genes *fecD* and *ceuE* in the ferret pathogen *Helicobacter mustelae*. Transcription of *fecD* and *ceuE* was iron- and Fur-independent and their inactivation did not affect cellular iron levels. Surprisingly, inactivation of *fecD* and *ceuE* resulted in a strongly reduced urease activity and cellular nickel content as measured by ICP-MS. Inactivation of either *nixA, tonb2* or *nikH* further diminished cellular nickel levels and urease activity of the *fecD* mutant. Inactivation of *fecD* and *nixA* also abolished nickel-dependent regulation of the urease systems. In contrast to NixA, metal acquisition by FecD seemed to be less specific for nickel, since *fecD* mutants also showed reduced cellular cobalt levels and increased cobalt resistance. We conclude that in *Helicobacter mustelae* the ABC transporter system FecDE-CeuE is likely to contribute to nickel and cobalt acquisition, and works independently of the previously described NixA, TonB2 and NikH system.

NT01/23 Copper homeostasis in *Listeria monocytogenes*

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Copper (Cu) is an essential element to many organisms, being particularly important for enzymes involved in oxidative phosphorylation due to its ability to undergo redox cycling. However, this property also allows Cu ions to participate in Fenton-like reactions resulting in the generation of deleterious hydroxyl radicals. Furthermore, the ability of Cu ions to bind avidly to sulfur and nitrogen donors results in the displacement of less competitive metals from proteins and enzymes, disrupting key metabolic processes. In order to cope with excessive Cu levels, bacteria employ a range of detoxification mechanisms. *Listeria monocytogenes*, a ubiquitous environmental saprophyte and important human pathogen, must cope with fluctuating levels of copper both in the environment and during infection. We have identified and characterized a Cu-responsive operon in *L. monocytogenes* EGD-e. Transcription of a copper-specific P_1-type ATPase, CopA, which detoxifies cytoplasmic copper, is controlled by the copper-responsive repressor CsoR. The third gene in the operon, a predicted copper chaperone, was shown to have a role in tempering transcription of the operon by reducing the level of copper available to CsoR. Further studies have investigated the role of the copper-responsive operon in the virulence of *L. monocytogenes* in both macrophage and mouse models of infection.

NT01/24 Atypical copper homeostasis in *Salmonella typhimurium*

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Bacteria employ a complex array of mechanisms to maintain cellular copper homeostasis. Copper toxicity is prevented by appropriate regulation of proteins which traffic and sequester excess copper. *Salmonella enterica* serovar Typhimurium (*S.* Typhimurium) has two metal-transporting P_1-type ATPases, CopA and GolT, whose actions largely overlap with respect to growth in elevated copper. Mutants lacking both ATPases are hypersensitive to copper and over-accumulate copper relative to wildtype or either single mutant. Such duplication of ATPases is unusual in bacterial copper tolerance. Although CopA and GolT serve to transport copper out of the cytosol, it is not known how copper is removed from the *S.* Typhimurium periplasm. Analyses of periplasmic copper-complexes identified copper-CueP as a predominant metal pool. Copper-CueP is a further atypical feature of copper homeostasis in *S.* Typhimurium and a slight increase in copper accumulation of *cueP* suggests a role in copper export. Elevated copper is detected by a reporter construct

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driven by the promoter of *copA* in wildtype *S.* Typhimurium during infection of macrophages. Double mutants missing both ATPases also show reduced survival inside cultured macrophages. It is hypothesized that elevated copper within macrophages may have selected for specialized copper resistance systems in pathogenic microorganisms such as *S.* Typhimurium.

NT01/25 The superoxide dismutase SodA is targeted to the periplasm of *Rhizobium leguminosarum* and other proteobacteria via a novel mechanism

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Superoxide is generated by the host during infection by pathogenic or symbiotic bacteria and is detoxified by various types of superoxide dismutase. Interestingly, the Fe/Mn superoxide dismutase SodA was detected in the periplasm of the plant symbiont *Rhizobium leguminosarum*, but does not carry the cleavable hydrophobic N-terminal signal peptide found in other proteins targeted to the periplasm. Instead, the N-terminus of SodA is highly hydrophilic and remains uncleaved. SodA translocation is unaltered in a *tatC* (twin-arginine translocation pathway) mutant. The export of SodA was also unaffected in *secDF* and *secB* mutants, but export was inhibited by azide, an inhibitor of SecA ATPase activity. The first 59 amino acids of SodA were sufficient to target a reporter protein to the periplasm, but export was also affected by changes to the C-terminus of SodA. Our results demonstrate a novel SecB- and signal peptide-independent pathway targeting proteins to the periplasm. Export of SodA to the periplasm was also observed in other proteobacteria. These findings force a re-evaluation of the role of SodA in the virulence of pathogens and have wider implications for the study of periplasmic targeting. Here we present our results towards the identification of this novel targeting mechanism.

NT01/26 An Ftr1p-like iron transporter in cyanobacteria

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Databases searches revealed that some cyanobacteria contain homologues of the Fet3p-Ftr1p iron transport system from *Saccharomyces cerevisiae*. These include an Ftr1p homologue (*ftr,* ferric permease) in *Synechocystis* sp. 6803 and *Nostoc* PCC 7120, and an Fet3p homologue (*mco*, multicopper oxidase) in *Nostoc* sp (*mco* is ~2kb downstream of *ftr*). Growth studies showed that Ftr-Mco from *Nostoc* can enhance the growth of *E. coli* under iron restriction whereas Ftr or Mco alone afforded little benefit. Ftr1-Mco displayed preference for ferrous iron and low pH, and required oxygen. *ftr* and *mco* are induced under low iron conditions in *Nostoc*, as was *ftr* of *Synechocystis*. These findings are consistent with a role for Ftr-Mco in ferrous iron uptake. Mco (All3942) of *Nostoc* has two cupredoxin domains (unlike Fet3p, which has three such domains), and thus is structurally distinct from Fet3p. Residues 1–31 are predicted to form a cleavable Tat-dependent secretion signal, suggesting that Mco is periplasmic but assembles in the cytosol. The *Nostoc* Mco protein was purified as a MalE-Mco fusion. UV-visible spectroscopic analysis of MalE-Mco showed a peak at 625 nm corresponding to the T1 copper site and a peak at ~300 nm which could correspond to a T3 copper site – such species are found in other multicopper oxidases. Metal saturation studies indicated the binding of copper, zinc and ferrous iron to MalE-Mco at molar ratios of six, ~one and ~three, respectively. Mco gave weak ferroxidase activity and appeared to turnover poorly suggesting a requirement for a ferric accepting partner.

NT01/27 YqjH from *Escherichia coli*: a novel role in intracellular iron scavenging?

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Since iron is essential for bacterial survival they employ efficient iron-acquisition systems to facilitate iron uptake during scarcity. Bacteria secret high-affinity iron chelating compounds, called siderophores, that bind Fe(III) to form ferri-siderophore complexes which are then taken up into the cell and in turn reduced to the ferrous state before utilization. A predicted 'siderophore interacting protein', YqjH of *Escherichia coli*, is encoded by the Fe–Fur repressed *yqjH* gene and is suspected to act in release of iron from intracellular ferric complexes. YqjH contains flavin adenine dinucleotide (FAD) as a co-factor which supports a likely role for YqjH in ferric reduction. Structurally, it consists of two domains resembling other members of the NAD(P)H:flavin oxidoreductase superfamily. Western blotting confirmed that YqjH levels are Fe–Fur repressed. Growth study suggests that YqjH can partly replace Fes in iron release from the siderophore enterobactin, supporting a role for YqjH in intracellular iron reduction. Additionally, an *entB yqjH* double mutant (*entB* mutants fail to produce the siderophore enterobactin) was found to exhibit a weaker growth than the corresponding single mutants under iron restriction, but had higher iron content. This suggests that YqjH is involved in countering iron restriction by facilitating the turnover of cellular iron.

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NT01/28 Severe zinc depletion of *Escherichia coli*: roles for high affinity zinc binding by ZinT, zinc transport and zinc-independent proteins

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Growing bacteria in a Zn-deficient environment is difficult given their impressive ability to acquire Zn from the environment. By Zn elimination we reduced the Zn content of *E. coli* growth medium to 60 nM or less. Cells grown in this Zn-deficient medium had a reduced growth rate and contained up to five times less cellular Zn. Microarray analysis of cells grown under Zn-replete and Zn-depleted conditions in chemostat cultures found nine genes to be up-regulated more than two-fold ($P<0.05$) in cells from Zn-deficient chemostats. One of these, *zinT*, was shown to be regulated by Zur. A mutant lacking *zinT* displayed a growth defect and 3-fold lower cellular Zn when grown under Zn limitation. The purified ZinT protein possessed a single, high affinity metal-binding site that can accommodate Zn or Cd. A further up-regulated gene, *ykgM*, is believed to encode a non-Zn finger-containing paralogue of the Zn finger ribosomal protein L31. The gene encoding the periplasmic Zn-binding protein *znuA* also showed increased expression. During both batch and chemostat growth, cells 'found' more Zn than was originally added to the culture. We show Zn elimination to be a more precise method of depleting Zn than by using the chelator TPEN.

NT01/29 Biotic sol-gel coating for the inhibition of corrosion and biofouling on metals in seawater

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A unique anti-corrosion coating that inhibits the biofouling of metals in seawater by combining sol-gel technology and micro-organisms has been developed as an alternative to existing environmentally-damaging biocide/antifouling strategies. Endospores of the corrosion-inhibiting bacterium *Paenibacillus polymyxa*, can withstand exposure to a wide range of solvent concentrations and acid pH levels during sol-gel formulation and, following immobilization within the sol-gel coating, provide enhanced corrosion inhibition when compared to that of sol-gel alone. Electrochemical measurements during an axenic experiment have shown the efficacy of the coating in inhibiting corrosion in the presence of *Desulfovibrio desulficurans*, a sulfate-reducing bacterium known to exacerbate corrosion in marine environments by forming corrosive metabolic by-products, including H_2S and *Pseudomonas aeruginosa*, which binds metal ions from alloys. A reduction in the formation of macroscopic biofouling and corrosion products has been observed, notably around prepared scratches on the surface of biotic sol-gel coated aluminium 2024-T3 panels immersed in estuarine seawater for three months when compared to those coated with the abiotic sol-gel. Scanning electrochemical microscopy is being developed as a technique to investigate spatial distribution of redox-active compounds within the coatings in order to understand the protection mechanism of the coating.

NT01/30 The role of zinc in marine cyanobacteria – development of methods in metallomics

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All organisms have a requirement for trace metals that perform essential catalytic and structural roles. In cyanobacteria trace metal utilization may be particularly high when compared to other prokaryotes due to their requirements for photosynthesis and carbon fixation, and examples include the use of Zinc in carbonic anhydrase, and Copper in plastocyanin. Significant progress has started to be made in understanding the requirements and handling of trace metals by cyanobacteria, particularly in relation to Iron. Zinc however has gone largely unstudied despite being only second to Iron in its abundance in life[1,2]. We have begun to develop new methodologies in metallomics and metalloproteomics to begin to unravel the requirements that various strains of marine cyanobacteria have for Zinc, and to try and gain further insights into how these organisms respond to both Zinc toxicity and Zinc limitation. Method development strategies have focused on culture conditions and cell fractionation techniques as well as the development of multidimensional chromatography to separate the metalloproteome to isolate Zn enriched fractions. Despite many challenges good progress has been made and a significant number of proteins found in Zinc enriched fractions have been identified.

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NT01/31 Regulation of methane oxidation in *Methylococcus capsulatus* Bath

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Methylococcus capsulatus Bath, an obligate methanotroph, oxidizes methane to methanol through two forms of the enzyme methane monooxygenase (MMO); a membrane bound particulate enzyme (pMMO) and a soluble cytoplasmic enzyme (sMMO). The expression of both enzymes is significantly affected by the availability of copper. The exact role of copper in regulation of MMO in *Mc. capsulatus* is still unclear. Therefore, the aim of this study was to shed some light on MMO regulation using the available genome sequence of this organism, together with mutagenesis and transcriptional regulation studies. A whole genome microarray identified 43 genes which were differentially expressed under low opposed to high copper growth conditions. Interestingly, a cluster of six genes upstream of the structural genes for the sMMO is up-regulated and may be involved in MMO regulation. Proposals for mutants in the light of microarray data are discussed. Mutants were constructed with deletions of genes suspected of involvement in copper transport or MMO regulation and were characterized. This identified *copA3* which is involved in copper uptake. Moreover, *nrpS-2* and *pk*, encoding a putative non-ribosomal peptide synthase and polyketide synthase, are involved in methanobactin production. Methanobactin is thought to be involved in copper acquisition in *Methylococcus capsulatus*.

NT01/32 Site-directed mutagenesis of MmoX: understanding substrate access to the di-iron core of soluble methane monooxygenase

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Soluble methane monooxygenase (sMMO) from *Methylosinus trichosporium* OB3b is a multicomponent non-haem iron oxygenase that naturally oxidizes methane to methanol and can also cooxidize a wide range of adventitious substrates, including mono- and di-aromatic hydrocarbons. Potential substrate trafficking routes into the di-iron centre have been proposed. However the restrictions which prevent the oxidation of larger substrates, including tri-aromatic hydrocarbons, have not been elucidated. Either increasing the volume of the active site pockets or altering the dimensions of the substrate channels may enable the formation of tri-aromatic hydroxylated products. Here, mutations were designed with a view to increasing accessibility to the active site, through disruption of ionic interactions at proposed substrate channel entrances, by altering -helical flexibility and by modifying the properties of two hydrophobic cavities adjacent to the di-iron centre. Mutations designed to disrupt certain ionic interactions diminished the turnover of aromatic substrates (E101L) and have varied minor affects on substrate specificity (E230L and R98L). Mutations designed to manipulate the hydrophobic pockets adjacent to the di-iron centre (F192M and F192I) gave altered regioselectivity, especially with di-aromatic substrates.

NT01/33 The relationship between heavy metals and antibiotic resistance in Scottish soils

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There are links between heavy metals and antibiotic resistance. In clinical settings, it has been known that co-resistance, where both genes are found on the same conjugative genetic elements, and cross-resistance, where one gene confers resistance to both toxicants, exists. However, little evidence of this relationship has been found in the environment.

Heavy-metal levels in the environment have increased in recent years due to industrial growth. What is problematic is that they can act as a more persistent selective pressure on bacteria, maintaining elevated antibiotic resistance gene levels. As such, heavy metals can fuel the multi-drug resistance problem that plagues clinical and agricultural health.

Here, we compared levels of heavy metals in soil samples from various locations in Scotland and, using qPCR, certain antibiotic resistance genes. The presence of copper has a statistically significant impact on the occurrence of two resistance genes (*tet*(M) and *bla*SHV). As there are no known homeostasis or resistance mechanisms involving both copper and these gene products, the results suggest co-resistance is occurring. The mechanism(s), by which this is occurring, is being investigated, but this forms a basis of on-going work, and suggests that lower levels of metals may impact environmental and public health than expected.

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NT01/34 The role of CorA in magnesium uptake in *Campylobacter jejuni*

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Magnesium (Mg^{2+}) is a cofactor of many enzymes which are essential for the viability of prokaryotes. CorA, a membrane integrated Mg^{2+} transporter, has been shown to play a vital role in the Mg^{2+} acquisition in other pathogens, namely *Salmonella enterica* serovar Typhimurium, *Escherichia coli* and *Helicobacter pylori* but no work has yet been done to *Campylobacter jejuni* to date.

A putative *corA* gene (Cj0726C) was identified in the genome sequence and a knock out mutant generated.

The *corA* mutant did not grow without Mg^{2+} supplementation (20 mM), indicating that Mg^{2+} acquisition by *corA* is essential for *C. jejuni* growth *in vitro* and that it might play a key role in adaptation to low-Mg^{2+} conditions. In addition, this phenotype suggests that CorA is the primary Mg^{2+} transporter. CorA is likely to be important for colonization and thus a CorA deficient strain may form the basis of possible vaccine strains of *C. jejuni*. The lack of CorA homologs in eukaryotes offers the possibility of using CorA as a target for the development of new *C. jejuni*-specific agents.

NT01/35 *Not presented*

NT01/36 Analysis of the microbial communities associated with a variety of seaweed species harvested from the south east of Ireland

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Seaweeds have been shown to accumulate significant amounts of heavy metals from ambient water through a combination of extracellular and intracellular mechanisms. Their surfaces are often colonized by diverse and complex bacterial communities. This study presents an investigation into the bacteria associated with seaweeds harvested from polluted and unpolluted areas in the south east of Ireland.

Culturable bacterial isolates were examined for tolerances to heavy metals such as Copper, Nickel, Lead and Chromium at concentrations of 0–100 mg l^{-1}. Furthermore, a number of these isolates were assessed for an ability to remove these metals from solution. Heavy metal uptake by these strains may indicate an additional functional mechanism contributing to the overall capacity of the seaweed to tolerate and accumulate metals from the environment.

The overall microbial diversity (incorporating culturable and nonculturable community members) associated with two seaweed species harvested from a relatively polluted river estuary was demonstrated by the construction of 16S rRNA gene libraries. Identifying bacterial populations utilizing this molecular approach has the benefit of being able to identify surface-bound bacteria that may not be readily culturable in the laboratory. Such strains may also possess significant traits pertaining to heavy metal tolerance and accumulation.

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NT01/37 Copper enhances organic hydroperoxide and hydrogen peroxide killing in *Xanthomonas campestris* pv. campestris

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Copper (Cu)-based biocides are important chemical controls for both fungal and bacterial diseases in crop fields. During plant microbe interaction, bacteria are exposed to reactive oxygen species produced by plant as a part of host defence responses. Hence, exposure to metals could modulate the oxidative stress response. Cu ions at a sub inhibitory concentration for growth (100 µM) enhances *t*-butyl hydroperoxide (*t*BOOH) and hydrogen peroxide (H_2O_2) killing of phytopathogen *Xanthomonas campestris* pv. campestris (Xcc). Addition of an anti-lipid peroxidation agent (α-tocopherol) and hydroxyl radical scavengers (glycerol and dimethylsulfoxide) partially protected the bacteria from the Cu-enhanced *t*BOOH and H_2O_2 killing, respectively. Furthermore, alkyl hydroperoxide reductase mutant is more sensitie to kiliing concentrations of $CuSO_4$. The phenotype could be partially suppressed by an H_2O_2 scavenger (pyruvate) and α-tocopherol. This suggests that Cu ions promote the formation of hydroxyl radicals, and lipid peroxidation which could be responsible for the Cu ion-enhanced H_2O_2 and organic hydroperoxide killing effects.

