SGM society for general Microbiology

Irish Branch Meeting

Mechanisms Of Bacterial Adhesion And Invasion

Department of Clinical Microbiology Trinity College Dublin

April 27th and 28th 2006

Institute for Molecular Medicine

Trinity Centre, St James's Hospital, Dublin 8

The Long Library Trinity College

Mechanisms of bacterial adhesion and invasion

Day 1

Session 1

April 27th

1.50 –2.00 pm Opening Remarks
Prof Thomas J Rogers
Professor of Clinical Microbiology, Trinity College Dublin.

Chair: Prof Fergal O'Gara
Professor of Microbiology
BIOMERIT and University College Cork

2.00 –2.35
Prof TJ Foster
Department of Microbiology Trinity College Dublin
Surface-anchored proteins of *Staphylococcus aureus* involved in nasal colonization

2.35 – 3.10
Dr J'O Gara
School of Biosciences University College Dublin
Regulatory pathways controlling the staphylococcal biofilm phenotype

3.10-3.45
Prof CJ Dorman
Department of Microbiology Trinity College Dublin
Control of type III secretion gene expression in *Shigella flexneri.*

Coffee and Poster viewing 3.45-4.15

Session 2

Chair: Dr Stephen Smith

Department of Clinical Microbiology Trinity College Dublin

4.15 – 4.50
Dr Ian Henderson
University of Birmingham UK
Autotransporters

4.50-5.25
Wolf-Dieter Schubert
Braunschweig, Germany
Structural biology of microbial pathogenesis

Short oral presentations 5.25 p.m. – 6.25 p.m.

(1) Role of Cortactin in pedestal formation by EPEC (ABSTRACT 46)
Narcisa Martinez-Quiles Ph.D.
Universidad Complutense de Madrid

(2) The Post-transcriptional Regulator RsmA plays a Role in the Interaction between *Pseudomonas aeruginosa* and Human Airway Epithelial Cells by Positively Regulating the Type III Secretion System (ABSTRACT 40)
Eoin P. O'Grady
BIOMERIT Research Centre, Department of Microbiology, University College Cork, Cork, Ireland

(3) Proteomic analysis of a sigma β-deficient mutant of *Listeria monocytogenes* **(ABSTRACT 1)**
Florence Abram
Bacterial Stress Response Group, Department of Microbiology, National University of Ireland, Galway, University Road, Galway, Ireland

(4) Molecular Analysis of the Interaction Between Staphylococcal Protein A and von Willebrand Factor ABSTRACT 44
Maghnus O'Seaghdha,.
Department of Microbiology, Moyne Institute of Preventive Medicine, Trinity College, Dublin 2

Wine reception and poster viewing 6.30 –7.30 At poster venue WMLT

Day 2

Session 3

Chair Prof Cyril J Smyth
Department of Microbiology Trinity College

9.00-9.35 a.m.

Dr Marguerite Clyne,

UCD School of Medicine and Medical Science Dublin 12, Ireland.

The Interaction of *Helicobacter pylori* with Trefoil Peptides

9.35-10.10 a.m.

Prof. M. Virji
University of Bristol U.K.
Susceptibility to *N. meningitidis* infections: target receptors at the cross-roads of colonisation and pathogenesis

Short oral presentations 10.10 –11.00

(5) Effects of secreted components of *Helicobacter pylori* On Epithelial Cell Tight Junctions (ABSTRACT 32)
Erica Mullaney
Institute of Molecular Medicine Trinity College, Dublin

(6) Structural Analysis of a Cell envelope protease (ABSTRACT 35)
Maurice O'Connell
Department of Chemical and Environmental Sciences, University of Limerick

(7) An in vitro cell-culture model and novel in vivo luciferase reporter system show internalin- and hemolysin-independent translocation of *Listeria monocytogenes* across M-cells. (ABSTRACT 9)
Sinead C. Corr
Department of Microbiology and Alimentary Pharmabiotic Centre University College Cork

Coffee and poster viewing 11.00-11.30

Session 4

Prof Joe Keane
St James's Hospital Dublin and the Institute for Molecular Medicine

11.30 – 12.05 p.m

Dr Roland Brosch
Unité de Génétique Moléculaire Bactérienne, Institut Pasteur Paris France.
Comparative and functional analyses of the tubercle bacilli - New insights into evolution and the ESAT-6 encoding immunogenicity and virulence locus

12.05 – 12.40 p.m.
Dr C. Buchreiser
Institut Pasteur Paris
***Legionella pneumophila* pathogenesis: lessons learned from genomics**

12.40 Closing remarks

2.00 pm Surviving your Ph.D
Robert Smith Lecture Theatre

MAIN SYMPOSIUM - ABSTRACTS

Surface-anchored proteins of *Staphylococcus aureus* involved in nasal colonization

Prof Timothy J Foster

Microbiology Department, Trinity College, Dublin 2, Ireland

Nasal colonization by *Staphylococcus aureus* is an important risk factor for infection. Bacteria adhere to desquamated epithelial cells in the nares. Several proteins including clumping factor B (ClfB) that are expressed on the bacterial cell surface promote adherence to squames. In addition to binding fibrinogen, ClfB also binds strongly to cytokeratin 10, one of the major proteins in squames. Molecular analysis identified the binding region for ClfB in keratin 10 as C-terminal “tail” region that is composed of quasi repeats of Tyr–$(Gly/Ser)_n$-Tyr. A synthetic peptide mimicking a typical glycine loop (YGGGSSGGGSSGGY) blocked adherence of ClfB-expressing bacteria to immobilized keratin 10. A ClfB–defective mutant colonized the nares of mice less readily than the wild-type. Active immunization with rClfB and passive immunization with anti-ClfB monoclonal antibody reduced nasal colonization in mice. This suggests that nasal colonization in humans might be reduced or prevented by vaccination.

Regulatory pathways controlling the staphylococcal biofilm phenotype

Dr James P. O'Gara

School of Biomolecular and Biomedical Science, Ardmore House, University College Dublin, Belfield, Dublin 4.

Gram-positive cocci and in particular *Staphylococcus* species, are predominant among the organisms responsible for infectious complications following surgical implantation of prosthetic medical devices. The pathogenesis of these device-related infections generally stems from the ability of the microorganisms to first adhere to the biomaterial surface and subsequently form a mucoid biofilm, originally referred to as "slime". Currently the best understood mechanism of staphylococcal biofilm development involves the synthesis and export of an extracellular polysaccharide termed polysaccharide intercellular adhesin (PIA) or poly-N-acetylglucosamine (PNAG) by *icaADBC* operon-encoded enzymes. Recent progress in elucidating the regulatory pathways controlling *ica* operon expression in both *S. epidermidis* and *S. aureus* will be summarized. Specifically, regulation of *ica* operon and biofilm expression by the transcription factors IcaR, SarA (Staphylococcal Accessory Regulator) and the alternative sigma factor σ^B will be described. In addition, recent findings on the role of the *araC*-type transcriptional regulator, Rbf (Regulator of biofilm formation) in *ica* expression and evidence for an *ica*-independent biofilm phenotype in methicillin resistant *S. aureus* (MRSA) will also be discussed

Control of type III secretion gene expression in *Shigella flexneri.*

Dr Elizabeth C Turner and Prof. Charles J Dorman*

Department of Microbiology, Trinity College, Dublin 2, Ireland.

The genes coding for the type III secretion system used by *Shigella flexneri* to invade human gut epithelia are located on a 230-kbp virulence plasmid and their expression is controlled in response to temperature, osmolarity and pH through a complex regulatory cascade. An AraC-like transcription factor called VirF is located at the top of the cascade and it activates transcription of a second regulatory gene called *virB*. The product of this gene is unlike any other transcription factor. Instead it closely resembles proteins involved in plasmid partitioning. VirB binds to the promoters of the operons that code for the type III secretion system and its effector proteins, where it overcomes the repressive activity of the H-NS nucleoid-associated protein. We will discuss the mechanism of the VirB-H-NS regulatory antagonism and consider its implications for the evolution of transcription regulatory mechanisms in Gram-negative bacteria.

Structural biology of microbial pathogenesis

Wolf-Dieter Schubert

Molecular Host-Pathogen Interactions, Division of Structural Biology, Helmholtz Centre for Infection Biology, D-38124 Braunschweig, Germany

The elucidation of the three-dimensional structures of bacterial virulence factors significantly contributes to our understanding of infectious diseases. Structures of these proteins ideally in complex with their host receptors are a prerequisite to explore host-pathogen interactions at the atomic level. *Listeria monocytogenes* is a Gram-positive human pathogen that causes severe disease mainly in immuno-compromized individuals. It utilizes a limited number of well-characterized virulence factors for adhesion, invasion and spread within the host.

We have determined the crystal structures of several virulence factors from *L. monocytogenes* and present structural and functional data on their interactions with host cell receptors. In particular, the structures of the leucine-rich repeat domain of internalin (InlA) in complex with the N-terminal domain of its human receptor E-cadherin has provided a wealth of information on the adhesion and invasion of the pathogen: We demonstrate that a single amino acid position of E-cadherin critically determines whether the mammalian host is susceptible towards *L. monocytogenes* infection via the intestinal route.

Susceptibility to *N. meningitidis* infections: target receptors at the cross-roads of colonisation and pathogenesis

Prof Mumtaz Virji

Department of Cellular and Molecular Medicine, School of Medical Sciences, University of Bristol, Bristol, BS8 1TD, UK.

Whilst most individuals are resistant to infections by meningococci, in the susceptible, the infection can be life-threatening. Epidemiological studies have shown that the risk of acquisition of meningococcal infection increases following certain viral infections. In addition, genetic polymorphisms may also contribute to such risks. Studies in my laboratory have focused on the host-bacteria interface, particularly on important bacterial ligands and host receptors involved in mutual interactions as well as the interplay between distinct ligand-receptor pairs.

One key receptor targeted by *Neisseria meningitidis* identified in our studies is the human CEA-related cell adhesion molecule (CEACAM). Further studies have shown that *Haemophilus influenzae* and *Moraxella catarrhalis* also bind to human CEACAMs. Besides being frequent colonisers of many healthy individuals, these bacteria have in common the tendency to cause opportunistic infections in susceptible hosts. The CEACAM-binding adhesins of these bacteria have been identified and were found be structurally unrelated. In the case of *N. meningitidis*, the adhesins belong to the Opa family of antigenically variable outer membrane proteins. We have analysed the binding requirements for Opa proteins on human CEACAMs using site-directed mutagenesis of the receptors.

To investigate the potential roles of CEACAMs in increasing bacterial load/invasion and thus host susceptibility, we have conducted several studies. One process by which host resistance to infection may be compromised includes modulation of receptor density that may occur following viral infections via the action of pro-inflammatory cytokines. Interestingly, CEACAMs are often expressed at low levels and can be upregulated in a variety of cell types. Another process could involve receptor polymorphisms that may lead to increased affinity of bacterial interactions and result in effective tissue penetration.

The seminar will address the intricacies of Opa-CEACAM interactions and host susceptibility in the context of modulations at the host-bacteria interface.