NT01/38 Roles of RirA and Irr in controlling iron uptake and the oxidative stress response of *Agrobacterium tumefaciens*

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Agrobacterium tumefaciens causes crown gall tumor disease in dicotyledonous plants. In order to survive and cause disease, phytopathogens have to successfully combat both iron deprivation and oxidative bursts, which are environmental stresses that are normally encountered during interaction with host plants. *A. tumefaciens* RirA (rhizobial iron regulator) functions in concert with Irr (iron response regulator) in controlling iron uptake. RirA repressed iron uptake under high-iron conditions thereby preventing iron overload-induced toxicity, whereas Irr acted as a positive regulator under low-iron conditions. Therefore, during iron starvation the expression of iron uptake genes were induced via derepression of RirA and activation through Irr that together help to increase efficiency of iron acquisition. Inactivation of the *rirA* led to deregulation of iron uptake genes. Consequently, the *rirA*$^-$ strain had increased sensitivity to H_2O_2-killing via the Fenton reaction. In contrast, an *irr* inactivation-mutant strain showed increased resistance to H_2O_2-killing, at least in part, due to the overproduction of a membrane bound ferritin (MbfA). The data indicated that RirA and Irr are important for adaptation to iron and oxidative stress in *A. tumefaciens*.

NT01/39 The 2-Cys peroxiredoxin, alkyl hydroperoxide reductase C binds haem and participates in its intracellular availability in *Streptococcus agalactiae*

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Haem is a redox-reactive molecule with vital and complex roles in bacterial metabolism, survival, and virulence. However, few intracellular haem partners were identified to date and are not well-conserved in bacteria. The opportunistic pathogen *Streptococcus agalactiae* (Group B *Streptococcus*; GBS) is a haem-auxotroph, which acquires exogenous haem to activate an aerobic respiratory chain. We identified the alkyl hydroperoxide reductase AhpC, a member of the highly conserved thiol-dependent 2-Cys peroxiredoxins, as a haem binding protein. AhpC binds hemin with a K_d of 0.5 µM and a 1:1 stoichiometry. Mutagenesis of cysteines revealed that hemin binding is dissociable from catalytic activity and multimerization. AhpC reductase activity was unchanged upon interaction with haem *in vitro* and *in vivo*. A GBS *ahpC* mutant displayed attenuation of two haem-dependent functions, respiration and activity of a heterologous catalase, suggesting a role for AhpC in haem intracellular fate. In support of this hypothesis, AhpC-bound hemin was protected from chemical degradation *in vitro*. Our results reveal for the first time a role for AhpC as a haem-binding protein.

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NT03 Streptococci

NT03/01 SzeF, SzeN and SzeP: three novel *Streptococcus zooepidemicus* superantigens

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The horizontal transfer of mobile genetic elements and their associated cargo genes between streptococcal species can significantly alter the virulence properties of recipient strains. Gain of prophage elements by *Streptococcus pyogenes* that contain superantigen-encoding genes has been linked with increased morbidity and mortality in humans. Similarly, the acquisition of Seq3 (containing *seeL*, *seeM*) and Seq4 (containing *seeH*, *seeI*) by *Streptococcus equi* have been suggested to represent key steps in the evolution of this host-restricted equine pathogen from an ancestral *Streptococcus zooepidemicus* strain. *Streptococcus equi* subspecies *zooepidemicus* is the most frequently isolated opportunistic pathogen of the horse. *Streptococcus zooepidemicus* also infects a wide range of other animal species including cattle, sheep, pigs, monkeys and humans.

Culture supernatants from several strains of *Streptococcus zooepidemicus* possess superantigen activity, but these strains lack known superantigen-encoding genes. Superantigens are bacterial toxins that trigger unspecific T-cell proliferation and overzealous inflammatory cytokine production in the host. We sequenced the genome of one of these putative superantigen-producing isolates, *S. zooepidemicus* strain BHS5 (*Sz*BHS5). The strain SzBHS5 has been recently associated with several cases of fatal haemorrhagic pneumonia in dogs. *Sz*BHS5 contains three novel superantigen-encoding genes, *szeF*, *szeN* and *szeP*, the products of which share 59%, 34% and 49% amino acid sequence identity with SpeH, SpeL and SpeM, respectively.

To determine the prevalence of *Streptococcus zooepidemicus* sAgs, we screened by quantitative PCR (qPCR) a panel of *Streptococcus equi* and *Streptococcus zooepidemicus* strains that are representative of the wider population as defined by MLST. This included 26 isolates of *Streptococcus equi* (representing 2 STs) and 165 isolates of *S. zooepidemicus* (representing 111 STs). Surprisingly, 35% (57 of 165) of *Streptococcus zooepidemicus* isolates examined contained *szeF*, 19% (31 of 165) contained *szeN* and 21% (35 of 165) contained *szeP*, with *szeN* and *szeP* almost always occurring together. The presence of superantigen-encoding genes was significantly associated with mitogenic activity in the *Streptococcus zooepidemicus* population ($P<0.000001$) and with isolation from cases of non-strangles lymph node abscesses ($P=0.000367$). Furthermore, 15 isolates from human cases of *Streptococcus zooepidemicus* infection were screened and 7 of which contained *szeF*, *szeN* and/or *szeP* and had mitogenic activity against equine PBMCs.

Each of the three novel sAgs were cloned and soluble protein expressed in *Escherichia coli*. Recombinant SzeF, SzeN and SzeP stimulated the proliferation of equine peripheral blood mononuclear cells, interferon γ (IFNγ) and tumor necrosing factor α (TNFα) production *in vitro*.

In conclusion, we report the identification of three novel sAgs: SzeF, SzeN and SzeP through genome sequencing of *Sz*BHS5, isolated from a case of acute fatal haemorrhagic pneumonia in a dog. *szeF*, *szeN* and *szeP* produce functional sAgs and were present in 50% of the diverse *Streptococcus zooepidemicus* population screened here. The presence of these sAgs was associated with isolation from equine cases of non-strangles lymph node abscessation. We conclude that horizontal transfer of these novel superantigens from and within the diverse *Streptococcus zooepidemicus* population is likely to have implications for animal health.

NT03/02 Pili of *Streptococcus equi* and *Streptococcus zooepidemicus*

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Objectives Pili are important virulence factors and potential vaccine targets. *S. equi* contains Fim1 also found in the sequenced *S. zooepidemicus* isolates, strain MGCS10565 and H70. However, the gene encoding the TetR-like repressor of this locus is a pseudogene in *S. equi*. Three other pilus loci are present in the *S. zooepidemicus* genomes: Fim1 and Fim2 in *Sz*MGCS10565 and Fim4 in *Sz*H70. This study determined the affect of regulatory loss of Fim1 in *S. equi* and identified pilus locus variation in a diverse population of *S. zooepidemicus* isolates.

Methods Allelic replacement mutagenesis was used to repair *tetR* in *S. equi* strain 4047 and transcription levels of the downstream pilus elements were quantified by RT-qPCR. 22 *S. zooepidemicus* genomes were sequenced and putative pilus loci identified.

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Results RT-qPCR of the Fim1 pilus genes showed no reduction in expression levels during *in vitro* growth on repair of *tetR*. Of the 22 *S. zooepidemicus* genomes examined, Fim1 was present in all strains. Fim2 was not found alone, Fim3 alone was present in four isolates, Fim2 and 3 were found together in five isolates and Fim4 was present in 10 isolates. Possession of Fim2 and 3 was mutually exclusive to Fim4. Interestingly, variation was greatest in the accessory pilin proteins that are predicted to enable pili to bind host tissue. One strain had inversion and disruption of the structural pilin genes.

Conclusions Repair of *tetR* did not decrease Fim1 expression *in vitro*. Genome analysis of 20 *S. zooepidemicus* genomes revealed no novel pilus loci, but confirmed the presence of Fim1 in all strains, possibly suggesting an important role in attachment and colonization. Concentration of variation in the accessory pilin proteins is suggestive of functional and/or immunological selection.

NT03/03 Characterization of multidrug and efflux pump inhibitor resistance in *Streptococcus pneumoniae* mediated by the PatAB ABC transporter

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Over-expression of the ABC transporter genes *patA* and *patB* has been shown to confer efflux-mediated multidrug resistance in laboratory strains of Streptococcus pneumoniae. However, the cause of the over-expression of these genes is unclear. In this study, the complete genome sequence of a reserpine-resistant laboratory strain, M184¸ was determined and compared to its parental strain, R6, to identify mutations. Fourteen genes contained mutations that altered their protein coding sequence. By excluding natural polymorphisms and only considering genes that are part of the core genome of *S. pneumoniae*, the number of candidate genes was reduced to nine. One of these genes, which contains a truncation mutation in M184, encodes a regulatory protein, and was also mutated in another reserpine-resistant mutant strain, M168. This suggests that this gene could be a regulator of *patA* and *patB* expression.

NT03/04 The intracellular survival of *Streptococcus agalactiae* in J774 macrophage-like cells

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Streptococcus agalactiae, otherwise known as group B streptococcus (GBS), is able to cause serious invasive infections in human newborns. Asymptomatic colonization of the genito-urinary tract of approximately one third of the female adult population provides a stable environment for GBS existence within humans. Up to 70% of the children born to colonized women acquire GBS *in utero* or during birth. Although the majority will not develop serious infection, in rare cases newborns are unable to control GBS colonization, resulting in dissemination and meningitis.

GBS can survive for prolonged periods following phagocytosis, but the extent to which this trait varies and/or correlates with clinical outcome is not known. To address this, we have screened 50 clinical GBS isolates from UK blood samples, representing many serotypes, MLST types and all three invasive origins (late onset, early onset and colonizing), for intracellular survival in J774 macrophage-like cells. In addition, we are now extending this approach to use transposon mutagenesis to identify GBS virulence factors that are required for intracellular survival.

NT03/05 Interactions of *Streptococcus* species with *Candida albicans*

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Streptococci are primary colonizers of oral cavity surfaces and are major components of oral biofilm communities. *Candida albicans* colonizes dental and mucosal surfaces, and is carried by up to 60% of human populations. *Streptococcus gordonii* attaches to *C. albicans* and influences the development of *Candida* biofilms, which are important in prostheses based infections. We hypothesize that streptococci recognize glycoproteins present on the cell surface of *C. albicans*, in particular those present on the hyphal form of growth. It has recently been shown that mutation in *MNT1*, encoding α1,2-mannosyltransferase involved in *O*-linked glycosylation, leads to reduced biofilm formation. In addition, *MNT1* mutant hyphae are defective in binding cells of *S. gordonii*. Fluorescence microscopy studies of hyphal-streptococcal interactions showed that five different species (8 strains) of *Streptococcus* were able to attach to wild-type strain hyphae to variable degrees. However, recognition of *ΔMNT1 ΔMNT2* mutant hyphae by all strains of streptococci was impaired. Levels of hyphal filament binding by streptococci correlated with production of AgI/II streptococcal surface protein adhesin. These results support the evidence that AgI/II polypeptide is a major mediator of inter-kingdom co-aggregation, and that *CaMNT1* is necessary for interactions of streptococci with *C. albicans*.

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NT03/06 *Candida albicans* cell surface glycoprotein Als3p is required for co-aggregation with *Streptococcus gordonii* and mixed-species biofilm formation

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Candida albicans is a dimorphic fungus that colonizes a range of body sites and may cause superficial or systemic diseases. Viridans group streptococci promote hyphal production by *C. albicans* leading to enhanced mixed-species biofilms. Within biofilms, *Streptococcus gordonii* adheres preferentially to hyphal filaments relative to blastospores. Previous work has suggested that *S. gordonii* adhesins SspA and SspB are required for binding *C. albicans.* We therefore explored the interactions of *S. gordonii* and SspB in binding hyphae and specifically to the hyphal-specific glycoprotein Als3p.

Als3p was required for early stage *C. albicans* mono-species biofilm formation. *C. albicans als3 /als*3 also failed to form a biofilm on *S. gordonii*-coated surfaces, indicating that Als3p was a potential receptor for *S. gordonii.* Confirming this, *S. gordonii* co-aggregation with *C. albicans* hyphae in suspension was abolished upon deletion of Als3p. *Lactococcus lactis* expressing SspB conferred on these bacteria the ability to adhere to *C. albicans* WT hyphae but binding was >80% reduced to *als3 /als*3 hyphae. Furthermore, *L. lactis* cells expressing SspB bound to the surrogate host *S. cerevisiae* expressing Als3p but not *S. cerevisiae* WT. *S. cerevisiae* Als3p were also unable to bind *L. lactis* WT, implying a direct interaction between surface proteins Als3p and SspB.

NT03/07 Antigen I/II family polypeptide in *Streptococcus pyogenes* M28 mediates biofilm formation and salivary protein interaction

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Streptococcus pyogenes M28 strains are associated with invasive disease, pharyngitis and child bed fever. These strains have horizontally acquired a 37.4 kb locus within which is encoded a protein with homology to the Ag I/II family of polypeptides. Ag I/II proteins have been shown to have a role in adhesion, biofilm formation and inter-species interactions in many oral streptococci. The aim of this study was to understand the role of Ag I/II like-protein AspA in biofilm formation in *S. pyogenes* M28. A range of strains were assessed for biofilm formation on saliva-coated coverslips, producing varying biofilm architectures. The *aspA* gene in two strains (H360 and MGAS6180) was inactivated by allelic replacement with *aad9* (spectinomycin resistance). These *aspA* mutants showed a significant reduction in biofilm formation on saliva-coated surfaces, while complementation restored biofilm formation to wild-type levels. Heterologous expression of AspA from strain H360 in *Lactococcus lactis* conferred a significant increase in biofilm formation and salivary aggregation compared to wild-type. Taken together, these data demonstrate that biofilm formation in M28 GAS may be strain-specific. However, in strain H360, AspA may have a significant role in biofilm formation, therefore contributing to GAS colonization and persistence.

NT03/08 Investigating the adjuvant activity of pneumolysin

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Pneumolysin (PLY) is a 53Kd cholesterol-dependent cytolysin that is largely conserved in all strains of *Streptococcus pneumoniae*, making it an ideal candidate for inclusion in a broad spectrum vaccine. We have shown that PLY is not only a protective immunogen but also has potent adjuvant properties. Both systemic and mucosal responses are achieved when PLY is used as an adjuvant which may prevent colonization and therefore provide non-serotype specific herd immunity. The cytolytic activity of PLY prevents its inclusion in a human vaccine; a non-lytic deletion mutant Δ6 was created for this purpose which retains adjuvanticity, albeit slightly reduced. Given the cytolytic properties of PLY it is possible that intracellular pattern recognition receptors are engaged; however, since the Δ6 mutant retains adjuvant activity this suggests that although pore formation may augment the immune response it is not required for adjuvanticity. IL-1β, IL-6 and KC have all been shown to enhance pneumococcal clearance and are induced in response to PLY stimulation of macrophages. Additionally, IL-1β and IL-6 are required for the induction of a Th17 mediated immune response which is known to protect against colonization with the pneumococcus. These three factors have been selected to investigate the functional differences between WT and Δ6 PLY in an attempt to elucidate the adjuvant activity of PLY, particularly the role of pore formation.

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NT03/09 Genetic regulation of pilus production in *Streptococcus pneumoniae*

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Streptococcus pneumoniae causes an array of diseases including otitis media, pneumonia, meningitis and septicaemia. A number of key virulence factors contribute to its ability to cause these diseases. These include the pneumococcal pilus, shown to be involved in attachment to lung epithelial and nasopharyngeal cells lines, aiding colonization and potentially allowing the bacterium to persist for longer periods. To date, four two-component signal transduction systems have been implicated in regulation of the *rlrA* islet that encodes the pneumococcal pilus.

A commercially available bioluminescent derivative of strain TIGR4 (Xen35) used for *in vivo* experiments showed decreased expression of the pilus at the protein and mRNA transcript level. Insertion of the *lux* genes required to produce bioluminescence altered the expression of genes (SP1914/SP1915) associated with the insertion site. No function has been allocated to these genes but homology searches suggest SP1915 is a DNA-binding protein.

Microarray analysis revealed a group of genes that were altered in expression compared to the parent TIGR4. Genome sequencing of Xen35 revealed several SNPs/ deletions that could be responsible for the decrease in pilus expression. Genes found to be different in their expression or sequence have not previously been found to be involved in pilus regulation, implying a more complex regulatory network surrounding production of the pneumococcal pilus.

NT03/10 A fusion-protein-based pneumococcal vaccine

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The Gram-positive bacterium *Streptococcus pneumoniae* is a major human pathogen, causing high morbidity and mortality worldwide. Currently there are two kinds of pneumococcal vaccines, the 23-valent polysaccharide (PS) vaccine and the PS-protein conjugated vaccine. The 23-valent PS vaccine has a limited efficacy in some high risk groups due to its weak immunogenicity. The PS conjugated vaccines are expensive for developing countries since it is difficult to manufacture. Also, strain and serotype switching can cause long-term vaccine failure. There is therefore a need to develop improved vaccines against pneumococcal diseases.

Pneumolysin (PLY) can act as a powerful mucosal adjuvant to induce both systemic and mucosal immunity to proteins genetically fused to PLY after intranasal vaccination. In this work, *S. pneumoniae* virulence factors, pneumoccocal surface adhesin A (PsaA), pneumococcal surface protein A (PspA), pneumococcal surface protein C (PspC) and pneumococcal histidine triad protein D (PhtD) have been genetically fused to PLY and Δ6 PLY, a PLY toxoid that lacks haemolytic activity but retains its immunogenic and adjuvant activity. The immune response to these fusion proteins have been measured by ELISA. The ability of these fusion proteins to stimulate protective immunity against infection with strains D39, TIGR4 and A66.1 was investigated using an intranasal invasive murine model.

Acknowledgements Support for this project was provided by PATH.