The Interaction of *Helicobacter pylori* with Trefoil Peptides

Dr Marguerite Clyne,

UCD School of Medicine and Medical Science, The Childrens Research Ctr. Our Lady's Hospital for Sick Children, Crumlin, Dublin 12, Ireland.

H. pylori is only found in vivo in association with gastric mucus secreting cells. The majority (~98%) of organisms are found living in the gastric mucus, with only a small proportion attached to the surface of gastric mucus secreting epithelial cells. Chronic colonisation of the gastric mucus layer is likely to be essential to the maintenance of a persistent reservoir of bacteria and subsequent cellular attachment and development of pathology. The localization of *H. pylori* in the stomach has been shown to match the expression of MUC5AC implicating MUC5AC or a molecule that is co-expressed with MUC5AC in the sequestration of *H. pylori* within the gastric mucus layer.

The trefoil peptides TFF1, TFF2 and TFF3 are a group of small cysteine rich proteins belonging to the trefoil factor family (TFF). They play a role in the process of restitution following mucosal injury and protect the integrity of the epithelial barrier. In normal gastrointestinal mucosal structures there is a general correlation between mucin glycoproteins and trefoil peptide gene expression. TFF1 segregates with MUC5AC in foveolar pit cells of the gastric body and superficial regions of the antral glands, TFF2 with MUC6 in the fundus and deep antral glands of the stomach and TFF3 and MUC2 are co-expressed throughout the large and small bowel mucosa.

In a recent study (Clyne M et al Proc Natl Acad Sci U S A. 2004 May 11;101(19):7409-14) we tested the hypothesis that TFF1 could act as a receptor for *H. pylori* possibly explaining why *H. pylori* only colonises the stomach and co-localises with MUC5AC. Recombinant monomeric and dimeric human TFF1, were coated onto latex beads and binding of five *H. pylori* strains was assessed by flow cytometry. All five strains bound to the TFF1 dimer but not to the monomer or BSA. Binding was inhibited by a TFF1 monoclonal antibody and following preincubation of the bacteria with TFF1. The BIAcore system based on the principle of surface plasmon resonance, uses sensor chip chemistry and an integrated flow system to allow "real-time" monitoring of molecular interactions at the sensor surface was used to further characterise the interaction of *H. pylori* with TFF1. *H. pylori* bound strongly to TFF1-coated dextran chips compared with uncoated chips. Preincubation of *H. pylori* with soluble TFF1 abolished binding. Preincubation of *H. pylori* with TFF1 promoted binding of the organism to porcine gastric mucin. TFF1, therefore appears to be a potent receptor for *H. pylori* which may explain the tropism of this organism for gastric tissue and the previously noted co-localisation of *H. pylori* with MUC5AC.

We have since further characterised the interaction of *H. pylori* with TFF1 using the BIAcore system. Binding of *H. pylori* to the dimeric form of TFF1 is inhibited by TFF3 dimer and vice versa. Glycosylated TFF2 has no effect on the interaction of *H. pylori* with TFF1 or TFF3. Incubation of *H. pylori* with the dimeric form of TFF1 and subsequent analysis by SDS PAGE and Western immunoblotting with a TFF1 antibody showed that *H. pylori* is capable of reducing dimeric TFF1 to the monomeric form. Degradation of dimeric TFF1 to the monomeric form could be an important virulence mechanism for the organism since the monomeric form of TFF1 has been shown to be biologically less active than the dimeric form. In this presentation I will discuss how we are attempting to characterise the interaction of *H. pylori* with TFF1 and with gastric mucus and suggest that the knowledge obtained may serve as a valuable reference system for studies on the interaction of other pathogenic organisms with mucus.

Legionella pneumophila pathogenesis: lessons learned from genomics

Dr Carmen Buchrieser

Unité de Génomique des Microorganismes Pathogènes, Institut Pasteur, France
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Legionella pneumophila, the causative agent of nearly 90% of Legionnaires' disease, replicates as an intracellular parasite of amoebae and persists in the environment as a free-living microbe. The analysis of the complete genome sequences of *L. pneumophila* strain Paris an endemic strain predominant in France, and strain Lens, an epidemic strain revealed a variety of astonishing features unique to *Legionella*, such as the extended array of eukaryotic-like proteins, promising candidates involved in interfering with phagocytic functions. However, only little is known about the transcriptional program of *L. pneumophila* during the infectious process. Gene expression experiments have so far only been carried out on some dozen single genes, and mostly under *in vitro* conditions. In the aim to decipher the molecular mechanisms used by *L. pneumophila* to replicate within and manipulate the eukaryotic host cell we analysed the genome-wide transcriptional programme of three *L. pneumophila* strains during their life cycles in the natural eukaryotic host *Acanthamoeba castellanii* as well as that of a *fliA* (σ^{28}) deletion mutant. This allowed to gain a better understanding of the regulatory cascade employed during the biphasic lifecycle of *L. pneumophila* and identified new traits of replication and transmission during intracellular growth of this pathogen.

Comparative and functional analyses of the tubercle bacilli - New insights into evolution and the ESAT-6 encoding immunogenicity and virulence locus

Dr Roland Brosch

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http://www.pasteur.fr/recherche/unites/Lgmb/

The genome sequence of the paradigm strain of tuberculosis research, *Mycobacterium tuberculosis* H37Rv revealed the existence of ~ 4000 genes in the 4.4 Mb sized genome, which may now be systematically tested for their contribution to the virulent phenotype of this highly successful human pathogen. To achieve this goal, comparative genomics of the tubercle bacilli and related technologies are of great help to uncover the molecular mechanisms involved in these processes and also to obtain insights in the evolution of the tubercle bacilli.

The findings of this study led to a complete revision of our understanding of the evolutionary pathway of the tubercle bacilli, and suggest that *M. tuberculosis* is closer related to the common ancestor of the tubercle bacilli than *Mycobacterium bovis*, which was for long time thought to be the progenitor of the tubercle bacilli. Recent analyses of strains showing smooth colony morphology that were isolated from tuberculosis patients in Djibouti-East-Africa, further extended these results, and allowed a progenitor species (M. prototuberculosis) to be proposed. Moreover, these studies defined new genetic markers as powerful diagnostic tools for the rapid and unambiguous identification and differentiation of the tubercle bacilli. Together with the enormous progress recently made in the field of genetic manipulation of the mycobacteria, focused functional analyses allow to investigate and confirm predictions made on the basis of comparative and functional genomics.

For example, functional studies of the region of difference 1 (RD1), absent in the attenuated vaccine strains *M. bovis* BCG and *Mycobacterium microti,* but present in all virulent strains of *M. tuberculosis* and *M. bovis*, have shown that proteins, like the 6 kDa early secreted antigenic target (ESAT-6), encoded in this chromosomal region are involved in pathogenesis and enhanced immune-recognition. As such, elucidation of the employed molecular mechanisms is important for the developement of both, new anti-tuberculosis drugs and improved vaccines.

POSTERS AND OFFERED PAPERS

Abstract 1 SHORT PRESENTATION

Proteomic analysis of a sigma b-deficient mutant of *Listeria monocytogenes*

Florence Abram* and Conor P. O' Byrne*
**Bacterial Stress Response Group, Department of Microbiology, National University of Ireland, Galway, University Road, Galway, Ireland*

In bacteria the general stress response is coordinated through the expression of numerous genes required for resistance to environmental and metabolic stresses. In the Gram-positive bacterial pathogen *Listeria monocytogenes* this redirection of transcription during stress is under the control of the alternative sigma factor Sigma B . An in-frame deletion mutant of *Listeria monocytogenes* 10403S, lacking the sigB gene was used in order to investigate how sigma^{B} contributes to stress resistance in this pathogen. We use a proteomic approach to identify proteins expressed in a sigma^{B}-dependent manner. Protein extracts were prepared from cells grown under stress conditions such as stationary phase and osmotic stress in order to identify differences in protein expression between the wild type and its corresponding deletion mutant. A number of proteins were identified whose expression is sigma^{B}-dependent. A combination of tryptic digestion and mass spectrometry was used to identify these proteins. The details of this study will be presented and we will suggest a model to account for the stress sensitivity of the sigB mutant.

Abstract 2

Measurement of cell surface hydrophobicity and adherence as indicators of potential biofilm formation by *Ralstonia pickettii*.

Catherine C. Adley, and F.M. Saieb F.M. Systems Microbiology Laboratory, Department of Chemical and Environmental Sciences, University of Limerick, Limerick. Ireland
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Experimental methodology to measure bacterial attachment has been widely studied. Adhesion and cell surface hydrophobicity (CSH) measurements, are two method which have been used by the scientific community. The CSH of bacteria is an important physicochemical feature and has been shown to be an important factor in the ability of opportunistic pathogens to adhere to surfaces, e.g. medical implants, dental surfaces. Adherence to surfaces is a first step in microbial colonization of new environments and is a key event in the pathogenesis of medically important microorganisms.

Microbial/bacterial adhesion to hydrocarbon (MATH/BATH) has been frequently studied as a test of cell surface hydrophobicity[1]. Liquid hydrocarbon is mixed with bacterial cultures, which disperses into droplets; these droplets may also adsorb microbial cells. When adhesion takes place, the cell-coated droplets rise to form a stable layer and the turbidity of the lower aqueous phase decreases. In the case of non-adherent cells, the droplets quickly coalesce, and the turbidity in the lower aqueous phase remains unchanged. This test was carried out on a selection of *Ralstonia pickettii* isolates from industrial and clinical environments, which produce homoserine lactones, known cell-cell signalling molecules of biofilm formation[3] in order to obtain a measure of their CSH as an indicator for biofilm formation.

Similarly, adhesion studies in microtiter plates using the method of Christensen *et al* 1985[2] were carried out. Attachment of cells to the microtiter plates can be read quantitatively after staining using an ELISA reader.

The adhesion studies provided positive indicators for biofilm development by *Ralstonia pickettii*. The CSH studies were inconsistent.

1. Rosenberg M. (1991) *Crit. Rev. Microbiol.* **18(2)**:159-173.
2. Christensen *et al* (1985). *J. Clin. Microbiol.* 22:996-1006.
3. Adley CC and Saieb FM (2005) *Ultrapure Water Journal* Feb/March: 14-17.