NT03/11 Cholesterol-dependent cytolysins as mucosal adjuvants

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Cholesterol-dependent cytolysins, (CDC), are a family of toxins produced by Gram-positive bacteria that produce large pores on cell membranes containing cholesterol. Over 20 species of bacteria produce CDCs including; *Streptococcus*, *Listeria*, *Clostridium*, *Bacillus* and *Arcanobacterium*. Even though the main action of the toxins is pore generation, the toxins also interfere with immune cell function as well as manipulating cytokine induction (Billington et al., 2000).

One prominent member of the CDC's is pneumolysin (PLY) which is produced from the pathogen *Streptococcus pneumoniae*. PLY is known to have various immunomodulatory properties and has recently been demonstrated to act as a ligand for TLR4. The TLR4 binding properties of PLY activates NF-κB and mitogen activated protein kinase pathways resulting in cytokine production (TNF-α, IL-1γ, IL-6 and IL-8) (Malley et al., 2003). It was recently shown that PLY is also able to act as a mucosal adjuvant when genetically fused to either eGFP or an *Streptococcus pneumoniae* antigen (Douce et al., 2010). It was demonstrated that both the native fully-active PLY and a detoxified version of PLY (Δ6PLY) were able to generate strong immune responses to test antigens to which they were genetically coupled.

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To establish if the immunogenic/self adjuvant characteristic displayed by PLY is unique, selected family members of the CDC's have been purified. The immunogenic and adjuvant activities of these proteins have been investigated by immunization of mice with purified toxin alone of with toxin fused to a second antigen.

The projects central aim is to determine whether the selected CDCs; suilysin (SLY), perfringolysin (PFO) and intermedilysin (ILY) possess similar immunogenic and adjuvant properties that PLY has been shown to possess and thus try to elucidate a possible mechanism for the adjuvant activity.

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NT03/12 *Not presented*

NT03/13 Effect of antibiotics on *Streptococcus mitis* virulence factors: a phenotypic and genotypic analysis

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Antibiotics used in the management of infective endocarditis (IE) at sub-minimum inhibitory concentrations (sub-MICs) modulate the expression of bacterial virulence determinants. This study has assessed the effect of penicillin, vancomycin and linezolid sub-MICs on the expression of virulence factors of *Streptococcus mitis*, a leading cause of IE.

The effect of 1/4× and 1/8× MIC of penicillin, vancomycin and linezolid on the neuraminidase activity and plasminogen binding of IE isolate *S. mitis* 881/956 was analysed throughout the bacterial growth, using colorimetric substrates. A genome-wide assessment of the effect of 1/8× MIC of penicillin was also performed at the mid-exponential growth phase of this organism, using microarray technology. Penicillin and vancomycin up-regulated the neuraminidase activity of *S. mitis* while, linezolid down-regulated its expression. The plasminogen binding ability of this organism was up-regulated by penicillin with no modulation being produced by the remaining antibiotics. Microarray analysis of *S. mitis* gene expression in response to penicillin revealed the modulation of the *rpsU* gene.

These results suggest that at sub-MIC levels, antibiotics act as signalling molecules, creating a stress response which modulates the expression of *S. mitis* virulence factors and housekeeping genes, in a manner independent of their mode of action.

NT03/14 Vaccination with vaccine fusions to pneumolysin confer protection in a murine model of colonization

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Streptococcus pneumoniae is a human pathogen that causes both mucosal and invasive diseases, including pneumonia, bacteremia, otitis media, and meningitis, throughout the world. Currently available vaccines are based on the capsular polysaccharide. As polysaccharide

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vaccines are poorly immunogenic in those most at risk of disease, new protein/polysaccharide conjugate vaccines have been developed. These vaccines are expensive to produce, limiting their use in the developing world where the burden of disease is greatest. New strategies are required that provide capsular and serotype independent protection. The approach taken here involved the genetic fusion of vaccine antigens to pneumolysin for the production of a serotype independent protein vaccine. MF1 mice were vaccinated intranasally with 4pmol of vaccine antigens and challenged with bioluminescent pneumococci in a murine colonization model. Here we show that proteins genetically fused to pneumolysin (PLY) become capable of generating an antibody response that is not seen when they are administered as a mix. In this study, pneumococcal surface protein A (PspA) or pneumococcal hisitidine triad protein D (PhtD) was fused to the N terminal of Ply and administered intranasally to mice. Serum IgG responding to PspA, PhtD and PLY were at a level comparable to those produced by parental administration. We also report the protective efficacy of these responses in a murine colonization model. Pneumococcal antigens genetically fused to pneumolysin are immunogenic when administered to mucosal surfaces and generate immunity that is protective in a colonization model.

Acknowledgements Support for this project was provided by PATH.

NT03/15 *Streptococcus pyogenes* virulence factor SpyCEP cleaves the entire repertoire of neutrophil chemoattractant chemokines

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Streptococcal necrotizing fasciitis lesions feature extensive necrosis with dense bacterial growth, yet are neutrophil poor. SpyCEP is an essential cell wall-associated and soluble virulence factor of invasive *Streptococcus pyogenes* (GAS), which cleaves and inactivates chemokines, abrogating phagocyte chemotaxis and activation. Although first identified as cleaving CXCL8 (IL-8), SpyCEP's full substrate range was unknown. Using SDS-PAGE, western analysis and mass spectrometry, a wide panel of CXCL chemokines were investigated to find substrates. CXCL1, 2, 6 and 8 were cleaved by cell-free GAS supernatants; CXCL3, 5 and 7 by live GAS cells. No cleavage was observed using an isogenic GAS strain lacking functional *cepA* (SpyCEP gene). The substrate range included all ELR+ CXC chemokines which ligate CXCR1 and CXCR2, the major neutrophil chemokine receptors, but no other chemokines. The chemokine cleavage site was located in a conserved tertiary structure, the second turn of the C-terminal α helix, and was unaffected by secondary structure. Unlike SpeB, a non-specific protease of GAS reported to cleave some chemokines, protease activity was not observed against a panel of other proteins. SpyCEP is hence a specific CXC ELR+ chemokine inactivator, allowing GAS to evade phagocytic killing. Experiments are underway to delineate enzyme regions that dictate substrate specificity.

NT03/16 Genetic variation as a mechanism for hyper-encapsulation in *Streptococcus pyogenes*

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Background Streptococcus pyogenes of the *emm*18 genotype are widely known to exhibit a hyper-mucoid phenotype, but the mechanism behind the unusual colony morphology has never been elucidated. The *emm*18 genotype is strongly associated with waves of highly transmissible pharyngitis and the recent resurgence of rheumatic fever; the hyper-mucoid phenotype is believed to underlie the disease association.

Results Quantification studies revealed that isolates of serotype *emm*18 produce more capsule at both the transcriptional and synthesis level than all other *emm*-types. A diffusible factor present in blood, to which *emm*18 isolates are uniquely sensitive, was found to induce upregulation of capsule production and enhance resistance to phagocytosis in human blood. A number of discrete genetic differences within the *emm*18 genome were identified, including two unique mutations in the Control Of Virulence (*cov*) two component Regulatory System, known to regulate transcription from the capsule operon. We have also identified sequence variation in the promoter region of the *hasABC* capsule operon, to which the CovR repressor protein binds, in combination with an insertion element able to transpose from this promoter within the *emm*18 genome.

Conclusions Varied and unique genetic differences contribute to the hyper-mucoid phenotype, and thus virulence, of serotype *emm*18 *Streptococcus pyogenes.*

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NT03/17 Investigation into a cluster of *Streptococcus pyogenes* post-partum sepsis

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Background A cluster of *Streptococcus pyogenes* (GAS) postpartum sepsis arose from one maternity unit leading to the deaths of two patients, significant infection of three others, and carriage in 3 healthcare workers. This cluster was unusual in terms of disease severity and rapid transmission.

Results Cluster-associated isolates demonstrated similar superantigenicity, expression of virulence factors and resistance to opsonophagocytosis as other GAS isolates from the UK. All the isolates were *emm*1 , the most common invasive *emm*-type in the UK, and had the same toxin and *covR/S* genotype. Sequencing of the highly variable streptococcal inhibitor of complement gene, *sic,* revealed that all the cluster-associated isolates had the same unique *sic*-type, with one exception, providing evidence that this was an outbreak, distinct from other *emm*1 strains in the UK. This was supported by whole genome sequencing which has enabled SNP frequency in GAS to be studied for the first time in humans during invasive clinical infection and transmission.

Conclusion This cluster of highly invasive *emm*1 strains was distinct from those strains causing invasive infection in the UK and distinct from strains circulating in the same town. Experiments are underway to determine the role of each unique genetic feature revealed in this study.

NT03/18 Characterization of the PrtM maturase of *Streptococcus equi*; a proven virulence factor in strangles

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Streptococcus equi subspecies *equi* is responsible for the prevalent and highly contagious equine respiratory infection known as strangles. Developing effective and universally accepted vaccines against this disease has been slow. The *S. equi* lipoprotein PrtM is a putative maturase, i.e. involved in the folding of proteins to be exported. It has been previously reported that PrtM is a virulence factor of *S. equi*, as demonstrated in both a mouse model and the equine host. Moreover, colonization of air interface organ cultures after inoculation with a maturase-deficient mutant strain was reduced compared to infections with the wild-type strain. In this study, we have performed proteomic analyses of cell-associated and secreted protein extracts from the maturase-deficient mutant strain and the wild type strain *S. equi* 4047. These data have revealed differentially expressed proteins. Specifically, we have found that FNE (a fibronectin binding protein) and IdeE2 (an immunoglobulin G endopeptidase) are present in the secreted protein extract of the wild type strain but not in the mutant strain. This suggests that proteolytic degradation of misfolded secreted proteins and that PrtM may not be linked solely to the folding of one specific substrate, but is likely a multi-substrate maturase. These data have allowed us to identify potential maturase substrates which warrant further investigation for their contribution to the virulence of *S. equi*.

NT03/19 Identification of proteins influenced by a virulence regulatory gene (*vru*) of *Streptococcus uberis*

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Intramammary infection with *Streptococcus uberis* is the most common cause of bovine mastitis in the UK. This disease significantly impacts on both animal health and welfare and the economics of milk production. A number of cell wall virulence determinants have been identified that are anchored by the sortase A enzyme and are essential for bacterial virulence in mastitis challenge studies. We have identified a virulence regulatory gene (termed *vru*) which potentially regulates one such anchored protein and a number of other putative virulence determinants using combinations of microarray and proteomic analysis.

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NT03/20 Declining serotype coverage of new pneumococcal conjugate vaccines relating to the carriage of *Streptococcus pneumoniae* in young children: a cross-sectional time-series analysis

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Background Seven-valent pneumococcal conjugate vaccine (PCV-7) was added to the UK's routine infant immunization programme in 2006 and led to a 41% reduction in invasive pneumococcal disease (IPD) by 2008. By 2009, the incidence of IPD in under 5's had increased to pre-PCV-7 levels and a new 13-valent pneumococcal conjugate vaccine (PCV-13) replaced PCV-7 in the UK infant immunization programme in April 2010. PCV-7 efficacy in invasive disease is widely documented, but reports on the contemporaneous effect of vaccine pressure on pneumococcal carriage are limited. We assessed the emergence of serotypes colonizing the nasopharynx of young children during PCV-7 implementation.

Methods Nasopharyngeal swabs were collected from children aged 4 years and under attending the outpatients department of a large UK teaching hospital during PCV-7 introduction (October 2006–February 2007) and in the two subsequent years (October 2007-February 2008 and October 2008-February 2009). Approximately 100 pneumococci were collected each year.

Results Total pneumococcal nasopharyngeal carriage remained stable during the three year period. We observed a significant 37.2% (95% CI, 23.7%-48.2%) decrease in nasopharyngeal carriage of PCV-7 serotypes and a concomitant increase in carriage of non-PCV-7 serotypes, the most prevalent of which were 6C, 11A, 19A and 22F. Of these, only serotype 19A is included in PCV-13. By March 2009, PCV-13 was predicted to cover only 33.3% (95% CI, 24.2–42.5%) of strains carried in the study population.

Conclusion We observed a significant decrease in the serotype-specific coverage of PCV-7 and PCV-13. The increase in the proportion of non-vaccine serotypes in our study and in current invasive disease underlines the importance of continued active surveillance of carriage and disease.

NT03/21 Bacterial cell wall cross-linking and antibiotic resistance: the role of the MurM protein in *Streptococcus pneumoniae*

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Streptococcus pneumoniae is the causative agent of several community-acquired infections and the emergence of multi-drug resistant strains is making treatment progressively more difficult. The organism has two mechanisms for evading penicillin: acquisition of low affinity penicillin binding proteins and the action of the MurM protein.

MurM is an aminoacyl ligase that adds either L-alanine or L-serine to the stem peptide lysine of peptidoglycan. It is the specific allele of *murM* within a particular strain of *S. pneumoniae* that determines relative levels of cross-linking and preference for serine or alanine. Deletion of *murM* causes reversion to penicillin sensitivity in strains that were previously resistant. In this study the MurM proteins from three strains of *S. pneumoniae* have been over-expressed and purified with the aim of comparing overall activity and substrate specificity. In addition to this, crystallography screens have led to the investigation and positive identification of MurM as a metalloenzyme.

NT03/22 The molecular basis of *Streptococcus agalactiae* DNase activity

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Streptococcus agalactiae (Group B streptococci, GBS) is the leading cause of invasive neonatal infections in industrialized countries. To establish an infection GBS have to avoid host immunity. Neutrophil extracellular traps represent an important mechanism of innate immunity that streptococci evade by the production of extracellular DNases. While the production of three distinct extracellular nucleases has been reported for GBS 30 years ago, the genetic basis of DNase production has not been characterized. Using an insertion mutant library, we screened for GBS mutants showing a diminished DNase production on DNA-methyl green agar. The screen led to the isolation of 33 mutants with diminished DNase activity. Genetic analysis of the insertion sites resulted in the identification of two putative DNase-encoding genes corresponding to the molecular sizes of the previously published 33 kDa and 26,5 kDa nucleases II and III of GBS. To prove DNase activity of the encoded proteins, both genes were cloned into the vector pET21a and expressed as recombinant His-Tag proteins in *E. coli*. Incubation of the purified proteins with various amounts of DNA resulted in the demonstration of intrinsic DNase activity for both genes, establishing a definite genetic link for DNase production in GBS.

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NT04 Acid stress: surviving & responding

↑CONTENTS

NT04/01 Survival of soft-rot-causing *Erwinia* against chemical control measures

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Erwinia carotovora subsp. carotovora (Ecc) is a devastating phytopathogen that causes major economic losses in agriculture due to soft rot disease in fruits and vegetables worldwide especially during storage. Presently, no chemical treatments are available for an effective control of this soft rot disease. The major limiting factors of controlling agent's efficacy include potency and swiftness of microbial attachment and growth during infection, low penetration in infection sites, internalization of microbial contaminants within plant tissues, and biofilm formation. Exhibition of injury and repair in soft rot causing *Erwinia,* may give a direct approach to assess safety measures to prevent disease. During control treatments the surviving population of pathogenic bacteria may have reduced cultivability either due to cell stress, cell injury or entry into altered physiological cell state. The minimum inhibitory concentration of different organic acids, organic and inorganic salts were determined. Cell injury and bactericidal effect of these chemical agents were studied by enumeration on non-selective medium and selective medium. Copper sulfate, sodium propionate, sodium benzoate, acetic acid, and sodium octanoate caused major cell injury in *Ecc* BR1 strain at sub-lethal doses. Resuscitation in *Ecc* BR1 strain cells injured with copper was observed on potato slice. Although characterization of injured cells is still ongoing, the first results indicates survival strategies of soft rot causing *Ecc* during control treatments become more important factor in determining effective control.

NT04/02 Acid-induction of flagellar gene expression in *Campylobacter jejuni* is linked to increased invasion of mouse intestinal epithelial cells on transwells

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Campylobacter jejuni is a major human foodborne pathogen in the developed world. However, how it causes disease is still poorly understood. Human infection commonly occurs via ingestion of contaminated poultry products. When the bacterium reaches the small intestine after gastric passage, it displays flagella-dependent invasion of intestinal epithelial cells, resulting in inflammation and gastroenteritis. Exposure to low pH is an inevitable stressor for *C. jejuni* during gastric passage, yet the effect of acidic pH on invasion has not yet been reported.

Microarray analysis of acid-shocked *C. jejuni* showed that several σ^{54}-responsive flagellar genes were induced at pH 5.0. We investigated the link between low pH and *C. jejuni* virulence by assessing the ability of acid-shocked or non-shocked *C. jejuni* to invade mouse intestinal epithelial (m-IC_{cl2}) cells. With m-IC_{cl2} monolayers grown on transwells, invasion of acid-shocked *C. jejuni* was often 10–100-fold increased. However, this increase in *C. jejuni* invasion was not observed when m-IC_{cl2} cells were grown on flat-bottomed wells. This suggests that *C. jejuni* may invade intestinal epithelial cells at the basolateral side after translocation through the monolayer. Acid shock may indeed enhance *C. jejuni* invasion, but this requires further study in the context of an appropriate invasion model.

NT05 Bioremediation of metals

↑CONTENTS

NT05/01 Bioremediation of plutonium-contaminated land

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The release of radionuclides from anthropogenic sources is of great scientific and public concern. The biogeochemical behaviour of Pu in contaminated soil was investigated in this study as microbial processes have the potential to mobilize Pu via the reduction of Pu(IV) to the potentially more mobile Pu(III). Microcosms containing Pu contaminated soil from an industrial site were constructed and the native bacterial community stimulated using glucose. Both fermentation and anaerobic respiration processes were stimulated in the microcosms and monitored over 118 days. A shift in the 16S rRNA gene profile was observed, with the dominant group changing from acidobacteria at time zero to betaproteobacteria at day 45. Clostridia, which are known fermentors and have been reported to facilitate

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the reduction of Pu(IV) to Pu(III) were not detected at time zero, but increased to 19% of the genetic profile by day 45. Despite the biogeochemical changes in the microcosms, negligible Pu was released into porewaters. This information is important for developing remediation options for Pu-contaminated soils, which may include managing legacy Pu in situ but not mobilization via the stimulation of metal-reducing bacteria *in situ*.