Abstract 3

Isolation and characterisation of the outer membrane components of *Helicobacter spp.*

Aoife M. Brennan and Anthony P. Moran
**Laboratory of Molecular Biochemistry, Department of Microbiology, National University of Ireland, Galway.*

Despite the recognised importance of the Gram-negative bacterium *Helicobacter pylori* in human gastroduodenal diseases, other *Helicobacter spp.* have remained relatively unstudied. Preliminary studies have indicated that other helicobacters, of both gastric and enterohepatic origin may be causative agents in other widespread chronic and inflammatory diseases in humans and various mammalian species. As bacteria that colonise the extreme environments of the stomach and intestine are likely to adapt their membrane architecture, the aim of this study was to isolate the outer membrane components of a number of different *Helicobacter spp*. and characterise these through the application of various biochemical and biological analyses. Lipopolysaccharide (LPS) was extracted using the hot phenol water technique from representative strains of 10 different species (Gastric: *H. canis*, *H. bizzozeronii*, *H. salomonis*, *H. pylori*, *H. felis* ; Enterohepatic: *H. bilis*, *H. hepaticus*, *H. pullorum*, *H. rappini*) and purified by enzymatic treatments before electrophoretic analysis (SDS-PAGE) with silver staining. Inter- and intra-species variation in LPS structure was observed. Serological probing for the presence of Lewis antigen mimicry, as seen in *H. pyori*, was carried out using both dot and Western blotting, and lectin analysis was used to determine sugar composition. Because of the differing membrane properties of strains, 4 extraction techniques were applied and evaluated for optimal extraction of outer membrane proteins (OMPs). Methods were evaluated on the purity and volume of proteins produced. The results show that gastric and enterohepatic *Helicobacter spp* express LPSs and OMPs of differing composition consistent with their varied ecological niches.

ABSTRACT 4

Proteomic and sub-proteomic analyses of *Aspergillus fumigatus*.

Stephen Carberry, Claire Neville, Kevin Kavanagh and Sean Doyle.
Department of Biology and National Institute of Cellular Biotechnology, National University of Ireland Maynooth, Co. Kildare, Ireland. Email: sean.doyle@nuim.ie

Aspergillus fumigatus is a recognised human pathogen, especially in immunocompromised individuals. The availability of the annotated *A. fumigatus* genome sequence will significantly accelerate our understanding of this organism. However, limited information is available with respect to the *A. fumigatus* proteome. Here, both a direct proteomic approach (2D-PAGE and MALDI-MS) and a sub-proteomic strategy involving initial glutathione affinity chromatography have been deployed to identify 54 proteins from *A. fumigatus* primarily involved in energy metabolism and protein biosynthesis. Furthermore, two novel eukaryotic elongation factor proteins (eEF1Bγ), termed ElfA and B have been identified and phylogenetically confirmed to belong to the eEF1Bγ class of GST-like proteins. One of these proteins (ElfA) has been purified to homogeneity, identified as a monomeric enzyme (molecular mass = 20 kDa; p*I* = 5.9 and 6.5) and found to exhibit glutathione transferase activity (specific activities (mean ± standard deviation, *n* = 3) of 3.13 ± 0.27 and 3.43 ± 1.0 μmol/min/mg, using CDNB and ethacrynic acid, respectively. Overall, these data highlight the importance of new approaches to dissect the proteome of, and elucidate novel functions within, *A. fumigatus* [1].

Reference:

1. Carberry, S., Neville, C.M., Kavanagh, K.A. and Doyle S. (2006) Analysis of major intracellular proteins of *Aspergillus fumigatus* by MALDI mass spectrometry: Identification and characterisation of an elongation factor 1B protein with glutathione transferase activity. Biochem Biophys Res Commun. 341(4):1096-104.

ABSTRACT 5

Bacterial cysteine proteases and their natural inhibitors

Roibeard Thorton, Jakki Cooney and Todd Kagawa
Dept of Chemical and Environmental Sciences & MSSI, University of Limerick, Limerick, Ireland.

The bacterial cysteine protease, SpeB, is from the Gram-positive pathogen *Streptococcus pyogenes*, and is implicated in bacterial virulence. The protease is produced as a zymogen which is exported from the cell via the Exportal system, where it is activated through an autoproteolytic event. We have recently identified a protein called Spi, which is a specific intracellular inhibitor for SpeB. RNA analysis shows that the *spi* gene is co-transcribed with the *speB* gene. Mining of bacterial genome sequences has identified SpeB homologues in a range of Gram-negative organisms. In several cases there are linked ORFs which could potentially encode specific inhibitors.

ABSTRACT 6

Occurrence of the *selu* gene in nasal carriage isolates of *Staphylococcus aureus* possessing the *egc* locus encoding enterotoxins and enterotoxin-like proteins

Mark M. Collery, John J.G. Tumilty, Jane M. Twohig, Cyril J. Smyth
Department of Microbiology, Moyne Institute of Preventive Medicine, Trinity College, Dublin 2, Republic of Ireland

Over the past decade the number of staphylococcal enterotoxins (SEs) and staphylococcal enterotoxin-like proteins (SEIs) has expanded to eighteen. An operon, designated the enterotoxin gene cluster (*egc*), was originally shown to comprise five *se* and *sel* genes (*seg, sei, selm, seln, selo*) and two pseudogenes, *ψent1* and *ψent2*, located between the *sei* and *seln* genes[1]. Subsequently an additional gene, designated *selu*, was described between the *sei* and *seln* genes[2]. The *selu* gene differs from the two pseudogenes by a 15-bp insertion in the *ψent1* pseudogene [2]. Among 24 *seg*- and/or *sei*-positive food isolates tested, 4 (16.6%) possessed the *selu* gene and the remainder the two pseudogenes[2]. Few other data are as yet available on the occurrence of the *selu* gene in *S. aureus*[3,4]. The lack of data on the frequency of occurrence of the *selu* gene in nasal carriage isolates of *S. aureus* prompted the present study. Fifty nasal carriage strains of *S. aureus* isolated over a ten-year period from healthy sophister microbiology students, dental students, student dental nurses, and student dental hygienists were screened using a series of multiplex PCRs and single gene PCRs for the presence of fifteen *se* and *sel* genes (*sea–see, seg–sei,* and *selj–selq*). Of these, 42 tested positive for the *egc* locus. Letertre *et al.* [2] described PCR primers to distinguish the presence of the *selu* gene and the *ψent1* and *ψent2* pseudogenes. Primer PSE2 is specific for the 15-bp insertion in the *ψent1* pseudogene. The primers PSE1 and PSE4 generate an amplimer of 1135 bp for *egc*-positive strains that are *selu*$^-$ (*ψent1*$^+$ *ψent2*$^+$) and a product of 1149 bp for strains that are *selu*$^+$. Primers PSE2 and PSE4 only generate a product for *egc*-positive strains that are *selu*$^+$ (790 bp), as do primers PSE2 and PSE6 (142 bp). Of the 42 *egc*-positive strains, 23 (54.8%) were positive for the *selu* gene, while 16 (38.1%) had the *ψent1* and *ψent2* pseudogenes. The 8 *egc*-negative strains yielded no PCR products. Three *egc*-positive strains were negative for both the *selu* gene and the *ψent1* and*ψent2* pseudogenes. These isolates are being further investigated to determine the structure of their *egc* loci. Two *selu*-containing *egc* loci have been described[5] [*selo, selm, sei, selu, seln* and *seg* – strain FRI137, accession no. AY205306; and *selo, selm sei*$_v$, *selu*$_v$, *seln*$_v$ and *seg*$_v$ – strain 382F, accession no. AY158703]. Whether variant genes *sei*$_v$, *selu*$_v$, *seln*$_v$ and *seg*$_v$ occur in *selu*$^+$ nasal isolates remains to be determined.

[1] Jarraud, S., Peyrat, M.A., Lim, A., *et al.* (2001). *J. Immunol.* **166**: 669-677.
[2] Letertre, C., Perelle, S., Dillasser F., Fach, P. (2003). *J. Appl. Microbiol.* **95**:38-43
[3] Fueyo, J.M., Mendoza, M.C., Rodicio, M.R., Muñiz, J., Alvarez, M.A., Martín, MC. (2005). *J. Clin. Microbiol.* **43**: 1278-1284.
[4] Bania, J., Dabrowska, A., Bystron, J., Korzekwa, K., Chrzanowska, J., Molenda, J. (2005). *Int. J. Food Microbiol.* [Epub ahead of print]
[5] Blaiotta, G., Ercolini, D., Pennacchia, C., *et al.* (2004). *J. Appl. Microbiol.* **97**: 719-730.

ABSTRACT 7

Influence of Growth Conditions on Polysaccharide Production by *Campylobacter jejuni*

A.T. Corcoran*, A.P. Moran
**Dept. of Microbiology, National University of Ireland Galway, 1 University Rd., Galway, Ireland*

Campylobacter jejuni is a leading cause of gastroenteritis worldwide and of importance as a food-poisoning agent in the developed world. Previously, *C. jejuni* was considered to synthesise high-molecular-weight lipopolysaccharide (LPS) only More recently, it was deduced that *C. jejuni* produces a capsular polysaccharide and low-molecular-weight lipooligosaccharide (LOS) only. This study aimed to elucidate the forms of polysaccharide-related compounds (PRC) produced by *C. jejuni* and to determine the influence of media and growth conditions on their production. *C. jejuni* NCTC 11168, the genome-sequenced strain, was grown on two different solid media and also in liquid culture, both in a chemostat and in batch culture. In addition, various physical conditions such as pH, temperature and osmolarity were varied. Using an established mini-phenol water technique, PRC were extracted and subsequently analysed by (i) electrophoresis (SDS-PAGE) with silver or Alcian blue staining, (ii) Western blotting with cholera toxin (CT) ligand and (iii) thin layer chromatography with diphenylamine staining, resorcinol staining and immuno-overlay with CT. Moreover, bacterial cells were lectin typed in a microtitre assay using a panel of 7 lectins. It was found that LOS was consistently produced under all conditions and also was CT-reactive upon Western blotting (indicative of GM_1 mimicry). A high-molecular-weight molecule, whose electrophoretic banding patterm resembled LPS, was produced in liquid culture whose characteristics were independent of growth phase. Polysaccharide production occurred in both liquid and on solid media, although polysaccharide characteristics were growth phase-dependent. The results indicated that NCTC 11168 produced a variety of PRC, which may be influenced by growth conditions and growth phase. Therefore, pathogenic studies investigating the role of the polysaccharides and glycolipids of *C. jejuni* should consider the ability of this bacterium to modify its production of PRC in response to growth conditions.

ABSTRACT 8

Structure of the Lipid Anchor of *Campylobacter jejuni* Polysaccharide

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Campylobacter jejuni is one of the most prevalent causes of gastroenteritis and food-poisoning worldwide. Despite this fact, the mechanisms of *C. jejuni* pathgenesis remain unclear. Previous investigations have shown that *Campylobacter jejuni* produces extracellular polysaccharides (PS) which have been structurally characterised and shown to be lipooligosaccharide/lipopolysaccharide (LOS/LPS)-independent. These *C. jejuni* PS were deduced to be capsular in nature; however, the capsular PS lipid anchor was not identified. The present study aimed to isolate and characterise a potential PS lipid anchor produced by *C. jejuni*. Purified PS was obtained principally from *C. jejuni* 81-176 (HS:23), but also from the HS:3 serostrain and 81116 (HS:6). The PS was obtained by ethanol precipitation and sodium acetate extraction, followed by treatments with RNase A, DNase II and proteinase K. Subsequently, gel permeation chromatography was used to purify the crude PS preparations. Initially, preparations were analysed electrophoretically (SDS-PAGE) with silver or Alcian blue staining, Western blotting with homologous antisera and thin layer chromatography using chemical staining and immuno-overlay development. The results of these analyses indicated that a high-molecular-weight PS containing phosphate and fatty acids had been isolated, indicative of a lipid moiety. Cleavage of the lipid moiety from the PS molecule was achieved using acid hydrolysis and phospholipase treatments. Subsequent chemical and structural studies using chemical degradative, methylation derivatization, and mass spectrometric techniques showed that the structure of the lipid moiety of *C. jejuni* PS was similar to the lipid anchor seen in *Escherichia coli* capsular PS and was phosphate-linked. These data conclusively show the occurrence of a lipid-linked capsular-like polysaccharide in *C. jejuni*.