NT05/02 Bioremediation of chromate (VI) using *Proteus mirabilis* bacteria

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The most toxic form of Chromium is chromate (VI), which usually occurs associated with oxygen as chromate (CrO_4^{2-}) or dichromate ($Cr_2O_7^{2-}$) oxyanions. It is known to be extremely toxic and harmful to human, animals and plants. Cr (VI) is widely used in the metal plating, tanning and other industries. As a result substantial amounts of Cr (VI) contaminated wastes are produced. Reduction of Cr (VI) produces Cr (III), which is less toxic, less soluble and less bioavailable.

Utilization of chromium reducing microbes and their products could enhance the efficiency of the process of detoxification of Cr (VI) to Cr (III). This poster presents an investigation into bioremediation of Cr (VI) using *Proteus mirabilis* (*P.m.*) bacteria, which are common in the environment. The chromate (VI) reduction activity has been investigated using colorimetric diphenylcarbazide assay technique. The results indicate significant rate of chromate (VI) reduction was observed. Further detailed analyses of reduction and speciation of chromium including measuring the total chromium during the bioremediation process are monitored using an inductively coupled plasma-optical emission spectrometry (ICP-OES), and separation of Cr (III) and Cr (VI) using an inductively coupled plasma-mass spectrometry (ICP-MS) connected with HPLC.

NT05/03 Biodegradation of aflatoxin B_1 by actinomycete cultures

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Aflatoxin B_1 (AFB_1) contamination of food and feed is the cause of serious economic and health problems. Different processes have been used to degrade AFB_1. In this study, biological degradation of AFB_1 by 3 Actinomycete species, (*Rhodococcus erythropolis* ATCC 4277, *Streptomyces lividans* TK 24, and *S. aureofaciens* ATCC 10762) was examined in liquid cultures. Four methods were used to assess the extent of the degradation; thin layer chromatography (TLC), high performance liquid chromatography (HPLC), liquid chromatography mass spectrometry (LC-MS) and Orbitrap LC-MS/MS. AFB_1 was effectively degraded by all of those test species. The biodegradation of AFB_1 by Actinomycete species was investigated under a variety of conditions in order to optimize its degradation. A range of temperatures (25 to 40 °C) and pH (4.0 to 8.0) were examined.

Preliminary studies in the degradative pathways used by these cultures to degrade AFB_1 may well indicate that different metabolic routes may have been used by these cultures. High resolution mass spectrometrical and differential expression analysis indicated that the degradation of AFB_1 was associated with the appearance of a range of lower molecular weight compounds and that these were not identical, for all three cultures. There has been major interest in the metabolite MW 236.1 in particular. Substantial amounts of this material were associated with the disappearance of AFB_1 in the cultures of *R.erythropolis*. There was a strong negative correlation between AFB_1 concentration and the level of MW 236.1. That is, while AFB_1 was decreasing the MW 236.1 was increasing. Based on this information, a possible mechanism of degradation of AFB_1 by liquid cultures of *Rhodococcus* was hypothesized. AFB_1 biodegradation was associated with the accumulation of intermediates of fatty acid metabolism, and glycolysis. |The effective degradation rate as well as the wide range of pH and temperatures under which degradation can proceed using the microbial strains in this study, may indicate the possible utility of these systems for application in food and feed process.

NT05/04 *Not presented*

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NT07 New insights into secondary metabolism

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NT07/01 *Not presented*

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NT07/02 A nucleoid-associated protein that modulates the transition phase in *Streptomyces*

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Secondary metabolism occurs after the main growth phase in *Streptomyces*. A 'transition phase' occurs to remodel global patterns of gene expression at the onset of physiological and developmental differentiation. Many different signals influence this transition phase, integrating, for example, information on nutritional status, growth rate, and stress responses. Several pleiotropic transcription factors that regulate the transition phase have been identified, but aspects of epigenetic control of gene expression are not well understood.

We are investigating the function of a novel gene in *S. coelicolor* (*tpmA* for *t*ransition *p*hase *m*odulator) encoding a protein that combines a histone-like domain with a DksA-like domain, the latter considered a ppGpp cofactor. The protein is important for integrating responses to both oxidative and osmotic stresses and also nutritional status. For example, a *tpmA* mutant is sensitive to oxidative stress at least in part due to reduced induction of the alternative sigma factor SigR. In addition, the mutant has a reduced induction of antibiotic production after osmotic stress, linked to a measurable change in DNA supercoiling. The histone-like domain of TpmA binds DNA non-specifically. Finally, over-expression of *tpmA* suppresses defects in secondary metabolism and differentiation of a *relA* mutant affected in ppGpp synthesis.

NT07/03 Effect of environmental factors on *Streptomyces coelicolor* secondary metabolite gene expression in soil: a transcriptomic and proteomic approach

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Streptomycetes are Gram-positive, soil inhabiting filamentous bacteria recognized for their production of secondary metabolites. Genome sequencing of *Streptomyces coelicolor* showed there to be 23 known or predicted secondary metabolite gene cluster ranging from antibiotics to siderphores, lipids and pigments amongst others. Further studies have shown additional putative secondary metabolite gene clusters including lantibiotics. Only a proportion of these gene clusters, however, are expressed under laboratory conditions. This study looks at the effect of different environmental conditions on the expression of these secondary metabolite gene clusters in *S. coelicolor* grown in soil microcosms. Environmental factors under investigation include nutrient amendments and the addition of nematodes and fungi to the microcosms. mRNA was used as an indicator of gene expression. Due to small copy numbers of the mRNA of interest, a RNA amplification protocol was optimized for RNA extracted from *S. coelicolor* grown in soil microcosms. Amplification up to 12500x was achieved. Reverse transcription PCR using specific primers for one secondary metabolite biosynthetic gene per cluster was used to detect changes in expression. Differential and constitutive expression was found for some secondary metabolite genes, which included expression of cryptic genes. Other secondary metabolite biosynthetic genes were not expressed under any of the soil conditions tested. Quantitative real time PCR is being used to show how genetic expression of the particular genes change over time in response to the different growth conditions. In a complementary approach, the microcosms have been subjected to novel protein extraction procedures to determine protein expression profiles in soil.

NT07/04 *Streptomyces coelicolor* osmoregulation: protein complex formation involving the atypical regulatory protein OsaC

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The Gram-positive soil dwelling bacterium *Streptomyces coelicolor* has a complex life cycle. The organism produces antibiotics in coordination with a switch from filamentous vegetative growth to reproductive sporulation. Osmoadaptation is critical for Streptomycete differentiation as sufficient turgor pressure must be generated to support this aerial growth.

The *S. coelicolor* osmoregulation gene *osaC* encodes a protein with an unusual domain composition. Initial database searches revealed only 12 other proteins sharing the same predicted domain composition, all from sequenced actinomycete genomes with 10 from Streptomyces Spp. *osaC* mutants are unable to produce aerial hyphae on media containing osmolyte.

OsaC modulates the regulation of *sigB* and *osaB*, genes encoding proteins that play an important role in the osmotic stress response. Indeed, the N-terminal kinase domain of OsaC functions as a SigB anti-sigma factor. Co-purification experiments have demonstrated association between SigB and OsaC. However, two-hybrid experiments with these proteins give no indication of direct binding of the proteins, suggesting an indirect association involving other accessory proteins.

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Further investigation using size exclusion chromatography and native PAGE suggests OsaC and SigB are both components of a large complex. 2-Dimensional PAGE and mass spectrometry have been used to identify potential components of this complex.

NT07/05 *Not presented*

NT07/06 *Not presented*

NT08 Respiratory & septic infections

↑CONTENTS

NT08/01 Are birds hot enough? Comparing parasitism of mammalian and avian macrophages by the fatal human fungal pathogen *Cryptococcus*

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Cryptococcosis is a fatal fungal disease of humans that causes death through meningitis. The major causative species, *Cryptococcus neoformans,* is a significant pathogen of the immunocompromised, especially AIDS patients. The major route of cryptococcal infection

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is through the lungs, where the yeast are phagocytosed by macrophages but then parasitize upon their host cell, thereby avoiding the host immune system. This parasitism includes being able to proliferate freely within macrophages and at least two unique non-lytic exit mechanisms: expulsion and lateral transfer. In addition, macrophages infected with *Cryptococcus* are thought likely to be the main vector for the spread of *Cryptococcus* to the central nervous system.

The role of birds, in particular pigeons, in the spread of *C. neoformans* is still not fully understood. Although there is plenty of evidence suggesting that the organism is frequently found amongst avian excreta, and can be isolated from birds themselves, there is very little evidence to suggest that birds contract systemic cryptococcal infections in nature. However, here we present data showing that *Cryptococcus* is able to parasitize avian macrophages including the first example of the expulsion of *Cryptococcus* from a non-mammalian host. Finally, we demonstrate that the higher body temperature is unlikely to be a fundamental barrier to cryptococcal growth in avian hosts, a result that calls into question existing dogma regarding the role of birds in fungal spread.

NT08/02 The major surface antigens of *Bacillus anthracis* are secreted via a SecA2-mediated pathway

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Bacillus anthracis, the etiological agent of anthrax, is a Gram-positive spore-forming bacterium. Infection is acquired through the uptake of *B. anthracis* spores from the soil or infected animal products by inhalation, ingestion or cutaneous abrasions. Between 5 and 10% of the proteins encoded by a bacterium are typically secreted across the cytoplasmic membrane, primarily *via* the Sec-dependent secretion pathway (Sec pathway). Since these include enzymes involved in cell wall synthesis and cell division, the Sec pathway is essential for growth and cell maintenance. In pathogenic bacteria, these secretory proteins include toxins and other virulence factors that contribute to pathogenesis.

A number of clinically important Gram-positive pathogens (*e.g. Staphylococcus aureus, Streptococcus gordonii* and *Strept. pneumoniae*) encode paralogues of components of the protein secretion apparatus, namely SecA (SecA1/SecA2) and SecY (SecY1/SecY2), implying that they have distinct primary and accessory protein secretion systems. Here we report the presence of paralogues of SecA and SecY in *Bacillus anthracis* that appear not to form a distinct accessory translocase. We provide evidence that the major surface-associated proteins of *B. anthracis*, namely Sap and EA1, are specific substrates of SecA2 and that their stability in the extracytoplasmic compartment of the cell is dependent on the presence of the lipoprotein chaperone PrsAB. Finally, we argue that the requirement for an additional copy of the SecY-like membrane-bound pore-forming protein is to sustain protein secretion at low growth rates, rather than to interact with SecA2 to form an independent substrate-specific Sec-like protein translocase.

NT08/03 Novel *in vitro* and *in vivo* models to study staphylococcal pathogenesis

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Staphylococcal virulence factors and pathogenesis are still incompletely understood. Vertebrate animal models are widely used to study *S. aureus* pathogenesis, but this approach has limitations including intensive management, high costs, Home Office restrictions and ethical implications. The aim of this study was to determine if human brain microvascular endothelial cells are a suitable model to study *S. aureus* pathogenesis *in vitro*, and to assess the validity of the desert locust (*Schistocerca gregaria*) as an *in vivo* model thus circumventing the need for legislative adherence. *In vitro* assays revealed that *S. aureus* exhibited high association (90% of the original inoculum) and invasion (24.25%) of brain endothelial cells compared with *S. epidermidis* which showed reduced association (15%) and invasion (0.08%). Both *S. aureus* and *S. epidermidis* were able to survive but not multiply intracellularly in brain endothelial cells. Adult locusts were injected intra-abdominally with ~1 or 3×10^6 Staphylococcal cells. *S. aureus* infected locusts showed increased mortality and morbidity compared to *S. epidermidis* infected locusts and controls. *S. aureus* was detected in the haemolymph, muscles and fat bodies, but not digestive tract or head ganglia. In contrast *S. epidermidis* neither survived in the haemolymph nor disseminated to other tissues. These findings indicate that brain endothelial cells and locusts could offer useful tools in which to study Staphylococcal interactions *in vitro* and *in vivo* respectively at an early stage, thus reducing the number of vertebrates required.

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NT08/04 Treating glue ear biofilms: an *in vitro* model

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Introduction Glue ear (otitis media with effusion, OME) is caused by biofilms with reduced susceptibility to conventional antibiotic therapy. Treatment with grommets removes the effusion, but does not address underlying biofilm infection. As a quarter of children require further surgery, better treatments based on understanding of OME aetiopathogenesis are required. This project aimed to develop an *in-vitro* model of biofilm infection, and test effectiveness of antibiotics against biofilms.

Methods Biofilms were established by incubating silicone discs in nutrient broth suspension of *Staphylococcus aureus* isolated from OME. Biofilms were exposed to rifampicin and clindamycin for different time periods and at various concentrations above minimum inhibitory concentration (MIC). Eradication was confirmed by removing the antibiotics and re-incubating the discs.

Results Biofilms could be distinguished from planktonic bacteria by their reduced susceptibility to antibiotics. Antibiotics were able to eradicate biofilms, but required treatment for several weeks at antibiotic concentrations between 100xMIC and 1,000xMIC. Importantly, biofilms did not re-grow once antibiotics were discontinued.

Conclusion Eradication of biofilms was possible, but required prolonged treatment with very high antibiotic doses. Delivering high-dose antibiotics by controlled-release biodegradable polymers directly into the middle ear is now being explored as a potential novel strategy to combat OME.

NT08/05 Investigation of the antibacterial effect of cationic antimicrobial peptides

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Objectives Gene encoded cationic antimicrobial peptides (CAPs) are naturally occurring antimicrobials produced by the innate immune system of eukaryotic and prokaryotic organisms. These constitutively expressed or induced, endogenous CAPs provide a fast response to bacterial invasion and rapidly neutralize a broad range of microbes. This investigation sought to examine the effect of a novel CAP on *Pseudomonas aeruginosa* NCTC 6750 with a view to elucidating mechanisms of action (MOA) of this polypeptide.

Methods Flame photometry, scanning electron microscopy (SEM) and flow cytometry were employed to investigate CAP antibacterial effects.

Results Flame photometry revealed incubation with 0.5mg/ml (2xMIC) of CAP1 induced minimal potassium loss in *P. aeruginosa*. Analysis of SEM bacterial images confirmed several published aspects of MOA attributed to CAPs. These include membrane blebbing, disruption of cell wall synthesis and inhibition of normal bacterial septation. Results from flow cytometry identified the inability of *P. aeruginosa* to withstand 0.5mg/ml (2xMIC) of CAP1, indicating less than a two-fold difference between minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC).

Conclusion Experimental data suggests MOA other than membrane disruption are responsible for the bactericidal action of CAP1. Several concentration dependent MOA were identified using SEM. Flow cytometry results confirmed practically synonymous MIC/MBCs for CAP1.

NT09 Extremophiles

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NT09/01 Genomic analysis of model salt-resistant bacteria: sequencing the 'smell of the sea'

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Halomonas are halophilic Gammaproteobacteria in the order Oceanospirilalles. *Halomonas* strain HTNK1 was isolated from the surface of the sea lettuce *Ulva,* a marine macroalga that makes the compatible solute dimethylsulfoniopropionate (DMSP). This isolate grew on DMSP and acrylate as sole carbon source, and, with the former, liberated the climate-changing gas dimethyl sulfide.

The genome sequence of *Halomonas* HTNK1 was determined and was compared to the complete finished sequence of *Chromohalobacter salexigens,* another member of the Oceanospirilalles. This has revealed insights into the basis of salt tolerance and other traits.

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NT09/02 *Not presented*

NT09/03 The impact of radiation fluxes on microbial cells

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Ionizing radiation leads to the production of significant quantities of reactive oxygen species and both oxidizing and reducing radicals, particularly in environments of relevance to nuclear waste storage facilities. These species have the potential to interact with a huge array of microbial biomolecules which may ultimately lead to deleterious physical consequences. In contrast, high radiation fluxes have been shown to be tolerated by a few specialist micro-organisms with highly active DNA repair mechanisms such as *Deinococcus species*. Furthermore, exciting new data suggest that ionizing radiation can have unexpected beneficial impacts on microbes such as fungi, which may be able to convert ionizing radiation into biochemical energy via pigments such as melanin. However, the mechanisms by which biological systems survive genetic and metabolic radical attack along with potential stimulation by ionizing radiation are poorly characterized. This research project will utilize highly focused metabolomic techniques to deliver fundamental physiological information on biochemical mechanisms that underpin cellular protection against radiation damage and novel energy metabolism. An initial phenotypic screen using Fourier transform infrared spectroscopy of cells exposed to a range of radiation doses will be utilized, followed by targeted mass spectrometry based methods.

NT09/04 Antarctica's ice-entombed cyanobacteria: diversity and distribution in cryoconite holes

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Cryoconite holes are vertically oriented, cylindrical shaped holes (10–100 cm in diameter and 20–60 cm deep) in the glacier surface that form when solar-heated surficial debris melts into the ice. They are solid frozen in winter but partially melt during summer though typically retain a solid ice-cover that isolates them from the atmosphere. Cryoconites and their associated microbial biota have received little attention, despite their contribution to the biodiversity and fixed nitrogen and carbon budgets of otherwise near-lifeless glacial catchments. Cyanobacteria are important primary producers in these ice-based ecosystems, but little is known about phylogenetics, geographic distribution and physicochemical factors influencing community composition. We investigated cyanobacterial diversity in cryoconites of the Diamond (Darwin Region, Lat 80°S) and the upper Koettlitz Glacier (Lat 78°S), Antarctica using a combination of morphological characteristics, cyanobacteria-specific automated ribosomal intergenic spacer analysis (ARISA) and 16S rRNA gene surveys. These sites provided both latitudinal separation and physicochemical gradients (e.g. pH 5.6–11.6). Cyanobacerial assemblages were comprised of taxa belonging to Chroococcales, Nostocales and Oscillatoriales. Statistical analysis showed no marked difference between cryoconites and close-by Antarctic land-based systems. Clone-library analysis (16S rRNA) provided further confirmation and resolution of these unique Antarctic ice-entombed cyanobacterial communities.