ABSTRACT 9 SHORT PRESENTATION

An in vitro cell-culture model and novel in vivo luciferase reporter system show internalin- and hemolysin-independent translocation of *Listeria monocytogenes* across M-cells.

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Relatively little is known about the precise route by which the foodborne pathogen *Listeria monocytogenes* gains access to intraepithelial lymphoid cells and mucosal lymphoid tissues. In order to determine the role of M-cells in listerial infection, we initially assessed colonization of Peyer's patch (P.P.) epithelium in BALB/c mice by *Vibrio cholerae* Eltor, wild-type *L. monocytogenes* LO28 and an isogenic hemolysin mutant (LO28Δhly). It was observed that both wild-type LO28 and LO28Δhly showed preferential colonization of P.P. epithelium in this model. Furthermore, a novel luciferase reporter system was used to show rapid site-specific localization of *L. monocytogenes* at intestinal Peyer's patches. Finally, to confirm the role of M-cells in transcytosis of *L. monocytogenes* we utilized an in vitro transwell model that mimics M-cell activity through differentiation of epithelial enterocytes via co-culture with murine Peyer's patch lymphocytes (P.P.L's). It was shown that *L. monocytogenes* transits M-cells at significantly increased rates compared to C2Bbe1 monocultures. In addition, M-cell transport occurred independently of bacterial hemolysin and internalin production. This study demonstrates rapid transcytosis of *L. monocytogenes* through M-cells, a process that occurs independently of the action of classical virulence factors.

ABSTRACT 10

Controlled expression and functional characterisation of SasG - a surface protein of *Staphylococcus aureus.*

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Staphylococcus aureus is a human pathogen that colonises the moist squamous epithelium in the anterior nares. This colonisation is mediated by cell surface proteins, such as clumping factor B. When genome sequences became available, ten novel surface proteins were identified. One of these proteins, SasG, stimulated adherence to desquamated nasal epithelial cells. A homologue of SasG, the plasmin sensitive (Pls) protein of *S. aureus,* masks bacterial binding to fibrinogen, fibronectin and IgG. As SasG shows significant sequence homology to Pls, it is possible that SasG functions similarly.

To enable controlled investigation of the function of SasG, DNA encoding the A domain was cloned into the plasmid pMUTIN4, which when introduced into the staphylococcal cell underwent homologous recombination with *sasG* in the chromosome resulting in insertion. This coupled the *sasG* gene to the IPTG inducible pSPAC promoter and simultaneously created a *sasG*::*lacZ* fusion for use in _betagalactosidase studies. The *sasG* gene was also cloned into the *E. coli/S. aureus* shuttle vector pALC2073, which placed *sasG* under the control of the tetracycline inducible promoter *xyl/tetO*.

Expression of SasG masked the ability of *S. aureus* expressing protein A (Spa), clumping factor B (ClfB) and the fibronectin binding proteins A and B (FnBPA and B) to bind to IgG, keratin and fibronectin, respectively. Binding of *S. aureus* was completely reduced to background levels by pALC::*sasG* and a notable decrease in binding was seen for the weaker expressing pALC::*sasG* uninduced and pMUTIN::*sasG* containing strains. The level of expression of Spa, ClfB and the FnBPs were similar to wildtype *S. aureus* as determined by Western immunoblotting using specific antibodies. Current work is focussed on the role of SasG in promoting adherence to desquamated epithelial cells.

ABSTRACT 11

Investigation of the factors influencing activation of the alternative sigma factor σ^B in *Listeria monocytogenes*

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The Gram-positive food-borne pathogen *Listeria monocytogenes* exhibits the ability to survive harsh environmental and energetic stresses through activation of a group of genes known collectively as the SigB (σ^B) regulon. Activation of this regulon is controlled by the alternative sigma factor σ^B, but the factors that influence the activation of σ^B are poorly understood in *Listeria*. This project seeks to define the mechanisms leading to the activation of σ^B in this pathogen. We will achieve this by generating antibodies against σ^B and two putative regulatory proteins of σ^B, RsbV and RsbW. These antibodies will then be employed to provide insight into both protein-protein interactions and cellular levels of these proteins in *L. monocytogenes*. Initial experiments have involved cloning each gene into a vector that incorporates and inducible promoter. Subsequent over-expression of the recombinant gene provided sufficient protein that was purified through use of Nickel affinity chromatography, a technique exploiting the polyhistidine motif integrated into each recombinant gene. With antibody generation initiated, preliminary experiments can now begin to investigate how these putative regulatory proteins associate with σ^B in *Listeria monocytogenes*.

ABSTRACT 12

Cloning of the murC Gene in order to Study its Putative Role in Attachment of *Lactobacilli* to Poultry GI Tract Epithelium

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Differential Display experiments have suggested that the *murC* gene in Lactobacilli appears to be upregulated in the presence of mucin. As this glycoprotein gel is normally found as a protective layer coating the GI tract epithelium this could indicate that *murC* plays a role in the attachment of Lactobacilli to the intestinal epithelium.
In order to confirm this hypothesis, the *murC* gene from a poultry GI tract strain of *Lactobacillus thermotolerans*, was cloned and its expression studied.
Degenerate primers based on known *murC* DNA sequences were used to generate a 700bp murC amplicon. DNA sequencing revealed an identity with a *murC* fragment previously identified by differential display as being upregulated by mucin. Northern analysis, using a model system based on MRS broth containing in increasing concentrations of porcine mucin and chicken intestinal mucus, was used to confirm the Differential Display data. A transcript of ~3000 nucleotides in length was observed suggesting the *murC* gene may be part of a polycistron. An attempt was made to identify the flanking regions of the *murC* gene using inverse PCR. Sequence data were consistent with *murC* forming part of a polycistron.
Experiments using Differential Display to study changes in gene expression in the presence of intestinal mucin have generated several other amplicons of interest, including proteins with mucus binding motifs.

ABSTRACT 13

Mechanism of how a bacterial pathogen develops resistance /tolerance to cationic antimicrobial peptides

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Pseudomonas aeruginosa is a gram-negative opportunistic pathogen. It is one of the main causes of nosocomial infections, infecting individuals with severe burns, AIDS, cancer and cystic fibrosis. *Pseudomonas* has also received attention due to its resistance to a wide variety of conventional antibiotics resulting from an intrinsically impermeable outer membrane and Multi Drug Resistance efflux pumps (Nikaido, 1998). This has lead to an increased interest for cationic antimicrobial peptide as potential therapeutic molecules.

Antimicrobial peptides are ubiquitous in nature. There are key components of the innate immune response and are the first lines of defence against infecting microbes (Zhang *et al*., 2005). They are known to interact with the outer membrane through binding to the LPS. Subsequent hydrophobic interaction with lipid A acyl chains is predicted to increase the outer membrane permeability, thus allowing the peptides to reach the target, leading to loss of integrity of the cell. Some *P. aeruginosa* and *Staphylococcus aureus* CF isolates produce a variant LPS that renders the bacterial cells less susceptible to CAMPs (Guo et al., 1998; Ernst, 1999). Various CAMPs resistance mechanisms probably exist and many remain to be identified. A dual strategy should allow us a greater understanding of the global mechanism by which *Pseudomonas* is able to evade cationic antimicrobial peptides.

Our approach is to use transcriptomic and Tn*5* mutagenesis to identify all *P. aeruginosa* genes whose expression is induced by subinhibitory concentration of CAMPs and all genes required for a basal sensitivity to CAMPs.

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ABSTRACT 14

Mechanisms of Opc-mediated cellular interactions with human endothelial cells

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Adhesion to and invasion of human endothelial cells by Opc-expressing *Neisseria meningitidis* (Nm) requires the presence of bridging proteins such as Fibronectin (Fn) and more importantly Vitronectin (Vn)[1,2]. This interaction is known to be integrin-dependent, since previous studies have revealed that binding was blocked by the presence of the tetrapeptide RGDS, but not RGES[2]. In this study, we investigated by flow cytometry the expression profiles of three integrins, namely αvβ3 (Vn and Fn receptor), αvβ5 (Vn receptor) and α5β1 (Fn receptor), in three distinct human endothelial cells: HBMECs (brain), HMEC-1 (dermal) or HUVECs (umbilical vein). In addition, since cell adhesion molecules expressed on the surface of endothelial cells are known to be modulated by cytokines, we examined the ability of the pro-inflammatory cytokines IFN-γ and TNF-α known to be present during meningococcal infection, to alter the integrin expression profiles in HBMEC and HMEC-1[3]. The effect of PMA, a Protein kinase C activator, was also investigated since it is also known to modulate the expression of integrins[4]. Studies have revealed that the various integrin receptors for Nm can be regulated independently, and that their expression can be either upregulated, downregulated or remain unaltered. In this regard, we have observed that IFN-γ and TNF-α caused no changes on the expression levels of αvβ3 in the cell lines studied, but that αvβ5 was upregulated in the presence of IFN-γ and downregulated in the presence of TNF-α. Stimulation of HBMEC and HMEC-1 with PMA caused a decrease in αvβ5 but an increase in αvβ3, confirming previous results[5]. To assess the effect of the observed changes in the integrin expression, we examined bacterial adhesion to and invasion of HBMEC and HMEC-1 following stimulation with the cytokines and PMA. The data showed that the observed integrin modulation does not entirely correlate with adhesion and invasion of Opc-expressing Nm to HBMEC and HMEC-1. For example, in the absence of overall upregulation of αvβ3, an increase was observed for Vn-dependent adhesion. This could be explained by changes in integrins other than their numbers. It is possible that integrin activation status changes thus affecting the affinity of ligand-receptor interactions. In further experiments, since Vn has been shown to better support the binding of Opc-expressing Nm to distinct human endothelial cells, the roles of αvβ3 and αvβ5 integrins (Vn receptors) have been investigated in greater detail.