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NT09/05 ***Actinobacteria*: major contributors to terrestrial rock weathering**

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Actinobacteria are widespread in nature, often constituting a significant fraction of the detected microbial diversity, especially in soil where they contribute to nutrient cycling and thus the terrestrial food web. It is unclear what role *Actinobacteria* may play in pedogenesis, particularly in the early stages of terrestrial rock weathering, an important global process affecting atmospheric CO_2 drawdown. Terrestrial volcanic rocks may be considered extreme environments, for reasons which include their low organic carbon content. PhyloChip (microarray) and 16S rRNA gene clone libray analysis revealed the presence of a diverse assemblage of *Actinobacteria* occurring within terrestrial volcanic rocks of different lithologies, and contributing as much as 43% of the total bacterial diversity. Traditional culture-based approaches revealed a dominance of *Actinobacteria* in volcanic glass, and the ability of many *Actinobacteria* isolates to grow using parent rock (basalt/palagonite) without nutrient amendment. Colonization experiments have revealed the ability of an actinobacterial isolate to rapidly colonize volcanic rock and enter cavities within the material. We suggest that given both the abundance of this phylum in terrestrial rocks, and the ability of isolates to establish growth independent of an additional nutrient source, *Actinobacteria* are likely to be major contributors to terrestrial rock weathering in the environment.

NT09/06 Bacterial calcium mineral precipitation under conditions past and present: the interior of calcium carbonates as an extreme environment

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The precipitation of calcium minerals by bacteria is a widespread phenomenon observed among many taxonomic groups and environments. During the precipitation process bacteria get entombed, which cuts off nutrients and creates an extreme microenvironment.

This study focuses on calcium carbonate precipitation in marine bacteria in order to understand the necessary parameters for precipitation. For this purpose bacterial strains, isolated from marine environments, were incubated in artificial seawater mimicking present day and Cretaceous (140 Ma ago) conditions by varying the magnesium/calcium-ratio and other medium components. Cultures were screened for precipitates using Scanning Electron Microscopy (SEM) and X-ray analysis.

For *Halomonas sp.* K2d-1, calcium mineral spheres were observed in the Cretaceous medium, exhibiting high calcium peaks which are typical for calcium carbonates. SEM imaging revealed micropores (bacterial prints) on the surface, which indicate that bacteria play a crucial role in precipitation. Analysis using a Focused Ion Beam (FIB) demonstrates that the micropores in the interior of the spheres were filled presumably with abiotic material, leading to a mixture of biotic and abiotic material.

This data yields new insights into the structure of biotic carbonates and the processes limiting bacterial growth in the extreme environments of carbonate sediments and precipitates, past and present.

NT09/07 Microbial diversity and processes at the critical zone in a volcanic environment

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The critical zone is the location at the rock weathering front where a variety of important earth system processes occur, such as the sequestration of CO_2 during silicate weathering and soil neogenesis from rock weathering. The critical zone is an important subsurface region of microbial activity in extreme environments because rocks dissolve and provide nutrients to microbial life. Yet the diversity and role of microorganisms at the critical zone is not understood. We examined microbial communities in vegetated and unvegetated rock-soil interface zones near Skorradalur Lake, Iceland, a site of previous geochemical analyses. Cultivation of microorganisms using organics-limited media produced isolates that would be expected from soils within cold environments (*Polaromonas* and *Psychrobacter* spp). Molecular analysis of the 16S rRNA gene clone libraries shows that the community is dominated by *Caulobacter* (58%). Our data show that the critical zone harbours a diverse community of bacteria, many of which may be adapted to cold-temperature critical zone environments and have as yet uncharacterized functions at rock interfaces. On-going work is focusing on characterizing these isolates, the molecular diversity of the active organisms in the critical zone and testing the rock weathering capability of the isolated organisms in the laboratory.

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NT09/08 *Spongiibacter arcticus* sp. nov., isolated from Arctic seawater

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A Gram-negative and motile bacterial strain, designated CL-AS9^T was isolated from polar seawater of the Arctic. Analysis of the 16S rRNA gene sequence of the strain showed an affiliation with the genus *Spongiibacter*, sharing 94.3 and 93.8% sequence similarities with the type strains of *Spongiibacter tropicus* and *Spongiibacter marinus*, respectively. Phylogenetic analyses revealed that strain CL-AS9^T formed a separate branch that was distinct from a clade comprising *S. marinus* and *S. tropicus.* Cells of strain CL-AS9^T were positive for oxidase and catalase activities. Other physiological and biochemical characteristics for strain CL-AS9^T will be provided to the poster presentation.

NT09/09 Prokaryotic abundances and compositions of marine aerosols above the East Sea, Korea, in August 2009 and May 2010

Byung Cheol Cho, CHUNG YEON HWANG & Gwang Il Jang

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Atmosphere is a hostile environment for living microorganisms due to its extreme and varying conditions including temperature, radiation and desiccation. Little has been known about marine airborne prokaryotes in open seas. To understand abundance and composition of marine aerosol prokaryotes, aerosol samples were collected both on 0.2 µm pore-size filters and in all-glass impingers (AGI-4) filled with a collecting medium (i.e. 0.2 µm filtered and autoclaved seawater) in the East Sea in August 2009 and May 2010. Surface seawater samples were also collected in each investigation. Prokaryotic abundances were assessed by using both real-time PCR and epifluorescence methods. Prokaryotic compositions were assessed by using denaturing gradient gel electrophoresis (DGGE) of PCR-amplified 16S rRNA gene fragments. Partial sequences of 16S rRNA gene from DGGE bands were dominated by bacterial groups of *Gammaproteobacteria* and *Bacteroidetes*, and an archaeal group of *Euryarchaea*. Majority of bacterial sequences in coastal and offshore aerosols were of marine origin and certain sequences co-occurred in both aerosols and seawaters, supporting that surface water is a main source of marine airborne bacteria. A potential of aerial transport of marine bacteria will be discussed in the oral presentation, along with the results of compositions of culturable bacteria and of viable prokaryotes based on RT-PCR-DGGE for aerosol samples in May 2010.

NT10 Industrial Biotechnology 2025

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NT10/01 Selective enrichment of microbial inocula to enhance biodegradative performance

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Bioaugmentation, one of the main approaches in bioremediation of recalcitrant organic chemicals, has met with mixed success. It is thought that the enrichment procedure is a crucial step that affects the performance of inocula and hence, the rate and extent of subsequent biodegradation. The feasibility of pre-conditioning the microbial inoculum was investigated by selectively enriching the inoculum with a range of chemicals that are expected to induce the enzymatic machinery responsible for hydrocarbon degradation. Hydrocarbon degradative profile was monitored using microtiterplates and a tetrazolium salt (INT) that enables quantifying bacterial respiration. Generally, in each of hydrocarbon sources (diesel, hexadecane, toluene and phenanthrene) used in this study, two to three enrichment substrates caused colour profile that is comparable or even significantly higher (Tukey's test; $P<0.05$) than that induced by diesel, a traditional enrichment substrate for inocula. Our results support the potential for conditioning inocula to enhance their hydrocarbon degradation efficiency.

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NT10/02 A high-throughput screen for the identification of a yeast strain capable of fermenting pentose sugars

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Fermentation of lignocellulosic biomass (LCM03s) is potentially a sustainable option for the production of bioethanol. LCMs contain fermentable hexose sugars and the currently non-fermentable pentose sugars; ethanol yield from lignocellulosic residues is dependent on the efficient conversion of available sugars to ethanol. One of the challenges for the production of ethanol is the lack of an organism, able to ferment these sugars into ethanol. Fermentative yeast, *Saccharomyces cerevisiae* is the preferred organism for conversion of sugars to ethanol but is unable to ferment pentose sugars. Naturally occurring yeasts, such as *Pichia* and *Kluyveromyces* spp. are known to ferment glucose and xylose to ethanol but the yields are poor. The aim of this study was to develop a high throughput screen to identify promising pentose fermenting strains of *Pichia* and *Kluyveromyces* with the intention of identifying strains that can be used as gene 'donors' in the development of recombinant ethanologens. Using spot test and Phenotypic Microarray PM plates (Omnilog) methods, we screened six *Pichia* and eight *Kluyveromyces* NCYC strains for their capacity to utilize hexose and pentose sugars (individually and in combination) under anaerobic and aerobic conditions.

NT10/03 New terpenoid production using pathway-engineered *Escherichia coli*

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Zerumbone, a sesquiterpene found exclusively in shampoo ginger (*Zingiber zerumbet* Smith) has been attracted attention as a spice and also as a lead compound of new bioactive substances. However, virtually nothing is known about its biosynthesis genes and pathway. In our previous study we have isolated a synthetase gene (*ZSS1*) of -humulene from shampoo ginger. In this study, we predicted a biosynthesis pathway from -humulene to zerumbone (Fig. 1), and have succeeded in the isolation of cytochrome P450 and short-chain dehydrogenase/reductase(SDR) genes.

Using RT-PCR with degenerate primers and RACE-PCR, we obtained full length of cDNA sequences of P450 and SDR, and named them *CYP71BA1* and *ZSD1*, respectively. Functional characterization of these genes revealed that *CYP71BA1* converted -humulene into 8-hydroxy- -humulene, and *ZSD1* converted 8-hydroxy- -humulene into zerumbone. As a result of gene expression analysis in leaf, stem and rhizome of the Shampoo ginger, *ZSS1* and *CYP71BA1* specifically were transcribed in a rhizome, but *ZSD1* was transcribed in all organizations. Expression of *ZSD1* coincided with a variation of zerumbone content in a rhizome. These results suggest that ZSS1, CYP71BA1 and ZSD1 involved in the zerumbone biosynthesis in shampoo ginger. We also succeeded in production of 8-hydroxy- -humulene through metabolic pathway-engineered *E. coli*.

NT10/04 Isolation and characterization of a novel thermophilic cyanobacterial isolate from Taian hot springs in Taiwan for high CO_2 mitigation and C-phycocyanin extraction

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The greenhouse effect has been increasing due to the accumulation of CO_2 released from the energy sector in the atmosphere. Thermophilic photosynthetic microorganisms have been developed for the CO_2 mitigation to adapt to the high temperature environments caused by sunlight and feeding flue gases. In the study, a thermophilic rod-shaped cyanobacterium TA-1 with 1.2–2.5 µm × 6.0–9.0 µm, preliminarily identified as *Thermosynechococcus* by 16S rRNA comparison, was obtained from Taian hot springs in Taiwan. TA-1 was examined with their growth characterizations and nutrient requirements. The results showed that TA-1 has the better growth at temperature 50°C, light intensity of 6000 lux, starting pH 7–9 and 40–50 mM NaCl. The preferred nitrogen source of TA-1 is $NaNO_3$ of which the minimal requirement is 10 mM. The purified C-PC from TA-1 is still kept native and active at a wide range of temperatures (4–60 °C) with a 65.65% activity even at 60 °C, as well as pH values from 4 to 9. In the study, the C-PC of TA-1 significantly shows with a good thermostability and acid-base stability, which imply the potential applications in the bioindustries due to their native and active forms (still sustaining the biological functions). In the merit of this strategy by integrating the production of C-PC from TA-1 biomass, mitigation of CO_2 from industrial exhaust gases could be more economically feasible.

Keywords Thermophilic cyanobacteria, *Thermosynechococcus*, thermostability, acid-base stability, CO_2 mitigation

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NT12 Protein folding and misfolding ↑CONTENTS

NT12/01 Genetic control of *de novo* formation of the yeast [*PSI*$^+$] prion

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The *de novo* formation of native prions in the yeast *Saccharomyces cerevisiae* requires the presence of at least one particular prion, [*PIN*$^+$]. In most stains [*PIN*$^+$] is the prion form of the Rnq1p protein and is the only prion so far reported to exist in natural (wild-type) strains. In [*PIN*$^+$] strains the *de novo* formation of [*PSI*$^+$], the prion form of Sup35p occurs spontaneously at a frequency between 10^{-6}–10^{-7} and is not usually associated with any genetic change either at the *SUP35* locus or at any other unlinked locus. However, we have identified several nuclear gene mutations that elevate the frequency of [*PIN*$^+$]-dependent, *de novo* formation of [*PSI*$^+$]. One strategy has been to identify proteins that physically interact with Sup35p and to determine whether, in their absence, the rate of *de novo* formation increases. This strategy has identified Ppq1p, a putative protein serine/threonine phosphatase. Deletion of *PPQ1* leads to an increase in the frequency of spontaneous *de novo* formation of [*PSI*$^+$] and when induced by overexpression of Sup35p. We have also identified two other genes whose products suppress *de novo* prion formation, namely the *TSA1* and *TSA2* genes. Tsa1p and Tsa2p are sequence-related antioxidants that protect cells against endogenous reactive oxygen species (ROS). A small fraction of the more abundant Tsa1 protein and majority of Tsa2p localize to ribosomes. The deletion of *TSA1* and *TSA2* leads to a significant oxidative damage-induced aggregation of Sup35p which leads to the formation of the transmissible [*PSI*$^+$] prion. These data support a model where peroxiredoxins function to protect the ribosomal machinery against oxidative damage, but when these systems become overwhelmed, [*PSI*$^+$] prion formation provides a mechanism for uncovering genetic traits which aid survival during oxidative stress conditions.

NT12/02 Cytochrome P450 bifunctionality and spectral difference in streptomycetes

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Streptomyces coelicolor A3(2) is a Gram-positive soil bacterium that contains a two gene operon involved in the biosynthesis of the antibiotic, albaflavenone. The second gene of this operon encodes a bifunctional P450 enzyme (CYP170A1): one active site producing albaflavenone, the other producing farnesene (at least *in vitro*). Bioinformatics shows this two gene operon is conserved across at least eight species of streptomycetes. The translated CYP170A DNA sequences were used in protein modelling to allow comparison of probable structures and to assess relatedness. The least related enzyme (compared to *S.coelicolor* A3(2)) was found in *Streptomyces albus*. To further investigate the functionality of both active sites in *S. albus* P450, the gene was cloned for expression and further characterization. CYP170A1 is unique among expressed P450 proteins, as it has a reduced CO Soret peak at 440nm. The newly expressed CYP170 from *S. albus* has a peak at 450nm. Protein modelling suggests a proline residue may be responsible for the difference in haem orientation between the two proteins. It is hoped that this work will allow greater understanding of the factors involved in P450 protein folding, with particular regards to the haem binding pocket, and contribute to our understanding of bifunctional P450 enzymology.

NT12/03 Is the autochaperone domain of Pet the sole folding determinator?

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The Plasmid encoded toxin (Pet) is a cytotoxin secreted via the type V (a) autotransporter pathway. The full-length protein consists of a N-terminal signal sequence that directs the protein for transport into the periplasm, followed by the effector domain and a C-terminal β-domain that facilitates transport through the outer membrane. The protein must remain unfolded during periplasmic and outer-membrane transit and only folds once it emerges from the C-terminal barrel into the extracellular milieu. The C-terminal part of the effector polypeptide emerges first, it then folds and acts as a folding platform for the rest of the polypeptide as it appears from the

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barrel. We have identified the internal autochaperone domain (AC) of Pet as the folding platform and found that even single point mutations in the core of the AC domain abolished folding of Pet. Experiments investigating whether the AC domain acts alone as the folding factor or if interactions with the β-barrel are needed will be presented.

NT14 Microbiology in the indoor environment

↑CONTENTS

NT14/01 Fungal colonization and contamination of cinematographic film: implications for film and archivists

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Cine film is composed of 3 generic layers: a polymer based base support, a photosensitive emulsion coating and a binder based on gelatine. Studies were carried out on 19 film reels using air sampling, to quantify spores released from contaminated film during a simulated inspection process, in order to assess exposure of archivists to spores. Organisms present were identified and screened for gelatinase production, since gelatine is the major substrate for fungal growth on the film. The majority of fungi present were *Aspergillus* and *Penicillium* species,16 out of 30 isolates of which produced gelatinase. For some films, released spore numbers exceeded the recommended safe exposure levels of 1000cfu/m^3. Some films appeared contaminated, but no fungal growth was detected post-inspection. However, hyphal growth was evident across film frames, indicating that the damage may have taken place in the past. This study indicated a need for detection of fungal contamination of film, the presence of viable fungal spores, and safe handling recommendations for film archivists.

NT14/02 Microbial ecology of sinks and drains: potential reservoirs of hospital-acquired infections?

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The hospital environment, with large numbers of vulnerable patients and widespread use of antibiotics, promotes development of resistant pathogens and facilitates spread of resistant infections. A pilot study of the environment of a well run and frequently cleaned intensive care unit previously revealed the presence and persistence of diverse microbial communities, a well as the presence, in sinks, of opportunistic pathogenic species that were frequently associated with patient infections. This study aimed to further investigate the microbial ecology of sinks, drains and taps. The laboratory was used as a model environment to investigate sinks and drain biofilm samples, taps and tiled splashbacks surfaces by cultural methods and compared with PCR-DGGE (via 16s rRNA genes). PCR detection of a range of antibiotic resistance genes including bla_{TEM}, bla_{SHV}, and $bla_{CTX\text{-}M}$ OXA and Metallo-β-lactamases such as VIM, KPC and IMP was established in this environment. Methods to study the potential spread of organisms from the sink to the wider environment were also established. Establishment of these analytical and experimental methods will facilitate our understanding of the significance of the hospital sink and as a reservoir of hospital acquired infection.

NT16 Bacterial vesicles

↑CONTENTS

NT16/01 Investigating the immunomodulatory effects of *Helicobacter pylori* membrane vesicles

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Helicobacter pylori (Hp) infects the stomachs of around half the world's population, causing persistent gastric inflammation that can lead to ulceration and cancer. Hp is considered to be non-invasive: the majority of bacteria exist free-swimming in the gastric mucus or adhere to epithelial cells, rather than penetrating the mucosal barrier. The life-long persistence of Hp infection despite vigorous host responses may be mediated by stimulating a high-level suppressive regulatory T-cell (Treg) response[1,2] via release of immunomodulatory proteins. How these reach host immune cells through the impermeable gastric epithelial layer is not yet known.

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We found that Hp culture supernatant potently stimulates release of the suppressive cytokine interleukin-10 from human Tregs *in vitro*. MALDI and fractionation analysis of this preparation showed that it contains several known immunomodulators (including GGT, VacA and Hsp60). Ultracentrifugation analysis showed that the secreted proteins are mainly membrane vesicle (MV)-associated rather than in a free soluble form. MV can readily pass through the gastric epithelial cell layer to exert effects on underlying immune cells. We are currently investigating whether MV have more potent effects on Treg than free soluble proteins.