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ABSTRACT 15

The role of *Campylobacter jejuni* glycoproteins during bacterial interactions with the human intestinal epithelia

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Campylobacter jejuni is the leading causative agent of bacterial acute gastroenteritis worldwide, with symptoms that range from mild diarrhoea to severe inflammatory enteritis. Recently a highly conserved 16 kb *N*-linked glycosylation locus, responsible for the glycosylation of more than 30 *C. jejuni* proteins, has been identified. Despite evidence for the role of this *N*-linked glycosylation locus in *C. jejuni* pathogenesis, very little is known about the exact biological role of these glycoproteins. We hypothesised that individual glycoproteins play critical roles in host–pathogen interactions, possibly by enhancing the ability of *C. jejuni* to adhere to and invade human epithelial cells or evade host immune defenses. The motility of individual *C. jejuni* 11168H glycoprotein mutants was assessed and three fully motile mutants (Cj0289/Cj0843/Cj0420) selected for further study. Adhesion to and invasion of Caco-2 cells *in vitro* and induction of IL-8 expression were investigated. A chick colonisation model was used to study colonisation ability *in vivo*. In comparison to the wild-type strain, the glycoprotein mutants were found to have different abilities to adhere to and invade Caco-2 cells. Intriguingly one mutant induced IL-8 expression at a lower level, one at similar levels and the other at higher levels compared to the wild-type strain. All three mutants showed reduced colonisation in the chick model. This work begins to increase our understanding of the role of glycoproteins in *C. jejuni* pathogenesis, raising the intriguing possibility that these glycoproteins may be involved in avoidance of the host innate immune defences.

ABSTRACT 16

Abstract title: Crystallization of SpeB-Spi complex – a novel protease–protease inhibitor pair from *Streptococcus pyogenes.*

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SpeB is a cysteine protease produced by the human pathogen *Streptococcus pyogenes*. Expression of SpeB is associated with an increase in virulence of the bacteria. SpeB is produced as a zymogen. The propeptide is released and after secretion producing a mature form of the protease. A recently described gene *spi* is located directly downstream of SpeB, codes for a protein which exhibits high sequence homology to the SpeB propeptide. Spi is not secreted. Spi was shown to have affinity for SpeB, suggesting that Spi is an intracellular inhibitor of SpeB and acts as a part of a control system for residual unsecreted SpeB. The aim of research is determination of the structure of SpeB in complex with Spi. Results will illustrate the nature of interaction between this protease-protease inhibitor pair. The structure of SpeB zymogen is known. It is thought that the Spi will bind to SpeB in similar fashion as SpeB propeptide however the ultimate evidence will come from the structure of the complex which will provide fine detail of the architecture of the protease-inhibitor interaction.

ABSTRACT 17

CYCLIC-DI-GMP SIGNALLING AND THE VIRULENCE OF THE PLANT PATHOGEN *XANTHOMONAS CAMPESTRIS*

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Xanthomonas campestris pathovar *campestris* (*Xcc*) is the causal agent of black rot disease, an important disease of cruciferous crops worldwide. The ability of *Xcc* to incite disease depends on several factors, including the synthesis of extracellular plant cell wall degrading enzymes, biofilm formation and dispersal and synthesis of the extracellular polysaccharide (EPS) xanthan. The *rpf* gene cluster of *Xcc* acts to positively regulate these phenotypes. RpfF, RpfG and RpfC are implicated in a regulatory system involving the diffusible signal factor DSF. The synthesis of DSF depends on RpfF and DSF perception and signal transduction is believed to involve RpfC/RpfG, a two-component system. RpfG is a novel regulator that contains an HD-GYP domain. Recent work from our laboratory has implicated this domain in turnover of the unusual nucleotide cyclic-di-GMP. Two other domains, GGDEF and EAL, have previously been implicated in cyclic-di-GMP metabolism. Proteins containing these domains may act in signal transduction systems that serve to link perception of different environmental cues to changes in cyclic-di-GMP levels. The aim of the work described here is to examine the role of these elements of the proposed cyclic-di-GMP regulatory network in the virulence of *Xcc* to Chinese radish. The mutants were created in the fully sequenced *Xcc* strain 8004 by either random insertional inactivation using Tn*5gus* followed by mapping or directed inactivation with the use of pK18*mob.* Mutants with disruptions in seven ORFs showed significant reduction in virulence compared to the wild type. Interestingly proteins which may be expected to be only involved in cyclic-di-GMP synthesis, (GGDEF domain alone) and only in cyclic-di-GMP turnover both appear to contribute to the ability of *Xcc* to incite disease. This and future studies of the *in vitro* phenotypes of the mutants should help establish the role of different elements of the proposed cyclic-di-GMP regulatory network in *Xcc*.

ABSTRACT 18

Retrospective detection of nosocomial outbreaks of CTX-M producing *E. coli*

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Objectives: The aim of this study is to determine the extent of clonal dissemination of CTX-M producing *Enterobacteriaceae* in Ireland.
Methods: One hundred and fifty confirmed ESBL producers received from clinical laboratories serving hospitals and communities throughout Ireland were examined for $bla_{\text{CTX-M}}$ using primers universal to all of the CTX-M phylogenetic groups. Positive isolates were then examined using primers specific to each of the groups, CTX-M-1, -2, -8, -9 and -25. Pulsed field gel electrophoresis (PFGE) using *Xba*I was performed on all isolates harboring a $bla_{\text{CTX-M}}$ gene.
Results: The $bla_{\text{CTX-M}}$ gene was detected in 88 isolates (63 $bla_{\text{CTX-M-group-1}}$, 24 $bla_{\text{CTX-M-group-9}}$ and 1 to be identified). The 63 isolates harboring a $bla_{\text{CTX-M-group-1}}$ gene were received from 8 laboratories between 2003 and 2005. PFGE analysis identified 30 major pulsed field profiles (PFPs). Ten CTX-M-group 1 producing *E. coli* isolates from one laboratory had indistinguishable PFPs. The isolates were collected from 8 patients, 2 male and 6 female ranging in age from 65 to 92 years. Six patients were associated with one hospital and 4 were associated with a single ward over approximately a 2 month period. The isolates were predominantly from urine specimens (80%). Another group of 9 closely related CTX-M group 1 producing isolates were identified from 4 hospitals in 3 cities.
Conclusions: These results confirm the significant risk of dissemination of CTX-M producing *E. coli* in a health care setting. The failure to recognize the outbreaks at the time suggests a need for greater awareness of the potential for nosocomial transmission of this group of pathogens.

ABSTRACT 19

NATURAL KILLER RECEPTOR^{+} (NKR^{+}) T-CELLS IN *HELICOBACTER PYLORI* INFECTION

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Helicobacter pylori (HP) is a primary cause of chronic gastritis, peptic ulcers and a cofactor in gastric cancer development. Although the nature of the host immune response influences the outcome of HP infection, the contribution of NKR^{+}T-cells is unclear, but they may protect tissues from damaging inflammatory-type responses and can control immune responses to infection and some tumours. The aim of this study was primarily the phenotypic characterisation of NKR^{+}T-cells in gastric mucosal tissues of HP- positive (HP^{+}) and -negative (HP^{-}) individuals and, secondly, an investigation of the stimulatory effects of HP lipopolysaccharide (LPS) on NKR^{+}T-cells. Biopsies obtained from the gastric antrum of HP^{+} and HP^{-} individuals were separated into their respective epithelial (EP) and lamina propia (LP) layers. Lymphocytes were stained with CD56, CD94, CD161 (NK markers), CD3 and V_24 (T-cell markers) and subjected to cytometric analysis. Significant populations of NKR^{+}T-cells occurred amongst EP and LP lymphocytes. In both, CD161^{+}T-cells were increased in HP^{+} individuals compared to HP^{-} individuals. Of the EP lymphocytes, CD56^{+} and CD94^{+}T-cells were decreased and of the LP lymphocytes, these populations were both increased in HP^{+} individuals, which suggests compartmentalised expansion and reduction respectively of these cells. NKR^{+}T-cell from peripheral blood or EP lymphocytes were cultured at 37^{o}C with three HP LPS preparations or *Salmonella typhimurium* LPS for 24h and then NKR^{+}T-cells were quantified using flow cytometry as above. Upon stimulation of EP lymphocytes with two of the three HP LPSs, CD56^{+}T-cells decreased whereas CD161^{+} and CD94^{+}T-cells increased. With the other HP LPS, all the categories of NKR^{+}T-cell increased. This suggests that dysregulation of NKR^{+}T-cell populations may be important in HP infection.

ABSTRACT 20

Clumping factor A of Staphylococcus aureus inhibits phagocytosis by human polymorphonuclear leukocytes

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Staphylococcus aureus is a major cause of nosocomial and community-acquired infection. *S. aureus* expresses several factors that promote avoidance of phagocytosis by polymorphonuclear leucocytes. Clumping factor A (ClfA) is a fibrinogen-binding surface protein of *S. aureus* that is an important virulence factor in several infection models. This study investigated whether ClfA is an antiphagocytic factor, and whether its antiphagocytic properties were based on its ability to bind fibrinogen. In *S. aureus*, ClfA was shown to be of equal importance to protein A, the antiphagocytic properties of which are well established. ClfA expressed in a surrogate Gram-positive host was also found to be antiphagocytic. A ClfA mutant that was unable to bind fibrinogen had a similar antiphagocytic effect to native ClfA in the absence of fibrinogen. ClfA inhibited phagocytosis in the absence of fibrinogen, and showed enhanced inhibition in the presence of fibrinogen.

ABSTRACT 21

CEACAM binding recombinant polypeptide confers protection against infection by respiratory and urogenital pathogens.

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The human specific pathogens *Neisseria meningitidis*, *N. gonorrhoeae*, *Haemophilus influenzae* and *Moraxella catarrhalis* share the property of targeting the Carcinoembryonic antigen (CEA)-related cell adhesion molecules (CEACAMs) expressed on human epithelia. CEACAMs are signalling receptors implicated in cell adhesion and regulation of several physiological functions. Their targeting by pathogens can lead to tissue invasion. Although the CEACAM-binding ligands of the bacteria are structurally diverse, they target a common site on the receptor. We have generated a recombinant polypeptide (rD-7) that blocks the interactions of mucosal pathogens with human epithelial cells and antibodies against it inhibit *M. catarrhalis* interactions with the receptor. As such, rD-7 is a potential anti-microbial agent to prevent infection via a strategy unlikely to promote bacterial resistance and a vaccine candidate against *M. catarrhalis*. In addition, the polypeptide could serve more widely as a novel research tool and as a potential therapeutic agent in CEACAM-based physiological disorders.

ABSTRACT 22

Molecular Characterisation of the *Salmonella*-specific Protein PagN

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Salmonellae are enteric pathogens that cause a range of illnesses, including gastroenteritis and enteric fever. During infection *Salmonella* adhere to and invade intestinal M-cells and epithelial cells. Once internalised, they are capable of multiplication and subsequent dissemination. The PhoP-activated gene *pagN* is broadly distributed within, and confined to the *Salmonella* genus. The gene-product is predicted to be a beta-barrel protein localised to the outer membrane. Furthermore, the primary sequence displays 58.75 % similarity to the *Escherichia coli* adhesin Tia. Expression of PagN is induced upon exposure to intracellular conditions such as low pH and low [Mg^{2+}]. This suggests that the protein may be a virulence determinant with a role in pathogenesis. Expression of PagN was directly correlated with the ability of recombinant *E. coli* to adhere to and invade epithelial cells. Adhesion and invasion was inhibited by addition of heparin, heparan-sulfate or chondroitin sulfate. Invasion was completely abolished in pgsA-745 cells; a proteoglycan-deficient CHO cell line. A *S. typhimurium* LT-2 *pagN* mutant showed a reduction in invasion phenotype, which could be restored with a low copy-number plasmid containing the *pagN* gene. *S. typhimurium* LT-2 over-expressing PagN failed to agglutinate erythrocytes. However, a *S. typhimurium galE* mutant, that expresses rough LPS, supported haemagglutination indicating a possible role for LPS in masking PagN.