References 1. Robinson *et al* (2008) *Gut* 57: 1375; 2. Jang (2010) *Pathology Res Pract* 206: 34.

NT17 Microbial models of human disease

NT17/01 The African trypanosome: a model for investigating assembly of the eukaryotic flagellum/cilium

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Eukaryotic cilia and flagella are evolutionarily conserved organelles that perform a variety of biological functions ranging from motility to sensory perception. Cilia/flagella are vital to human health and defects in cilium/flagellum function underpin an array of inherited human disorders; including respiratory disease and retinal degeneration. Assembly of functional cilia/flagella requires the accurate targeting, processing and transport of proteins from the cytoplasm to the cilium/flagellum compartment via the basal body; a canonical microtubule organizing centre that orchestrates cilium/flagellum formation. As the major structural component of eukaryotic cilia/flagella is a microtubule-based axoneme, ciliated/flagellated cells are required to target significant quantities of the heterodimeric protein α/β-tubulin to the basal body. In eukaryotes formation of functional α/β-tubulin depends upon a conserved tubulin folding pathway involving 5 specific tubulin cofactors (TBC); TBCA-E. In addition to these canonical cofactors most ciliated/flagellated eukaryotes also encode a distinct flagellum-specific isoform of TBCC. Our studies on the flagellated protozoan parasite *T. brucei* (which is an excellent model for studying flagellum assembly) provide direct experimental evidence that this isoform is specifically targeted and tethered at the basal body where it carries out an essential and specific role in axoneme formation. We have designated this protein TbRP2. TbRP2 is a homologue of the human protein XRP2; mutations in which lead to certain forms of the hereditary eye disease X-linked retinitis pigmentosa. Using the trypanosome as a model we are investigating how TbRP2 is targeted to the basal body and its role in tubulin processing. These studies will provide further insight into the molecular basis for X-linked RP as well as promote a greater understanding of how proteins are specifically targeted to the cilium/flagellum compartment.

NT17/02 Functions of chymotrypsin-like protease (CTLP) in oral *Treponema*

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Treponema denticola and *Treponema vincentii* are oral spirochetes associated with human periodontal disease. Several virulence factors have been described for *T. denticola*, among them a surface localized prolyl-phenylalanine-specific chymotrypsin-like protease (CTLP) with a broad repertoire of cytopathic activities. The CTLP has been reported to degrade host proteins, hydrolyze bioactive peptides and help spirochetes penetrate epithelial cell monolayers. In this study we have first compared the CTLP gene locus of *T. denticola* with the corresponding locus in *T. vincentii*. Southern hybridization, PCR experiments, and recent availability of the genome sequence, have shown that *T. vincentii* contains an incomplete CTLP gene locus. To better understand the function of the CTLP complex we investigated the abilities of *T. denticola*, *T. vincentii*, and a CTLP^{-} mutant of *T. denticola* to interact with fibrinogen (a substrate for CTLP) or with *Porphyromonas gingivalis* (a co-populator of diseased tissues). Only the CTLP-expressing strain of *T. denticola* was found to degrade synthetic substrate SAPFNA, bind fibrinogen, and form co-aggregates with *P. gingivalis*. CTLP was also implicated in co-aggregation of *T. denticola* with a range of other oral pathogenic species. Blocking of CTLP activity with excess fibrinogen, or with protease inhibitor, did not affect co-aggregation of *T. denticola* with *P. gingivalis*, suggesting that enzymic activity was not required for the co-aggregation process. In conclusion, CTLP is necessary for *Treponema* adherence to fibrinogen and for co-aggregation of *Treponema* with other oral bacteria.

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NT17/03 *Not presented*

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NT17/04 Molecular epidemiology of human Parechovirus type 1 in clinical samples from children with gastroenteritis using RT-PCR

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Background and objective Human parechovirus type-1 (HPeV-1) is a genus of picornaviridea which form a diverse group of small, single stranded positive sense RNA viruses. In general it seems to be responsible for more gastrointestinal and respiratory syndromes and less central nervous system (CNS) symptoms. Since there is no accurate information about epidemiology of HPeV-1 in Iran. Therefore this project was designed to describe the frequency of human *Parechovirus* type-1 in stool samples from children with gastroenteritis using RT-PCR.

Methods RNA was isolated from 472 stool samples from children (under 4 years old) with diarrhea; cDNA was prepared and amplified using specific primers from 5 untranslated region (5 UTR). In order to confirm PCR products and avoid false positive, RT-nested-PCR was performed. Amplified DNA products were analysed on 1% agarose gel and a single band of 265 bp was obtained, which was confirmed by DNA sequencing. We also performed a comparison between the cell culture and RT-PCR for detecting HPeV1.

Results From 472 samples examined during last two years, 112 samples were HpeV-1 positive (23.7%). The results showed that the prevalence of this virus in children under one year old with diarrhea (P=0.036), was in spring and autumn (P<0.001). Boys had more positive cases than the girls (P<0.001). From 20 samples which were found positive by RT-PCR only three of them showed the same result by cell culture method after a week.

Conclusion The results revealed that RT-PCR is a more practical and sensitive technique for HPeV-1 detection directly from clinical samples, which is valuable for epidemiology. Also, the rapid detection of HPeV1 by RT-PCR can decrease both the unnecessary use of antibiotics and the costs in clinical practice.

Keywords Gastroenteritis, Epidemiology, Parechvirus, RT-PCR, 5' UTR

CMM Clinical & medical microbiology

CMM/01 Towards a fusobacterial multilocus sequence typing system

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Fusobacterium nucleatum is a common inhabitant of the oral cavity playing a key bridging role between coaggregating partner bacteria in the formation of dental plaque. The species is often associated with periodontal disease and acute oral infections such as dentoalveolar abscesses. *F. nucleatum* is divided into five subspecies, but discrimination between these subspecies and the closely related *F. periodonticum* using 16S rRNA-based methodologies is hampered due to heterogeneity within sequences of different gene copies within strains. The aim of this study was to investigate the potential for developing a multilocus sequence typing system for fusobacteria by

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PCR amplifying and sequencing different housekeeping genes from a collection of 40 clinical isolates identified as either *F. nucleatum* or *F. periodonticum* from appropriate samples from patients presenting with periodontal disease, dentoalveolar abscesses, pericoronitis and otitis media. PCR primers were designed from sequences within genomes of three *F. nucleatum* subspecies. Of those tested the most successful for PCR amplification from all strains were adenylate kinase, enolase, triosephosphate isomerize and *recA* genes. Sequence analysis and dendogram construction shows evidence that isolates from patients with periocoronitis are *F. periodonticum*, and those from patients with otitis media are commonly *F. nucleatum* subspecies *polymorphum*. In conclusion, fusobacterial typing through comparison of housekeeping gene sequences shows considerable promise.

CMM/02 Modulating release of antibiotics from bone cement

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Background Antibiotic – loaded bone cement 'spacers' are used as an adjunct to treatment in 2-stage arthroplasty revisions. If release of the correct choice of antimicrobials is optimized, systemic therapy might be curtailed and emergence of resistance minimized.

Aims To determine the elution period of antimicrobials from bone cement with and without a copolymer, polyvinylpyrrolidone (PVP).

Methods Triclosan, gentamicin and clindamycin with and without (PVP) in CMW bone cement, was tested against six bacteria using serial plate transfer.

Results While there was little difference between clindamycin and clindamycin with PVP, and between gentamicin and gentamicin with PVP, there was marked enhancement of release of triclosan with PVP. Resistance developed when antimicrobials were used singly but not when used in combination.

Conclusion The addition of water soluble PVP was expected to enhance elution of antimicrobials from bone cement. This occurred with triclosan, a poorly water-soluble agent, but there was no significant difference for gentamicin and clindamycin, which are preferentially water-soluble. Other copolymers are being explored in an attempt to enhance their release.

CMM/03 Co-operation and conflict in *Staphylococcus aureus* populations and the implications for virulence

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Staphylococcus aureus causes significant morbidity in hospital environments. It coordinates production of many virulence determinants using a cell-to-cell signalling mechanism termed quorum sensing (QS), which involves the production and sensing of autoinducing peptide (AIP) signal molecules by the agr locus. This type of cooperation is costly to perform for individual cells and social evolution theory predicts that such behaviours are vulnerable to invasion by non-cooperating 'cheats'.

We experimentally tested the theory by introducing mixed populations of RN6390B wildtype: agr mutant (QS cheat) *S. aureus* into a waxworm (*Galleria mellonella*) virulence model. Results indicate that the agr mutants invaded the cooperating WT populations during infections and that cheat invasion also leads to a reduction in infection severity. We have demonstrated for the first time that QS in Gram-positives is a social trait and that *S.aureus* cooperating populations can be invaded by cheats in an animal model resulting in the reduction in the overall virulence of an infection. The work suggests that asocial cheats could be developed to treat infections, which has major implications for the treatment of antibiotic resistant organisms such as *S. aureus.*

CMM/04 The role of glyceraldehyde 3-phosphate dehydrogenase (GapA-1) in *Neisseria meningitidis* adherence to human cells

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N. meningitidis is an obligate human commensal which causes life-threatening infections such as septicaemia and meningitis. Glyceraldehyde 3-phosphate dehydrogenases (GAPDHs) are cytoplasmic glycolytic enzymes, which although lacking identifiable secretion signals, have also been found localized to the surface of several bacteria (and some eukaryotic organisms), where they contribute to the colonization and invasion of host tissues. The aim of this study was to characterize the putative role of GapA1 in the pathogenesis of meningococcal disease. GapA1 null-mutants were generated in strain MC58 and *SiaD* background. Sub-cellular

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fractionation and flow cytometry were used to investigate outer-membrane localization and surface exposure of GapA1. The wild-type, mutant and complemented strains were compared in association using human brain microvascular endothelial (HBME) or larynx carcinoma (HEp-2) cells. Cell fractionation experiments reaffirmed that GapA1 is indeed localized both to the cytoplasm and to the outer membrane. Flow cytometry data demonstrated that GapA-1 could be detected on the cell surface, but only in a *siaD*-knockout (capsule-deficient) background. Loss of GapA1 did not affect the growth of the bacterium *in vitro*, however, a GapA1-deficient mutant showed a significant reduction in adhesion to HEp-2 and HBME cells compared to the wild-type and complemented mutant. In summary, GapA-1 is localized to the cell surface and enhances *in vitro* association of meningococci with human cells.

CMM/05 The moonlighting protein fructose bisphosphate aldolase of *Neisseria meningitidis*: surface localization and role in cell adhesion

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Neisseria meningitidis is an obligate human nasopharyngeal commensal, which has the capacity to cause life-threatening meningitis and septicemia. Fructose-1, 6-bisphosphate aldolases (FBA) are cytoplasmic glycolytic enzymes, which despite lacking identifiable secretion signals, have also been found localized to the surface of several bacteria where they bind host molecules and exhibit non-glycolytic functions. The aim of this study was to determine whether FBA is surface exposed in meningococci and can influence the interaction between meningococci and host cells. *cbbA* knockout and complemented derivatives of *N. meningitidis* MC58 were generated. Sub-cellular fractionation and flow cytometry were used to investigate outer-membrane localization and surface exposure of FBA. The wild-type, mutant and complemented strains were compared in association assays using human brain microvascular endothelial (HBME) or larynx carcinoma (HEp-2) cells. Despite lacking a recognizable export signal, cell fractionation experiments showed that meningococcal FBA is localized both to the cytoplasm and the outer membrane. Flow cytometry further demonstrated that outer-membrane-localized FBA was accessible to FBA-specific antibodies. An FBA-deficient mutant was not affected in its ability to grow *in vitro*, but showed a significant reduction in adhesion to HBME and HEp-2 cells compared to its isogenic parent and complemented derivative. In summary, FBA is a surface-exposed protein that is required for optimal adhesion of meningococci to host cells.

CMM/06 High intensity 405 nm light inactivation of MSSA and MRSA strains of *Staphylococcus aureus*

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Photodynamic-inactivation of microorganisms is a research area gaining interest due to the emergence of resistance to control methods such as antibiotics and disinfectants. The present study demonstrates the visible-light wavelength sensitivity of meticillin-sensitive and meticillin-resistant *Staphylococcus aureus* (MSSA & MRSA) to 400–450 nm light, and subsequently, bacterial inactivation using 405 nm High Intensity Narrow Spectrum (HINS) light. To determine the wavelength sensitivity of the test bacteria, this study utilized filtered light from a continuous xenon white-light source combined with a range of narrow-band optical filters (10 nm FWHM) to expose bacterial suspensions. Results demonstrated that the bacteria showed sensitivity to wavelengths of light within the visible region, with the peak wavelength for inactivation being 405±5 nm. Exposure to wavelengths longer than 430nm did not cause significant bacterial inactivation. Following identification of 405±5nm as the most bactericidal wavelength band, the study then investigated the use of high-intensity 405-nm light, generated from light-emitting diodes (LEDs). Results demonstrate that when the inactivation kinetics of MSSA and MRSA were compared using the 405nm HINS-light LED array and using the 405nm filtered light, similar results were found for each strain of bacteria, indicating that the applied dose of 405nm light was the important factor, not the light source.

CMM/07 The effects of *Staphylococcus aureus* on osteogenesis: mechanisms underlying osteomyelitis

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Osteomyelitis (OM) is a progressive inflammatory bone infection caused predominately by *Staphylococcus aureus*. OM patients have a higher tendency to experience pathological fractures as a result of weakened weight bearing bones from the infection. This study investigates the effect *S. aureus* has on proliferation, maturation and osteogenesis. Osteoblast proliferation demonstrated that *S. aureus* potently inhibited osteoblast growth ($P<0.0001$) over a 21 day period. Additionally, uninfected osteoblasts possessed high levels of alkaline phosphatase (AP) (a well established biochemical indicator of bone formation) after 21 days whereas, *S. aureus* infected

osteoblasts inhibited AP production (P<0.01) during the same time period. Uninfected osteoblasts mineralized normally over 21 days, however, *S. aureus* infected osteoblasts displayed no evident phosphate mineralization. Furthermore, uninfected osteoblasts also displayed signs of mineralization by calcification over a 21 day period but in the presence of *S. aureus*, osteoblasts lacked the ability to calcify. Biochemical quantification of calcification verified the inability of *S. aureus* infected osteoblasts to undergo calcification (P<0.0001). In addition, osteogenic gene markers – collagen type-I, osteopontin and osteocalcin were all inhibited (P<0.01–0.001) in the infected cells during the 21 days. In conclusion, *S. aureus* inhibits osteogenesis, consequently leading to weakened bones and pathological fractures in osteomyelitis patients.

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CMM/08 *Not presented*

CMM/09 Comparative study for virulence of *Mycobacterium avium* isolates from patients with nodular-bronchiectasis-type and cavitary-type diseases

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Mycobacterium avium (Mav) lung infections called nodular-bronchiectasis (NB)-type *M. avium* complex (MAC) disease are globally increasing. To elucidate whether there are unusual populations of Mav, causing NB-type disease rather than cavitary (CA)-type disease, we compared the virulence of Mav isolates from patients with NB-type (NB-Mav) and those from CA-type diseases (CA-Mav) based on intracellular growth in various types of human cells. Five each strains of NB-Mav and CA-Mav were compared with each other for their invasiveness and ability to intracellularly replicate in various types of cultured cells of human origin. The two types of Mav isolates showed a similar ability in average to replicate in macrophages and lung epithelial cells. Moreover, they showed a similar ability to induce the production of reactive nitrogen intermediates and reactive oxygen intermediates by macrophages and susceptibility to antimicrobial molecules. Therefore, it appears that there is no essential difference in virulence in terms of infectivity to human macrophages and lung cells between Mav strains isolated from NB-MAC disease and those from CA-MAC disease. These findings indicate the importance of further studies to elucidate mechanism for the establishment of NB-type MAC diseases based on host immunological conditions rather than pathogenic nature of MAC organisms themselves.

CMM/10 Properties of immunosuppressive macrophages generated by *Mycobacterium avium* complex infection induced in *bcg*^{s} and *bcg*^{r} genotype mice

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Immunosuppressive macrophages (Mϕs) induced in the spleen of *Mycobacterium avium* complex (MAC)-infected mice (MAC-Mϕs) exhibit suppressor activity against T cell mitogenesis. In this study, we examined profiles of MAC-induced generation of immunosuppressive Mϕs in MAC-susceptible BALB/c (*bcg*^{s}) or -resistant CBA/JN (*bcg*^{r}) mice and found the following. First, MAC

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infection induced more potent immunosuppressive Mϕs in BALB/c mice, compared to CBA/JN mice. Second, the MAC-Mϕs induced in BALB/c mice exhibited much greater ability in generating reactive oxygen intermediates than those induced in CBA/JN mice, indicating that the former was more activated than the latter. Third, MAC-Mϕs induced in BALB/c mice were found to consist of two distinct subpopulations on the basis of their adherence to plastic wells. The strongly adhesive Mϕ population (SA-Mϕs) exhibited marked suppressor activity. Meanwhile, the weakly adhesive Mϕ population (WA-Mϕs) exhibited modest suppressor activity. Fourth, both SA-Mϕs and WA-Mϕs possessed a $CD11b^{+}$ $F4/80^{+}$ $SR\text{-}AI^{+}$ $CD14^{+}$ $CD206^{+}$ phenotype characteristic of matured Mϕs, and suppressed IL-2 receptor expression by concanavalin A-stimulated T cells. These findings indicate that the *bcg* gene may be related to the generation of MAC-Mϕs in host mice. In addition, MAC infection in BALB/c mice generates two types of immunosuppressive Mϕs with different levels of suppressor activity.

CMM/11 ***Staphylococcus aureus*** **nitroreductase protects against nitrosative stress**

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Staphylococcus aureus is a Gram-positive opportunist bacterium that lives on the skin and mucous membranes of warm-blooded animals. However, in immunocompromised individuals *S. aureus* can cause serious infections. The dramatic increase in the number of infections caused by antibiotic-resistant *S. aureus* represents a major public health threat that requires a quick response.

Nitrofurans are efficient antimicrobials agents against a large range of pathogens including *S. aureus*. For the action of these antibiotics it is essential the function of nitroreductases, that activate nitrofurans though a reduction process. In this work, the role of a putative nitroreductase of *S. aureus* encoded by the gene SA0UHSC_00833 (*ntrA*) was studied. A strain lacking the gene was constructed and the expression and purification of the protein was performed. The *S. aureus ntrA* mutant shows an increase in resistance to nitrofurazone and nitrofurantoin and enhanced sensitivity to S-nitrosoglutathione (GSNO). The increased resistance of *ntrA* mutant to nitrofurans correlates with the lower nitroreductase activity determined in the cell extracts of the mutant strain. The biochemical characterization of the recombinant NtrA reveals that this enzyme possesses a dual activity since it is able to reduce nitrofurantoin and nitrofurazone (K_m of 18.8 and 25.7 μM, respectively) as well as GSNO (K_m=181 μM). A phylogenetic analysis suggests that NtrA is a member of a novel family of the nitroreductase enzymes. In summary, we show that *S. aureus* NtrA is a nitroreductase that contributes to the activation of nitrofurans. Moreover, NtrA is found to be a bifunctional enzyme since it also metabolizes GSNO. Hence, in the absence of nitrofurans NtrA will defend the bacterium by decomposing the endogenously GSNO formed when *S. aureus* is exposed to nitrosative stress generated by the immune system.

Reference Tavares, A.F. *et al.* (2009) *J Bacteriol* 191:3403–3406.

CMM/12 *Not presented*

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CMM/13 *Not presented*

CMM/14 *Not presented*

CMM/15 *Not presented*

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ENV Environment

↑CONTENTS

ENV/01 Biological verification of novel technologies for chemical remediation of contaminants in the protection of groundwater

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The protection of groundwater from soluble pollutants is a global priority. To prevent pollutant leaching from soils, there is a need to develop and apply techniques that are able to complex metals and render them less soluble. In this study the performance of kaolinite, biochar, lime and calcium polysulfide (CaS_x) were compared. In addition to detailed chemical analysis, biosensors were used to confirm the efficacy of the applied technology.

Microbial biosensors are rapid and responsive tools that enable the relative pollutant dose to be placed in a hazard/ biological context. This enables a significant step towards quantitative risk assessment and appropriate sustainable solutions.

At low doses, all techniques were adequate but at elevated doses CaS_x outperformed the others. Although CaS_x rendered the least metal to be mobile, there was a poorer (than predicted) response from the microbial biosensor demonstrating that although the metal was effectively mitigated there were indirect impacts on the water quality.

While the initial work was conducted with laboratory amended soils, the results were found to translate directly to historically impacted soils. In this study, biosensors were an ideal complementary tool to confirm the most suitable dose of ameliorant toad to soil for the protection of groundwater.

ENV/02 *Not presented*

ENV/03 The impact of silver nanoparticles on aquatic microbial communities involved in hydrocarbon degradation

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Increasing production of engineered nanoparticles (e.g. silver) has raised concerns about their potential environmental risks. Silver nanoparticles (AgNP) are known antimicrobial agents, and in aquatic environments they may disrupt key microbial processes such as hydrocarbon degradation. This study examined the effect of AgNP on hydrocarbon degradation by microbial pure cultures and sediment communities. Characterization of capped and uncapped AgNP (by DLS, AFM and FFF) showed the capped AGNP were smaller in diameter than the uncapped (35 nm ± 0.55, and 165.7 nm ± 15.59 respectively), and were more resistant to

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aggregation in media. Cell growth and viability of pure cultures incubated with different AgNP concentrations (0.5–70 mg L^{-1}) were measured by CFU counts and fluorescent staining, and showed a 10 fold decrease in cell growth in the presence of AgNP (from $6x10^{11}$ to $3x10^{10}$ cells ml^{-1}, P<0.01). GC-MS analysis revealed that hydrocarbon degradation by sediment microorganisms was also reduced by different AgNP concentrations. PCR-DGGE analysis of a 16S rRNA gene fragment was performed and identified shifts in the microbial community structure during hydrocarbon degradation. These results are important as they suggest that AgNP may have a detrimental effect on environmental microorganisms and their processes, following either accidental or intentional release.

ENV/04 Analysis of the RDX-degrading gene cluster in *Rhodococcus rhodochrous* 11Y

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Hexa-hydro-1,3,5-trinitro-1,3,5-triazine (Royal Demolition Explosive – RDX) is a widely used explosive and a serious environmental pollutant. We have isolated the genes *xplA* and *xplB* from *Rhodococcus rhodochrous* 11Y, which encode an RDX degrading cytochrome P450 system. This unusual P450 system has now been identified in RDX degrading bacteria from geographically distinct locations including the United Kingdom, Australia, Israel and North America and interestingly the genes show near sequence identity suggesting that *xplA/B* may have been rapidly distributed across the globe by horizontal gene transfer. We have found that the XplA/B P450 system is encoded on an operon, which also contains a putative permease transporter and transcriptional regulator. To investigate whether the permease and regulator play a role in RDX metabolism, an unmarked gene deletion system (pK18mobsacB) was used to delete both the permease and regulator genes from *R. rhodochrous* 11Y. Analysis using whole cell assays showed that there was no significant difference in the rate of RDX removal between the wild type and permease deleted strain; however, real time PCR revealed that expression of *xplA* was 1.7-fold higher in the putative regulator deleted strain compared to the wild type when grown on RDX as the sole nitrogen source. This putative regulator was found to display close homology to the regulator protein MarR, a multiple antibiotic resistance repressor. This suggests that the putative regulator might be acting as a repressor of *xplA* expression.

ENV/05 *Not presented*

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FB Fermentation & bioprocessing (industry)

FB/01 Optimizing fermentation under stress conditions of high specific gravity

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Saccharomyces cerevisiae has been employed in brewing, whisky and bioethanol fermentations because this species has superior fermentation characteristics. Strains are selected on the basis of their efficiency in the conversion of fermentable sugars to ethanol and on their capacity to tolerate fermentation stresses. Each of these industries have adopted high gravity (high sugar) fermentation practices to maximize product formation from fixed fermenter capacities and minimize the use of energy and process water inputs. This approach increases ethanol concentrations at the end of fermentation eliciting potential stress conditions for the fermenting strain. The impact of high ethanol concentrations on cell integrity and the capacity to ferment have not been fully elucidated. To address this omission the relative tolerance of several laboratory, distilling and bioethanol *S. cerevisiae* strains to ethanol in anaerobic conditions normally associated with mid to late fermentation was assessed. The assessment criteria were: the capacity of cells to recover and grow on YPD ethanol spot plates; the capacity to maintain cell viability in ethanol dosed suspensions; and the impact of ethanol exposure on genome stability. All three criteria are key attributes for fermentation optimization. Not surprisingly bioethanol strains were the most tolerant and could be ranked with respect to their capacity to withstand up to 25% (v/v) ethanol. Distilling strains could also be ranked with respect to tolerance and laboratory strains were the most sensitive to this stress. For the strains assessed, a negative correlation between concentration of ethanol to which the strains were exposed and genome damage leading to the formation of petites was observed. A hypothesis to explain this observation will be presented.

FB/02 Construction and characterization of *Clostridium sporogenes fldZ* enoate reductase knock out mutant

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Enoate reductases are members of 'Old Yellow Enzyme' family and are found in bacteria, lower fungi and plants. Enoate reductases are used in industrial biocatalysis for stereospecific reduction of enoates, enals, enones and unsaturated nitro compounds. *Clostridium sporogenes* was previously shown to catalyze hydrogenation of C=C double bond of *(E)*-1-nitro-2-phenylpropene. The reaction was proposed to be catalysed by enoate reductase, however previous attempts to purify this enzyme were unsuccessful.

As an alternative strategy to identify the enzyme, *C. sporogenes* enoate reductase knock-out mutants were prepared. The ClosTron gene knock-out system was designed to inactivate enoate reductase gene, based on the genomic sequence of enoate reductase *fldZ* gene in the closely related *C. botulinum* A str. Hall. Mutated *C. sporogenes* showed decreased biomass production in media containing glucose, phenylalanine or tryptophan. The mutants were not able to reduce *(E)*-1-nitro-2-phenylpropene, which confirmed the hypothesis that enoate reductase was responsible for reduction of this substrate.

Primers were designed to the *C. botulinum fldZ* gene, and genomic DNA was extracted from *C. sporogenes* and used as a template for PCR. The amplified product showed the presence of an open reading frame for 665 amino acids with high homology to *C. botulinum* fldZ enoate reductase.

FB/03 A novel yeast isolated from Taiwan having potential use in biodiesel production

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The climate change caused by greenhouse gases and the projected energy shortage in the next few decades make the finding of alternative energy sources a matter of urgency. Of all renewable energy sources, biodiesel produced from micro-organisms is considered as a great potential candidate. In this study a microbial isolate, TN-1, rich with oil accounting for about 50% of dry cell weight was obtained from Tainan coast in Taiwan. Examined with 18S rRNA, D1/D2 region of 26S rRNA, and internal transcribed spacer of rRNA

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genes (ITS), the isolate TN-1 is identified as a novel yeast. The isolate TN-1 also demonstrated different patterns of nutrient utilization by analysing physiological and biochemical characterizations, comparing with known yeasts. From the results, TN-1 grew better and generated oil in medium containing 1% seawater at 20°C and pH=8. While TN-1 uses sucrose as a carbon source, about 70% of dry cell weight is found and its major fatty acids contain 17.34% of C14:0, 20.40% of C16:0, 4.69% of C18:0, 40.47% of C18:1, 11.47% of C18:2, 2.41% of C18:3, and 0.97% of C20:4. In term of renewable energy the novel yeast TN-1 has a great potential application in the biodiesel industry.

SC Systems & cells

↑CONTENTS

SC/01 *Not presented*

SC/02 Structure–activity relationship of the *Pseudomonas quinolone* signal

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Pseudomonas aeruginosa is a Gram-negative opportunistic human pathogen capable of producing a welter of highly-active secreted virulence factors. The production and secretion of these virulence factors is centrally controlled by the quorum sensing (QS) system of *P. aeruginosa*. The *Pseudomonas* quinolone signal (PQS) is a key component of this cell-cell communication mechanism, and has been shown to coordinate a wide range of complex cellular activities from virulence to biofilm formation and secondary metabolite synthesis. The PQS molecule itself is a member of the alkyl quinolone family (specifically, 2-heptyl-3-hydroxy-4-quinolone). Consequently, PQS and its natural derivatives are exceptionally hydrophobic and are packaged for transfer between cells into membrane vesicles (MVs), colloquially known as 'communication balls'. Remarkably, PQS is also known to *stimulate* MV production / fusion. To investigate the structural basis for this and for the other activities of PQS, we have recently synthesized a diverse variety of PQS analogues and tested these for their ability to influence a range of virulence-related phenotypes *in vivo*. The specific structural features of these analogues that facilitate MV formation (as well as their interaction with specific protein receptors) will be discussed. Our data suggest that the architecture of the PQS molecule is constrained by multiple factors, one of which is its ability to interact with the LPS layer of the cell envelope.

GM General microbiology

↑CONTENTS

GM/01 Population shifts and polymicrobial culture of oral TM7 bacteria

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Molecular analyses of the oral microflora have facilitated identification and detection of bacteria which have not yet been cultured in the laboratory. Amongst these are TM7 bacteria some of which are associated with progression from health to periodontal disease. In this

study we aimed to investigate population shifts of TM7 bacteria in dental plaque of patients undergoing orthodontic treatment, and to attempt *in vitro* culture. Proportions of TM7 bacteria in gingival margin plaque samples from 20 consenting patients were shown by real-time PCR to increase during treatment for bond methods of orthodontic appliance attachment. Investigation of plaque by Denaturing Gradient Gel Electrophoresis, and cloning, sequencing and subsequent phylogenetic analyses, indicated oral carriage of up to seven TM7 phylotypes within an individual with diversity changing during orthodontic treatment. Some TM7 phylotypes were able to grow anaerobically in mixed culture with other plaque bacteria. Fluorescence *In Situ* Hybridization and live/dead experiments confirmed the presence of large, viable TM7 cells in mixed culture, and suggested intimate interactions between TM7 and other strains. In conclusion, during orthodontic treatment, TM7 bacteria increase in proportion and diversity in dental plaque. Some TM7 phylotypes could be cultured *in vitro* in the presence of other oral bacterial species.

GM/02 Characterization of the *Campylobacter jejuni* metal-responsive peroxide-stress regulator PerR

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Campylobacter jejuni is the most common cause of food-borne bacterial gastroenteritis with an estimated 400,000 cases per year in the UK. *C. jejuni* is a microaerophilic organism requiring lower oxygen and increased carbon dioxide concentrations for growth. But it is also able to survive the oxygen-exposed transmission from its avian host into the human host, where it is exposed to reactive oxygen species produced by the human immune system. One of the regulatory proteins involved in the protective response of *C. jejuni* to oxidative stress is the metal-responsive regulatory protein PerR, which controls transcription of peroxidases in *C. jejuni*. While targets of PerR are known, the mechanism by which it senses oxidative stress are not known. To investigate this, we have expressed an un-tagged version of *C. jejuni* PerR in *E. coli*, and have purified this 16 kDa protein to 80% purity using size exclusion chromatography and a heparin column. *E. coli* proteins present in the sample do not have DNA binding domains, and hence are unlikely to interfere. The purified PerR will allow for future experiments including further biochemical and genetic characterization, including metal cofactor determination, crystallization assays and *in vitro* protein-DNA interactions.

GM/03 *Not presented*

GM/04 TspA interacting proteins of *Neisseria meningitidis*

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Neisseria meningitidis is a human commensal that resides in the nasopharynx. It occasionally gains access to the blood causing bacteraemia and meningitis. T-cell stimulating protein (TspA) is a conserved, immunogenic protein that stimulates T-cells and B-cells. It is required for optimal adhesion of meningococci to host cells. TspA has a periplasmic N-terminus and an inner membrane spanning domain that links it with a cytoplasmic domain. Hence, TspA was thought to play an indirect role in the adhesion process by interacting with protein(s) in the meningococcal cell envelope.

To identify proteins TspA-interacting proteins, overlapping recombinant fragments were expressed and purified as fusion proteins with glutathione-S-transferase protein. A receptor activity-directed affinity tagging protocol was employed to identify TspA-interacting meningococcal proteins. ELISA and SPR were also used to investigate interactions between TspA and candidate proteins.

Four putative TspA-interacting proteins were identified: PilQ and PilT (components of the type IV pilus machinery); the major outer-

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membrane protein, PorA, and the protein chaperone, ClpB. Moreover, the N-terminus of TspA was identified as the interacting domain with PorA. These results show that periplasmic domains of TspA interact with several outer-membrane proteins which have a key role in meningococcal pathogenesis and adhesion to host cells.

GM/05 The interplay between quorum sensing and metabolism in *Pseudomonas aeruginosa*

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The medically significant human pathogen *Pseudomonas aeruginosa* is responsible for causing a broad-spectrum of mild to potentially life threatening conditions. The ability of *P. aeruginosa* to cause disease is controlled by a cell density-dependent regulatory network termed quorum sensing (QS). *In P. aeruginosa*, QS achieves co-ordinated cell-cell communication through the production of *N*-acyl-L-homoserine lactones (AHLs) and the *Pseudomonas* quinolone signal (PQS). Production of these quorum sensing signal molecules requires precursors including fatty acids, *S*-adenosylmethionine (SAM) and aromatic amino acids. SAM is derived from the activated methyl cycle (AMC) that functions as an important pathway dedicated to the degradation of the toxic metabolite *S*-adenosylhomocysteine (SAH). Through removing genes encoding AHL synthases RhlI and LasI from the complex hierarchical system of *P. aeruginosa* by expressing them in the heterologous host, *Escherichia coli*, this study has measured the influence of AHL production upon bacterial metabolism. AHL profiles were broader than previously reported, correlated with a reduction in the intracellular concentrations of several metabolites, and were more pronounced in the *E. coli* strain producing the LasI synthase than the RhlI enzyme. Production of foreign QS signal molecule (QSSM) synthases had a knock-on effect on the native *E. coli* QSSM, autoinducer-2 (AI-2). AI-2 production was significantly reduced, probably since it requires AMC metabolites. The influence that these metabolic perturbations had on cell fitness was manifest through reduced growth in minimal media. Complementation of growth by exogenously added metabolites confirmed our hypothesis that QSSM synthesis creates a drain on metabolite levels with consequences for cell fitness. Site-directed mutagenesis of key catalytic residues in the QSSM synthases is underway to directly prove that the effects we are seeing are due to the function of the synthases, and not the production of a heterologous protein. Moreover, complete profiling of *P. aeruginosa* PA01 AHL synthase mutants is unravelling the interrelationship between metabolism and cell-to-cell communication in *P. aeruginosa*.

GM/06 Distribution of *Acanthamoeba* spp. in water treatment plants in England

VYDEKI SHANMUGANATHAN[1,2] & Naveed Ahmed Khan[1]

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Background and objectives of investigation *Acanthamoeba* spp. is a protozoan pathogen that can cause painful, sight-threatening keratitis. Up to 93% of infected patients are users of contact lenses. Several lines of evidence reported tap water as a major risk factor in contracting *Acanthamoeba* keratitis. The objective of this study was to determine the presence of *Acanthamoeba* spp. at different stages of water treatment at two water treatment plants in England.

Methods Water samples from each stage of water treatment process were analysed by membrane filtration and were plated on to bacterial-seeded agar plates. The presence of A*canthamoeba* spp. was determined using morphological characteristics and molecularly by employing polymerase chain reaction using genus-specific DNA probes.

Results Acanthamoeba spp. were detected in 100% of raw surface water samples analysed. Amoebae were not detected post-filtration or in the final treated water. There was no seasonal distribution in the occurrence of *Acanthamoeba* spp. in water treatment plants.

Conclusion The water treatment processes used in England typical of those from this study are effective at removing *Acanthamoeba* spp. The *Acanthamoeba* spp. detected in tap water is likely to be from older plumbing systems or storage tanks within the domestic property.