The *pagN* gene directs the production of an outer membrane protein. PagN promotes adhesion to and invasion of epithelial cells through interactions with host cell surface proteoglycans. In particular, PagN binds to the heparin moieties of glycosaminoglycans.

ABSTRACT 23

Platelet activation by *Staphylococcus aureus*

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Staphylococcus aureus is the leading cause of infective endocarditis (IE), a serious life-threatening infection of heart valves. Platelet activation promoted by *S. aureus* resulting in aggregation and thrombus formation is thought to be an important step in the pathogenesis of IE. Isogenic *S. aureus* mutants defective in the expression of one or more surface proteins were used to identify factors responsible in mediating platelet activation. The fibrinogen-binding protein clumping factor A (ClfA) and the fibronectin-binding proteins (FnBPA, FnBPB) were shown to be potent activators of platelet aggregation when expressed by *S. aureus*. Both ClfA and FnBPA were capable of stimulating rapid platelet activation and aggregation when expressed on the surface of the non-aggregating surrogate host *Lactococcus lactis*. Aggregation was inhibited by function-blocking monoclonal antibodies recognising either the platelet fibrinogen receptor (GPIIb/IIIa integrin) or the platelet IgG Fc receptor (FcγRIIa), indicating an important role for these receptors in the activation process. Using plasma-depleted platelets, it was demonstrated that plasma bridging molecules (fibrinogen, fibronectin) promoted efficient, GPIIb/IIIa-dependent adhesion of platelets to ClfA- or FnBPA-expressing bacteria. Immunoglobulin (Ig) G in plasma was an essential co-factor for ClfA- and FnBPA-promoted activation, which is consistent with the requirement of a functional platelet FcγRIIa receptor. Furthermore the IgG had to contain specific antibodies recognizing the staphylococcal antigens that were engaging the resting platelets. A model is proposed whereby the expression of surface proteins that are capable of binding a platelet-reactive factor from blood, such as fibrinogen (ClfA, FnBPA) or fibronectin (FnBPA), promote bacterial-platelet binding through specific plasma protein bridging to GPIIb/IIIa on resting platelets. This is followed by recognition of specific antibodies bound to the activating bacterial protein by the platelet low-affinity IgG Fc receptor FcγRIIa, resulting in signalling events leading to platelet activation and aggregation.

ABSTRACT 24

Mutagenesis of the Rns Regulator of Enterotoxigenic *Escherichia coli*

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Enterotoxigenic *Escherichia coli* (ETEC) is a major cause of diarrheal disease in travellers and children in the developing world. ETEC accounts for approximately 210 million episodes of diarrhea and 380,000 deaths annually. Adherence of ETEC to the human intestine is an important event in the establishment of infection and is mediated by fimbriae such as CFA/I and the CFA/II group that includes CS1 and CS2 fimbriae. The expression of CS1 and CS2 fimbriae is positively regulated by the 30 kDa protein Rns, a member of the AraC family of transcriptional regulators. Currently little is known about what domains of the Rns protein are required for this activity.

Using pentapeptide insertion mtagenesis a pool of 5000 Rns mutants was screened and from this 16 independent insertion mutants were found to have altered ability to trans-activate the CS1 promoter. 6 of the mutants were truncates, however the remainder could be grouped into three classes: pentapeptide insertions within the C-terminal region of Rns (the putative DNA binding domain), insertions within the N-terminal domain (to which no function has been ascribed), and insertions in the leader sequence of the *rns* mRNA transcript. Mutants from the first and second group were assessed for their ability to bind to the CS1 promoter.

It was found that both the C-terminal region and the N-terminal region of the Rns protein are required for trans-activation of the CS1 promoter. It was also noted that the sequence of the 5' end of the leader sequence of the *rns* transcript is important in determining the copy number of Rns.

ABSTRACT 25

Three overlapping yet distinct Integration Host Factor (IHF) regulons in *Salmonella enterica* serovar Typhimurium.

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Nucleoid-associated proteins (NAPs) provide a dynamic protein scaffold which provides both structure to the bacterial nucleoid, and allows for the fine tuning of gene expression. This is done chiefly by facilitating or impeding the interaction between other transcriptional regulators and their target nucleotide sequences. Integration host factor, IHF, encoded by *ihfA* and *ihfB*, is a dimeric protein. The composition of the dimer changes with growth phase, and IHF abundance increases through the cell cycle. Mutants of *Salmonella enterica* serovar Typhimurium which lack the genes *ihfA, ihfB,* or both *ihfA* and *ihfB* were made, and the transcriptional profiles of these mutants were examined using DNA microarray technology.

The respective regulons were found to contain many similar elements and encoded functions important in diverse areas of bacterial metabolism, such as substrate utilisation, cellular motility and locomotion, and in the expression many of the virulence determinants of *S.* Typhimurium. However, variations in gene expression patterns in individual IHF mutants were identified showing that the three forms of the protein control three distinct yet overlapping regulons. Loss of the ability to produce either homodimer affected gene expression patterns differently; an inability to produce IHFα_2 exerted effects chiefly early in the growth cycle, whereas loss of IHFβ_2 most perturbed transcriptional patterns in later phases of growth. Genes whose transcription were most profoundly affected by the loss of heterodimeric IHF were also subject to regulation by RpoS, closely implicating IHF in the management of stationary phase gene expression.

ABSTRACT 26

The role of Lrp in regulation of the *fim* operon of *Salmonella enterica* serovar Typhimurium

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Salmonella enterica serovar Typhimurium (*S*. Typhimurium) is a Gram-negative broad host-range pathogen that causes a self-limiting gastroenteritis in humans and a systemic typhoid-like fever in mice. Bacteria that survive the acid pH of the stomach predominantly colonise the Peyer's patches of the small lumen of the intestine. Host cell adhesion is mediated by fimbrial surface appendages, of which *S*. Typhimurium has 13 putative operons. Type 1 fimbriae of the *fim* operon mediate adhesion to a wide variety of cells, including murine enterocytes. The leucine-responsive regulatory protein (Lrp) is a global regulator of gene expression found in many diverse bacterial species, including *E. coli* and *Salmonella*. Lrp is known as a global regulator of metabolism, but has also been shown to affect expression of fimbrial operons in both *E. coli* (*fim, pap*) and *S.* Typhimurium (*pef*). In this study, DNA microarray and RT-PCR analysis of the Lrp regulon of *S.* Typhimurium revealed a positive regulatory role for Lrp on *fimA,* encoding the major subunit of *S.* Typhimurium *fim* type-1 fimbriae, and other genes of the *fim* operon. The interaction of Lrp with the promoter regions of various genes of the *fim* operon was investigated by electrophoretic mobility shift assay. In addition, mannose-sensitive haemagglutination of erythrocytes was investigated and found to be absent in the *lrp* mutant strain. This phenotype was restored in a complemented strain. This work demonstrates an essential positive regulatory role for Lrp in the regulation of type-1 fimbriae of *S*. Typhimurium.

ABSTRACT 27

Investigation of the molecular basis for the inhibition of threonine deaminase by homocysteine in *Escherichia coli*

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Isoleucine biosynthesis in *Escherichia coli,* as well as in other organisms, is initiated by the conversion of L-threonine to α-ketobutyrate and ammonia. This reaction is catalyzed by threonine deaminase. Previous studies by our group have shown that this enzyme is inhibited by the sulphur-containing amino acid, homocysteine (Hcy). The question of how Hcy might inhibit the activity of threonine deaminase *in vitro* remains to be determined. To achieve this objective we have cloned and over-expressed threonine deaminase in *E.coli*. The enzyme was purified via the histidine tag (6x) engineered onto the C-terminus. After purification on Ni^{2+} columns, activity levels were determined using colorimetric assays. These assays showed that threonine deaminase rapidly lost activity following elution from the column. SDS-PAGE revealed that the purified enzyme migrated as two bands. Current studies are aimed at stabilizing the enzyme so that kinetic studies can be performed. These will shed light on the mechanism by which Hcy inhibits threonine deaminase

ABSTRACT 28

Haem transfer between components of the haem-uptake system in *Streptococcus equi*

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Iron is an essential element for microbial growth. To overcome the low availability of iron in mammalian hosts, pathogens have developed an array of iron uptake systems including the use of the iron-rich reservoir of haem-containing proteins. The group C streptococcus, *Streptococcus equi* subsp. *equi,* is an important upper respiratory tract pathogen of horses. Analysis of the *S. equi* genome sequence has identified a 10-gene cluster likely to be involved in haem uptake. Of the proteins encoded by this operon, Sehp possesses features of a membrane-bound protein, whereas HtseABC are likely to form a typical ABC transporter consisting of a ligand-binding lipoprotein, a permease and an ATPase, respectively. Recombinant Sehp and HtseA have been over-expressed and purified from *Escherichia coli*. Ligand binding experiments have shown that both of these proteins can bind haem and haemoglobin. In this study, we show by ELISA and by *in vitro* pull down assays that Sehp can interact with HtseA and based on analysis of spectral shifts that Sehp can capture haem from haemoglobin and transfer it to HtseA.

ABSTRACT 29

The role of Clumping factor B of *Staphylococcus aureus* in platelet activation

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Staphylococcus aureus can stimulate activation and aggregation of platelets which is thought to be important in the pathogenesis of infective endocarditis mediated by surface-located proteins. The fibrinogen-binding protein clumping factor A and the bifunctional fibrinogen and fibronectin binding proteins FnBPA and FnBPB stimulate rapid activation (1-2 min). This requires a fibrinogen or fibronectin bridge between the bacterial surface protein and the low activity form of the platelet integrin GPIIb/IIIa. In addition, ClfA or FnBP specific antibodies are requited to link the bacterial surface protein to the platelet Fc receptor FcγRIIa. Other surface proteins stimulate activation by a slower mechanism that requires specific antibody and the stimulation of complement fixation. Bacterial cells bind to platelets via a complement receptor and FcgRIIA. In both cases activation is triggered by clustering of FcγRIIA which stimulate signal transduction.

Clumping factor B (ClfB) is a surface-associated fibrinogen-binding protein that causes platelet aggregation with a longer lag time (6-7min). In order to investigate whether a fibrinogen or complement mechanism or a combination are utilised by ClfB, the protein was expressed in the surrogate host *Lactococcus lactis*. A non-fibrinogen binding mutant of ClfB (ClfB Q235A) still activated platelets without dramatically increasing the lag time. Inhibitors of platelet receptors GPIIb/IIIa and FcγRIIa caused complete inhibition of platelet activation by ClfB, as did a monoclonal function-blocking anti-ClfB antibody. Bacteria expressing the wild-type protein required only fibrinogen and IgG to stimulate activation. However the mutant protein required complement as well as IgG and fibrinogen. These experiments suggest a mechanism in which both fibrinogen and complement contribute to platelet activation promoted by ClfB.

ABSTRACT 30

Monitoring *Listeria monocytogenes* gene expression. Turning on the lights *in vivo*

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Here we describe the development of a novel luciferase based reporter system for *Listeria monocytogenes,* which allows exact translational fusions between the reporter and promoter of interest. To validate the *in vitro* and *in vivo* use of bioluminescence to measure bacterial gene expression, we have utilized the well characterized hemolysin (*hlyA*), *essential* for intracellular vacuolar escape, and the pre-protein translocase (*secA*), which plays an essential role in the translocation of unfolded proteins from the cytoplasm, across the cell membrane. The utility of the system in exemplified by the ability to monitor bacterial gene expression in a murine model of listeriosis.

Bron, P. A., I. R. Monk, S. C. Corr, C. Hill, C. G. M. Gahan. (2006) Novel luciferase reporter system for *in vitro* and organ-specific monitoring of differential gene expression in *Listeria monocytogenes*. Appl. Environ. Microbiol. *In press* (April)

ABSTRACT 31

Genetic tools for investigating *Pseudomonas aeruginosa* infection in animal models.

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Pseudomonas aeruginosa is an important opportunistic pathogen, capable of causing both acute and chronic infection. In the cystic fibrosis (CF) lung, chronic infection by *P. aeruginosa* leads to progressive respiratory damage and is the most common cause of morbidity and reduced life expectancy in CF patients
The pathogenicity of *P. aeruginosa* is attributable to the production of numerous virulence factors, the regulation of which is tightly controlled. Although a number of factors are recognised as playing a role in colonisation, little is known regarding the mechanisms responsible for the co-ordinated regulation and expression of genes involved in infection. Animal models of infection provide remarkable insights into the infection process and allow in-depth evaluation of the role of particular bacterial genes, via knockout mutants, in establishing infection in vivo.
The aim of this work was to generate luminescently tagged PAO1 and isogeneic mutant strains of *P. aeruginosa* for use in an animal model of acute pneumonia. The use of luminescently labelled bacteria allows better visualisation and tracking of the infection process in living animals, non-invasively and in real-time. A Tn7 based (Choi et al 2005) expression system was chosen because of its site and orientation specific insertion at a neutral intrageneic site. A modified lac promoter, $P_{A1/04/03}$ (Lanzer and Bujard, 1988) was cloned upstream of the lux CDABE operon. PAO1 and isogenic mutant strains of *P. aeruginosa* were successfully tagged and will be screened in a mouse model of acute pneumonia.

References

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ABSTRACT 32 SHORT PRESENTATION

Effects of secreted components of *Helicobacter pylori* On Epithelial Cell Tight Junctions

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INTRODUCTION: The gastrointestinal epithelium represents the first cellular barrier against intraluminal insults. The human epithelial cell line T84 differentiates in vitro into polarised monolayers with functional tight junctions. Tight junctions prevent the passage of molecules and ions through the space between cells. T84 cells therefore represent a good in vitro model for studying the physiological response to the gastrointestinal pathogen *Helicobacter pylori.*
AIM: To compare the effects of *H. pylori* outer membrane vesicles (OMV) and *H. pylori* soluble secreted proteins (HPE) on polarised epithelia.
MATERIALS AND METHODS: T84 cells were grown in the prescribed manner and seeded onto semi-permeable membranes. Polarisation and differentiation were monitored by measuring transepithelial electrical resistance (TER). Once confluence was reached, the cells were treated with OMVs, HPE or media alone. The cells were then processed for immunofluoresence staining.
RESULTS: HPE induced a dramatic reduction in TER after 1 to 2 hours of exposure, while OMV did not induce a significant reduction in TER after 24 hours exposure. To determine if a decrease in TER was associated with changes at the molecular level of the tight junctions, we analysed expression of the tight junctional protein ZO-1. In control monolayers, ZO-1 was expressed exclusively at the tight junctions. However, in HPE treated monolayers, there was a complete disruption of tight junctions and a redistribution of ZO-1 throughout the cell. There was no change in the expression of ZO-1 in monolayers treated with OMVs.
CONCLUSIONS: The decrease in TER and concomitant disruption of tight junctions caused by secreted components of *H. pylori* demonstrates the negative interaction between the bacterium and the epithelium.

ABSTRACT 33

Mechanisms of adhesion and invasion of *Burkholderia cepacia* of lung epithelial cells *in vitro*.

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Burkholderia cepacia complex (Bcc) are group of Gram negative bacteria which are opportunistic pathogens in cystic fibrosis patients. Nine species have been identified to date, two of which are considered most virulent*, B. cenocepacia* and *B. multivorans*. One aspect of these pathogens is that they cross the epithelial barrier of the lung and cause bacteraemia in a subgroup of patients. We have examined the invasion of six Bcc species in different lung epithelial cell models: A549, 16HBE-14o- and Calu-3 cells, in order to investigate the role that Bcc-lung cell interactions play in virulence. The latter two cell lines form well-differentiated monolayers when grown on semi-permeable supports, allowing us to study the effects that Bcc strains have on epithelial integrity and tight junction proteins. We have shown that the invasion process is saturable, indicating it is receptor mediated. In addition, invasion of Calu-3 and 16HBE14o- cells by strains of *B. multivorans,* was reduced when the cells were grown as tight monolayers compared with that on plastic, suggesting basolateral receptors are required for the process. In contrast, four strains of *B. cenocepacia,* showed comparable invasion of both cell lines irrespective of culture model. We are currently investigating the identity of the receptors involved in adhesion using glycosidases and other enzymes. All species of Bcc examined reduced the transepithelial resistance of Calu-3 cell monolayers and readily translocated across the epithelial monolayer. Overall, it is apparent that the different species within the complex have different mechanisms of invasion. However, it is also clear that the potential to cause bacteraemia is not limited to the two most commonly found species.

ABSTRACT 34

Investigating the impact of human bronchial epithelium on *Pseudomonas aeruginosa* protein expression

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Pseudomonas aeruginosa is gram-negative bacterium capable of colonizing a diverse number of niches. Therefore, successful adaptation to a new host environment, like that of the lung, relies on the ability of the organism to respond to alterations in the surrounding environment. Most lung pathogens initially interact with their host at epithelial surfaces where they adhere and then proceed to colonize, multiply locally, or invade deeper into host tissues. Epithelial cells are the first physical line of defense against infection and are remarkable for their ability to provide critical physiological functions in response to frequent microbial attack.
It is hypothesised that *P. aeruginosa* builds up an array of adaptive responses, including, altered gene and protein expression, which allows this bacterium to counteract the epithelial defense mechanism and establish infection. Our objective is to characterize the initial adaptation of *P. aeruginosa* to host tissue. Using a proteomics approach we plan to gain an insight into the initial stages of *P. aeruginosa* infection and to identify bacterial genes and proteins regulated in response to respiratory epithelial cells.
Following *P. aeruginosa* infection of human bronchial epithelial (HBE) cell lines, total bacterial protein was isolated. Initial results indicate altered expression of a number of bacterial proteins in response to HBE cells. Identification of these proteins is currently underway. Preliminary data indicates that a proteomics approach may be very useful in identifying mechanisms of the *P. aeruginosa* human infection process, as well as having the potential to propose new therapies to halt these infections.

ABSTRACT 35 SHORT PRESENTATION

Structural Analysis of a Cell envelope protease

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C5a peptidase is a highly specific cell envelope protease (CEP) produced by *Streptococcus pyogenes* and is thought to aid the bacterium evade the host immune system. C5a peptidase specifically cleaves human complement component C5a at the C terminus between residues His67 and Lys68 releasing a seven amino acid peptide. Removal of the C terminus fragment considerably reduces the ability of C5a to act as a chemoattractant for polymorphonuclear lymphocytes (PMNL's). C5a peptidase exhibits high sequence similarity with other members of the CEP's such as those found in *Lactococcus lactis*. These CEP's cleave casein in a specific pattern and are used extensively in the food industry to create dairy products with specific flavours and tastes. This study is a structural analysis of CEP's. We have crystallised the surface exposed domain of C5a peptidase and X-ray diffraction studies are on going. Elucidation of the structure of the structure of C5a peptidase will give an insight into the proteolytic mechanism of CEP's. This will facilitate the modulation of cheese and other dairy products using related CEP's. Additionally it will reveal the structural reasons for the high specificity of C5a peptidase and its role in *S. pyogenes*, an important human pathogen, in its evasion of the human immune system.

ABSTRACT 36

The *qnrA* gene was not detected as a mechanism of quinolone resistance in *salmonella* in Ireland in 2001-2005

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OBJECTIVES: To screen non-typhoid *Salmonella enterica* isolates from Ireland for the plasmid encoded quinolone resistance determinant *qnrA*.

METHODS: Five thousand and ninety isolates of non-typhoid *Salmonella enterica* from humans and animals were received by the National Salmonella Reference Laboratory of Ireland between 2001 and 2005. Susceptibility to nalidixic acid and ciprofloxacin was performed in accordance with Clinical Laboratory Standards Institute disk diffusion methods. Ciprofloxacin MICs were determined by Etest for all nalidixic acid resistant isolates. Isolates with a CIP MIC of ≥ 0.25 mg/ml were screened for the presence of *qnrA* by PCR using specific primers previously described. A *qnr*A positive isolate was includes as a positive control.

RESULTS: Sixty seven (1.3%) isolates had a ciprofloxacin MIC ≥ 0.25 mg/ml. The *qnr*A positive isolated yielded a product of expected size. All isolates tested were negative for the *qnrA* gene

CONCLUSION: The plasmid mediated quinolone resistance determinant *qnrA* identified in non typhoid *Salmonella enterica* isolates in other countries was not detected in human or animal isolates from Ireland in the period 2001 to 2005

ABSTRACT 37

The role of Fis and DNA supercoiling during intracellular survival of *Salmonella enterica* serovar Typhimurium

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Salmonella enterica serovar Typhimurium causes human gastroenteritis and a typhoid-like disease in mice. The ability to invade and survive within host cells is essential for infection. Survival of *Salmonella* during macrophage infection is associated with a relaxation of DNA supercoiling in the bacteria. The DNA binding protein Fis has been shown to regulate many virulence genes during *Salmonella* infection including the SPI-2 genes involved in macrophage survival. In this study we investigate the roles played by DNA supercoiling and Fis in the expression of the SPI-2 genes. Reporter plasmid topoisomer analysis showed that a decrease in negative supercoiling occurs during infection of macrophages but not during infection of epithelial cells. In addition *fis* mutants were unable to induce a similar relaxation of supercoiling during infection of macrophages. Flow cytometry revealed that *fis* gene expression is induced intracellularly during macrophage infection but not during intracellular survival in epithelial cells. The SPI-2 regulatory gene *ssrA* was induced by relaxation of DNA supercoiling following DNA gyrase inhibition with Novobiocin or following induction of Fis expression *in vitro* or during infection of both macrophage and epithelial cell lines. We report that the *ssrA* gene is induced by relaxation of negative supercoiling but that this process (DNA relaxation) does not occur in epithelial cells. In addition we report that the Fis protein plays a role in this process suggesting that this protein can induce transcription of *ssrA* directly by binding at the promoter and indirectly by affecting the overall level of DNA supercoiling.