GM/07 Heat-shock protein 70-peptide complexes as a novel vaccine candidate against mycobacterial infection

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Mycobacterium tuberculosis is the main cause of tuberculosis (TB) in humans. According to the World Health Organization tuberculosis causes 2 million deaths per year [1]. The Bacille Calmette Guerin (BCG) vaccination has varying efficacy and the recent resurgence of

TB highlights the need for more efficient vaccine agents. Evidence has shown that heat shock proteins (Hsp) are highly immunogenic and some are involved in chaperoning peptides for antigen presentation and the generation of potent T cell responses [2]. With a view to developing a new TB vaccine, this study examines the ability of mycobacterial Hsp70 to deliver bacterial peptides to the murine immune system. Using a prime-boost vaccination strategy in C57B/6 mice we show that mycobacterial Hsp70-peptide complexes induce a Th1-type immune response in T-helper cells and that this response is directed against mycobacterial antigens other than the Hsp70 chaperone.

References [1] Global Tuberculosis Control, report by the World Health Organization (2009). [2] J. Babak, P. MacAry & P. Lehner, Structure and function: heat shock protein and adaptive immunity, *The Journal of Immunology* 179 (2007), 2035–2040.

GM/08 Identification and functional characterization of a *Staphylococcus aureus* TIR-domain-containing protein

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Toll-like receptors (TLRs) are type I transmembrane pattern recognition receptors that possess varying numbers of extracellular N-terminal leucine-rich repeat motifs, followed by a cysteine-rich region, a transmembrane domain, and an intracellular Toll/IL-1 R (TIR) domain, which is responsible for initiating signaling and adaptor molecule interaction. TLRs are vital components of the innate immune system which recognize foreign Pathogen Associated Molecular Patterns and initiate downstream signaling cascades. This results in activation of transcription factors which produce inflammatory cytokines, chemokines and adhesion molecules. Recently a diverse range of bacteria, including *Escherichia coli*, *Brucella melitiensis* and *Salmonella* spp., have been found to express TIR-domain containing proteins or Tcps. Some of these bacterial Tcps have been shown to have a subversive role, inhibiting TLR signaling and thereby dampening the immune response. Here, we describe the identification of a Tcp in strains of *Staphylococcus aureus*, isolated from clinical and sub-clinical cases of bovine mastitis, including a meticillin-resistant *S. aureus* strain (MRSA) found in humans, pigs and cattle. Furthermore, we provide evidence for the interaction of this Tcp with TLR2. We are investigating this Tcp as a potential virulence factor of *S. aureus* strains that cause bovine mastitis.

GM/09 The role of TLR2 and dectin-1 in phagocytic and inflammatory response to antigen expressed on yeast surface

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The pattern recognition receptors toll like receptor 2 (TLR2) and dectin-1 are part of the innate immune system and are well documented in the recognition of yeast antigens. In the current project, we express a viral envelope protein on the surface of the yeast *Saccharomyces cerevisiae* (*S. cerevisiae*) and analyse the innate immune response induced by binding of recombinant *S. cerevisiae* to TLR2/dectin-1 to determine if expression in yeast enhances the immunogenicity of the viral protein. In addition, bovine TLR2 and dectin-1 have been cloned and expressed as dye-tagged molecules and are used for receptor-ligand studies by confocal microscopy. The recombinant *S. cerevisiae* stimulated production of the chemokine CXCL-8 in primary bovine macrophages possibly through activation of NF-kB, however, failed to stimulate production of reactive oxygen species in the same cells. Additionally, bovine macrophages primed with *S. cerevisiae* expressing viral envelope proteins had a greater capacity for stimulating proliferation of naïve CD4+ T-cells compared to macrophages primed with envelope protein alone or *S. cerevisiae* without envelope protein expression. Heat inactivation of recombinant *S. cerevisiae* increased ROS production and capacity to stimulate CD4+ T-cells in macrophages but did not alter CXCL-8 release compared to the live form. On-going work now aims to elucidate the relative roles played by TLR2 and dectin-1 in the immune response to recombinant *S. cerevisiae*.

GM/10 Real-time PCR detection of Fe-type nitrile hydratase genes from environmental isolates suggests horizontal gene transfer between multiple genera

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Nitriles are organo-cyanides that are present in the environment due to biological and industrial activity; they are produced by plants and present in industrial effluents. Despite their toxicity, many microbes can metabolize nitriles as an energy source via nitrilase or

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nitrile hydratase/amidase enzymes. Microbial nitrile hydrolysis has significant industrial potential for bioremediations – the removal of nitrile contamination from the environment, and more importantly biotransformations/green chemistry – the production of enantiopure pharmaceutical intermediates and products. The objective of this study was to develop a real-time PCR detection method for the screening of environmental isolates for Fe-type nitrile hydratase (NHase) genes. Seaweed and soil samples from Ireland, Poland and Australia were sampled and enriched with various nitriles, leading to the isolation of 256 nitrile-degrading bacterial isolates. These isolates were screened for Fe-type NHases resulting in positive detection from 46, which were identified by 16S rRNA gene sequencing. The α and β subunit genes were amplified by conventional PCR and sequenced from the isolates also. Identical and near-identical NHase genes have been identified from bacteria of numerous genera, such as *Microbacterium*, *Rhodococcus*, *Bacillus*, *Curtobacterium*, *Arthrobacter*, *Klebsiella*, *Streptomyces*, *Rahnella*, *Burkholderia*, *Buttiauxella* and *Paenibacillus*, and from very different environmental isolation sites, such forest soil sampled in Poland and seaweed sampled in Ireland. A novel NHase that shows only 88% DNA sequence homology to other NHase genes in the database has been identified in isolates of four different genera isolated from Irish seaweeds and Australian soils.

The geographical distribution and conservation of the NHase genes across the genera provides strong evidence of horizontal gene transfer.

GM/11 Developing a multiple displacement amplification technique with digital PCR applicable to biological processes

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More than 99% of micro-organisms found in environments are uncultivable in the laboratory and this makes it difficult to investigate microbial communities in natural environment.

The aim is to investigate limitations of currently used techniques for identification and detection of microorganisms in natural microbial communities; and then to improve the reliability and the speed of microbial detection using digital PCR and Multiple displacement Amplification technique (MDA).

We showed in this project a method, digital PCR (dig-PCR) and MDA technique, to amplify and analyse various genomes obtained from single uncultivated bacterial cells. We isolated single cells by dilution, and MDA was used for amplification of single microbial cells, thereby enabling genomic analysis of microorganism without culture. Single cells were sequenced using DNA amplified by MDA. Genomic DNA amplified from single cells originating from uncultured bacteria has 93–97% similarity to 16S rRNA genes of cultivated bacteria.

Combining MDA technique with dig-PCR has the ability to detect and obtain information of functional genes and phylogenetic information of microorganisms directly (even the uncultivable ones); and these will be useful in degradation and bioremediation in the environment and industries. Also, combining single cell sequencing with metagenomic data will be powerful in assembly of multi-species shotgun sequences into their individual genomes.

GM/12 Uncovering bacterial esterase activity for use in the enantioselective synthesis of tertiary alcohols

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Enantiopure tertiary alcohols (TAs) are versatile building blocks which find application in organic synthesis and in the preparation of high value pharmaceutical compounds. The organic synthesis of enantiopure TAs in most cases requires unfavourably harsh conditions; however the enzymatic hydrolysis of bulky tertiary alcohol esters (TAEs) offers a route towards preparation of enantiopure TAs. To find esterases with suitable activity for the targeted synthesis of pharmaceutical active precursors, two different strategies were followed. By directed isolation, bacteria from activated sludge of a communal sewage treatment plant possessing activity towards the simplest tertiary alcohol ester *tert*-butylacetate have been enriched and characterized using 16S rRNA analysis. In contrast to directed isolation, selected bacteria from a strain collection that were enriched with linear or branched chain alkanes and alkylbenzenes which do not posses ester functionality have also been studied. By knowing degradation pathways for several hydrocarbons, esterase activity was described as a basic requirement necessary for growth, utilization and biotransformation of those model compounds by the tested strains. Using a pH-based screening for the detection of tertiary alcohol esterase activity in cell extracts we identified a number of bacterial strains which possess interesting activity and enantioselectivity towards the kinetic resolution of TAs.

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GM/13 *Not presented*

Diversity of extremely halophilic *Bacteria* and *Archaea* which grow at elevated temperature and alkaline pH

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Whereas the majority of extremophiles are characterized by one or in some cases by a combination of two extremes, a literature search was done to compare the combined optimal growth conditions of extremely halophilic micro-organisms with respect to alkaline pH and elevated temperature. Very few bacteria among the extreme halophiles (defined as taxa growing optimally at Na^+ concentration corresponding to 10% w/v) had both a pH optimum above 8.5 and a temperature optimum above 50°C. The bacteria with the most extreme optimal growth conditions were the recently published *Natranaerobius* and *Natronovirga* species. These micro-organisms belong to a novel family and novel order within the Phylum *Firmicutes*. Similarly, among the archaeal extreme halophiles the novel isolated species *Natronolimnobius* '*aegyptiacus*' exhibited the most extreme optimal growth conditions for the combination of alkaline pH, temperature and salt concentration. However, in contrast to the *Bacteria*, a much larger percentage of the *Archaea* were extreme halophiles, and many of these micro-organisms tolerated more than 4 M (up to and including saturated levels) of NaCl. Among the halophilic alkalithermophilic bacteria, only the yet unpublished *Natranaerobius* '*grantii*' tolerated up to saturation of Na salts (about 1.6 M of the sodium is from sodium carbonate which is required for the pH control).

The surveys were done to stimulate further research in isolating polyextremophilic extreme halophiles and to find the physico-chemical boundaries of life for a combination of stressors: alkaline pH, elevated temperature and high salt concentration. As previously shown for *N. thermophilus*, some of these micro-organisms are also resistant to UV radiation. It will be of interest whether other isolates can be found which are, in addition to UV-resistant, also resistant to desiccation and high levels of gamma radiation.

Community structure in two geochemically distinct hydrothermal pools in Uzon Caldera, Kamchatka, far east Russia

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Contemporary hydrothermal features are of interest as microecological systems. Two thermal pools in the Uzon Caldera, Arkashin Schurf (Arkashin) and Zavarzin Spring (Zavarzin), were sampled to characterize geochemical conditions and bacterial and archaeal community structure, including elemental composition, lipid composition, 16S rRNA sequencing, and C, N and S stable isotope ratios. Sediments in Arkashin relative to Zavarzin had higher concentrations of elements associated with magmatic source water. The archaeal glycerol dialkyl glycerol tetraether (GDGT) profiles from Arkashin were dominated by GDGT-4. Up to 350 μg g^{-1} of phospholipid fatty acids (PLFA) were recovered from Arkashin and included biomarkers for Aquificales. In Arkashin, 206 Bacterial 16S sequences were assigned to 19 operational taxonomic units (OTUs). The most abundant were related to the '*Sphingobacteria*', *Hydrogenobaculum* (*Aquificales*) and *Variovorax* (*β-Proteobacteria*). In Zavarzin, relative abundance among archaeal GDGTs was more evenly distributed than in Arkashin. Nintety-one archaeal 16S rRNA sequences were assigned to 31 OTUs representing the *Crenarcheota* and '*Korarchaeota*'. Approximately one quarter of the Zavarzin archaeal sequences appeared to represent unique clades of *Archaea*. Biomarkers in up to 455 μg g-1 of PLFA recovered from Zavarzin included those for *Chloroflexi* as well as for heterotrophic bacteria. The 300 Bacterial 16S sequences analyzed from Zavarzin were assigned to 59 OTUs. The most abundant sequences were related to *Rosiflexus*, followed by *δ-Proteobacteria* and clostridia. These data, coupled with stable isotope ratios, indicate that *Hydrogenobaculum* is a primary producer in Arkashin and allocthonous inputs may be an important C source in Zavarzin. Thus, the distinct geochemical conditions give rise to unique community structures in each pool.

Spatial and physicochemical correlations to the genetic diversity of *Thermoanaerobacter uzonensis* isolates from geothermal springs of Kamchatka, RFE

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Strain similar to *Thermoanaerobacter uzonensis* (≥97% 16S rRNA sequence similarity) were isolated from 11 different geothermal springs located in the Kamchatka Peninsula, Russian Far East. From 123 *T. uzonensis* isolates portions of eight universally conserved protein coding genes, *gyrB*, *lepA*, *leuS*, *pyrG*, *recA*, *recG*, *rplB* and *rpoB*, were PCR amplified, sequenced, and analysed to assess the interspecies diversity and the genetic structure of the population. All of the protein coding genes were variable, although the quantity and type (synonymous compared to non-synonymous substitutions) of variation differed. The number of protein coding gene sequence alleles varied from 3 (*rplB*) to 46 (*recG*) and appeared to be under different levels of evolutionary selection. Within the Uzon Caldera population, the low index of association value (I_a = –0.03), suggests that recombination occurs between the populations. However, sequence types represented by multiple strains were, in all cases, found in isolates derived from the same spring in the Uzon Caldera. Significantly, the same sequence types were found in isolates derived from samples collected in 2005 and 2006 from the same geothermal springs. The sequencing and analysis of a set of protein coding genes from *T. uzonensis* strains has revealed that

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↑CONTENTS

Diversity of extremely halophilic *Bacteria* and *Archaea* which grow at elevated temperature and alkaline pH

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Whereas the majority of extremophiles are characterized by one or in some cases by a combination of two extremes, a literature search was done to compare the combined optimal growth conditions of extremely halophilic micro-organisms with respect to alkaline pH and elevated temperature. Very few bacteria among the extreme halophiles (defined as taxa growing optimally at Na^+ concentration corresponding to 10% w/v) had both a pH optimum above 8.5 and a temperature optimum above 50°C. The bacteria with the most extreme optimal growth conditions were the recently published *Natranaerobius* and *Natronovirga* species. These micro-organisms belong to a novel family and novel order within the Phylum *Firmicutes*. Similarly, among the archaeal extreme halophiles the novel isolated species *Natronolimnobius* '*aegyptiacus*' exhibited the most extreme optimal growth conditions for the combination of alkaline pH, temperature and salt concentration. However, in contrast to the *Bacteria*, a much larger percentage of the *Archaea* were extreme halophiles, and many of these micro-organisms tolerated more than 4 M (up to and including saturated levels) of NaCl. Among the halophilic alkalithermophilic bacteria, only the yet unpublished *Natranaerobius* '*grantii*' tolerated up to saturation of Na salts (about 1.6 M of the sodium is from sodium carbonate which is required for the pH control).

The surveys were done to stimulate further research in isolating polyextremophilic extreme halophiles and to find the physico-chemical boundaries of life for a combination of stressors: alkaline pH, elevated temperature and high salt concentration. As previously shown for *N. thermophilus*, some of these micro-organisms are also resistant to UV radiation. It will be of interest whether other isolates can be found which are, in addition to UV-resistant, also resistant to desiccation and high levels of gamma radiation.

Community structure in two geochemically distinct hydrothermal pools in Uzon Caldera, Kamchatka, far east Russia

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Contemporary hydrothermal features are of interest as microecological systems. Two thermal pools in the Uzon Caldera, Arkashin Schurf (Arkashin) and Zavarzin Spring (Zavarzin), were sampled to characterize geochemical conditions and bacterial and archaeal community structure, including elemental composition, lipid composition, 16S rRNA sequencing, and C, N and S stable isotope ratios. Sediments in Arkashin relative to Zavarzin had higher concentrations of elements associated with magmatic source water. The archaeal glycerol dialkyl glycerol tetraether (GDGT) profiles from Arkashin were dominated by GDGT-4. Up to 350 μg g^{-1} of phospholipid fatty acids (PLFA) were recovered from Arkashin and included biomarkers for Aquificales. In Arkashin, 206 Bacterial 16S sequences were assigned to 19 operational taxonomic units (OTUs). The most abundant were related to the '*Sphingobacteria*', *Hydrogenobaculum* (*Aquificales*) and *Variovorax* (β-*Proteobacteria*). In Zavarzin, relative abundance among archaeal GDGTs was more evenly distributed than in Arkashin. Nintety-one archaeal 16S rRNA sequences were assigned to 31 OTUs representing the *Crenarcheota* and '*Korarchaeota*'. Approximately one quarter of the Zavarzin archaeal sequences appeared to represent unique clades of *Archaea*. Biomarkers in up to 455 μg g–1 of PLFA recovered from Zavarzin included those for *Chloroflexi* as well as for heterotrophic bacteria. The 300 Bacterial 16S sequences analyzed from Zavarzin were assigned to 59 OTUs. The most abundant sequences were related to *Rosiflexus*, followed by δ-*Proteobacteria* and clostridia. These data, coupled with stable isotope ratios, indicate that *Hydrogenobaculum* is a primary producer in Arkashin and allocthonous inputs may be an important C source in Zavarzin. Thus, the distinct geochemical conditions give rise to unique community structures in each pool.

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Spatial and physicochemical correlations to the genetic diversity of *Thermoanaerobacter uzonensis* isolates from geothermal springs of Kamchatka, RFE

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Strain similar to *Thermoanaerobacter uzonensis* (≥97% 16S rRNA sequence similarity) were isolated from 11 different geothermal springs located in the Kamchatka Peninsula, Russian Far East. From 123 *T. uzonensis* isolates portions of eight universally conserved protein coding genes, *gyrB*, *lepA*, *leuS*, *pyrG*, *recA*, *recG*, *rplB* and *rpoB*, were PCR amplified, sequenced, and analysed to assess the interspecies diversity and the genetic structure of the population. All of the protein coding genes were variable, although the quantity and type (synonymous compared to non-synonymous substitutions) of variation differed. The number of protein coding gene sequence alleles varied from 3 (*rplB*) to 46 (*recG*) and appeared to be under different levels of evolutionary selection. Within the Uzon Caldera population, the low index of association value (I_a = –0.03), suggests that recombination occurs between the populations. However, sequence types represented by multiple strains were, in all cases, found in isolates derived from the same spring in the Uzon Caldera. Significantly, the same sequence types were found in isolates derived from samples collected in 2005 and 2006 from the same geothermal springs. The sequencing and analysis of a set of protein coding genes from *T. uzonensis* strains has revealed that recombination occurs, that portions of the genome are under different evolutionary selection pressures, while different *T. uzonensis* ecotypes may exist within and between the spatially close geothermal springs of the Uzon Caldera. While these results reinforce the view that extreme environments, such as terrestrial geothermal springs, indeed exhibit interesting island dynamics, these results also point to how different micro-organisms within these environments may have significantly different spatial diversity patterns.

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