ABSTRACT 38

Molecular characterisation of citrate hydroxamate and hydroxamate type siderophore acquisition systems by *Pseudomonas aeruginosa*.

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Pseudomonas aeruginosa produces two siderophores, pyoverdine and pyochelin, but is also capable of utilising several xenosiderophores including citrate hydroxamate and hydroxamate type siderophores. Analysis of the *P. aeruginosa* PA01 genome sequence revealed the presence of two genes, *chtA* and PA1365, encoding proteins displaying significant similarity to the aerobactin outer membrane receptor, IutA, of *Escherichia coli*. The *chtA* and PA1365 genes were mutated by insertional inactivation and it was demonstrated that *chtA* encodes the outer membrane receptor for aerobactin and the structurally similar siderophores rhizobactin 1021 and schizokinen. Expression of *chtA* was demonstrated to be iron regulated; however in contrast to previously characterised siderophore receptors from *P. aeruginosa*, ChtA was not induced in the presence of its cognate siderophore.

Analysis of the *P. aeruginosa* genome sequence revealed the presence of relatively few inner membrane siderophore transport systems with respect to outer membrane siderophore receptors. *In* silico analysis of the *P. aeruginosa* genome sequence resulted in the identification of a putative inner membrane siderophore transporter and it was demonstrated that expression in a *Sinorhizobium meliloti* siderophore transport mutant conferred upon the strain the ability to utilise the hydroxamate type siderophores, ferrioxamine B and ferrichrome. A *P. aeruginosa* mutant was unaffected in ferrioxamine B and ferrichrome utilisation suggesting redundancy of transport across the inner membrane.

ABSTRACT 39

Evidence for positive regulation of intercellular adhesion (*ica*) operon transcription by the araC-type transcriptional regulator Rbf in Staphylococcus aureus.

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Staphylococci are important pathogens in biofilm-associated infections of implanted medical devices. Currently the *ica* operon-encoded polysaccharide intercellular adhesin (PIA/PNAG) is the best understood mechanism of biofilm development in staphylococci. Recently Lim et al. (J. Bacteriol. 186:722-9) reported that an *araC*-type transcriptional regulator, designated *rbf* (regulator of biofilm formation) was required for biofilm development in *Staphylococcus aureus*. Overexpression of the *S. epidermidis rbf* homologue, and to a lesser extent the *S. aureus rbf* gene, induced macroscopic cell aggregation in liquid cultures of both *S. epidermidis and S. aureus*. Rbf-induced cell aggregates were dispersed by treatment with sodium metaperiodate but not proteinase K suggesting that the extracellular matrix mediating intercellular adhesion was carbohydrate in composition. Consistent with this, Rbf-induced cell clustering in *S. aureus* was *icaADBC*-dependent. In addition this phenotype was also dependent on *sarA*, which is known to be required for PIA/PNAG-mediated biofilm but was σ^B-independent, which is not required for biofilm development in *S. aureus*. Interestingly overexpression of *rbf* strongly activated *ica* operon expression thus revealing the genetic basis for increased intercellular adhesion. However this finding contrasts with previous data which revealed that mutation of the *rbf* gene had no significant effect on *ica* transcription. Taken together these data indicate that *rbf* controls biofilm development in *S. aureus* via *ica*-dependent and *ica*-independent mechanisms.

ABSTRACT 40 SHORT PRESENTATION

The Post-transcriptional Regulator RsmA plays a Role in the Interaction between *Pseudomonas aeruginosa* and Human Airway Epithelial Cells by Positively Regulating the Type III Secretion System

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Pseudomonas aeruginosa is an opportunistic Gram-negative bacterium capable of causing infections in plants, animals and humans. Successful adaptation of the bacterium to its host environment relies on the ability of the organism to tightly regulate gene expression. RsmA is a small RNA-binding protein that plays an important role in the post-transcriptional regulation of a large number of virulence-related genes in *P. aeruginosa*. However, the role of *P. aeruginosa* RsmA in bacterial-host interactions has not yet been investigated.

This study showed that during the interaction between *P. aeruginosa* and airway epithelial cells, an *rsmA* mutant failed to induce morphological changes in host cells such as cell rounding and actin depolymerization and failed to cause cytotoxicity of epithelial cells. The *rsmA* mutant displayed significantly increased invasion of *P. aeruginosa* into airway epithelial cells over a wide range of multiplicities of infection (MOI), while adhesion was not significantly affected at the majority of the MOI levels investigated. As these cellular phenotypes have previously been associated with a defective type III secretion system (TTSS), regulation of the TTSS in *P. aeruginosa* was examined. Immunoblotting showed that the *rsmA* mutant was defective in the secretion of the type III effector proteins ExoS and ExoT and in the synthesis of proteins involved in the translocation apparatus of the TTSS. Furthermore, the *rsmA* mutant showed decreased transcription of several regulators of the TTSS. Taken together, these data show that RsmA plays a novel role in regulating the virulence-associated TTSS with important consequences on the interaction between *P. aeruginosa* and airway epithelial cells.

ABSTRACT 41

Cold anaerobic microbiological degradation of pentachlorophenol

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Microbiological waste treatments include aerobic and anaerobic processes. Aerobic treatment is efficient but is energy-demanding and produces excess biomass requiring safe disposal. Anaerobic processes, although often reducing waste by only c.85%, consume less energy and produce biogas. Furthermore, psychrophilic (<18°C) anaerobic digestion (PAD) may offer increased cost-efficiency and sustainability.

Two expanded granular sludge bed-anaerobic filter (EGSB-AF) bioreactors, designed by the authors, were seeded with anaerobic granular biofilms (sludge) and used to treat pentachlorophenol (PCP)-contaminated (1-15 ppm) wastewater at 9-15°C at PCP loading rates of 1-10 g m^{-3} d^{-1} for 436 days.

Batch assays were performed at 15°C (psychrophilic) and 37°C (mesophilic) to measure microbial activity, *in vitro* PCP-depletion and toxicity under aerobic or anaerobic conditions using biomass from an aerobic treatment facility or temporal granular/fixed-film samples from the EGSB-AFs, respectively. Aerobic and anaerobic activities were determined by CO_2 production and specific (acetate/hydrogen-induced) methanogenic activity (SMA) measurements, respectively.

Efficient chemical oxygen demand removal (≥90%), biogas methane composition (60-70%) and PCP removal were achieved by the EGSB-AFs when the PCP influent concentration was 1-10 mg l^{-1}, with reduced efficiencies only after the addition of wastewater containing 15 mg l^{-1}.

SMAs and toxicity thresholds differed between granules and fixed-films – thus, bioreactor design may facilitate spatially-specific development of different functional communities – but were highest in 37°C-hydrogenotrophic assays (>800 ml CH_4 g [biomass]$^{-1}$ d^{-1}). Thus, most methane was channelled through H_2/CO_2, rather than acetate, and methanogens remained mesophilic – although psychrotolerant –rather than psychrophilic. PCP-degradation in, even low-temperature, anaerobic batch assays compared favourably with those in aerobic tests.

From our data, PAD appears feasible, and more advantageous than aerobic treatment, for PCP mineralization.

ABSTRACT 42

Characterisation of point mutations influencing the binding of Spi to SpeB

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Streptococcal pyrogenic exotoxin B (SpeB) is an extracellular cysteine protease produced by *Streptococcus pyogenes*.

SpeB is an important virulence factor of *S. pyogenes* and "knock-out" mutations in this gene have resulted in strains with reduced pathogenicity. It is produced as a zymogen. The mature protease is generated by auto proteolysis of the prosegment preventing ectopic cleavage of the bacterial proteins by SpeB.

Downstream of the *speb* gene is a gene, which encodes a specific, intracellular protease inhibitor termed Spi. The proposed function of Spi is protection of the intercellular space from the action of residual unsecreted mature SpeB and thus is essential for cell survival.

The aim of this study is to characterize residues essential for the interaction between Spi and SpeB. Target residues in Spi have been mutated using a PCR based method. Recombinant mutated proteins were expressed in *Escherichia coli* DH5_ using the expression system pProEXHT-b and the protein purified using IMAC.

The purified proteins have been used in Kinetics Assays to determine their ability to inhibit SpeB. Data suggests that several of these mutant forms of Spi have an altered binding affinity to SpeB as compared to wild type Spi.

ABSTRACT 43

Genetic analysis of the biofilm phenotype in methicillin resistant *Staphylococcus aureus*

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Biofilm development by meticillin resistant *Staphylococcus aureus* (MRSA) represents an additional pathogenic mechanism in its already impressive arsenal of virulence determinants. Enzymes encoded by the *ica* operon synthesise an extracellular polysaccharide adhesin implicated in biofilm formation. However, preliminary research in our laboratory revealed that glucose-induced biofilm development in MRSA isolates appears to be *ica*-independent. In this study we investigated the contribution of *ica* and other genes associated with biofilm development in clinical isolates of MRSA. Bacteriophage 80 _ was used to transduce *ica* operon deletion mutations into MRSA strains displaying a glucose-dependent biofilm phenotype. Mutations in the *ica* operon had no effect on biofilm development in seven clinical MRSA strains but did result in a biofilm negative phenotype in the laboratory strain *S. aureus* RN4220. Bacteriophage 80_ was also used to generate deletion mutations in the global regulators of virulence SarA (staphylococcal accessory regulator) and Agr (accessory gene regulator), which are known to influence the staphylococcal biofilm phenotype. Deletion of *sarA* in two clinical MRSA isolates and in *S. aureus* RN4220 resulted in a biofilm negative phenotype. In contrast, deletion of the *agr* locus did not affect the biofilm phenotype in ten clinical MRSA strains tested or in *S. aureus* RN4220. These findings support the existence of an *ica*-independent biofilm phenotype amongst clinical strains of MRSA and further reveal that this MRSA biofilm phenotype is *sarA*-dependant and *agr*-independent.

Abstract 44 SHORT PRESENTATION

Molecular Analysis of the Interaction Between Staphylococcal Protein A and von Willebrand Factor"

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Protein A of *Staphylococcus aureus* (Spa) is a cell surface protein comprising 4-5 immunoglobulin-binding domains, each arranged in an anti-parallel three-helix bundle. In addition to binding the Fcγ region of IgG, each Spa domain can bind to the Fab region of immunoglobulin bearing V_H3 heavy chains. Indeed, the structures of individual Spa domains in complex with the Fcγ and V_H3-Fab of IgM have been solved. Protein A also binds to von Willebrand factor (vWF), a large multimeric plasma protein that is essential for haemostasis. vWF binds to damaged blood vessel walls and can immobilise platelets to this site of damage through a high affinity interaction with the platelet receptor GpIb, initiating the formation of a clot. To investigate the Spa-vWF interaction, a series of recombinant Spa truncates fused to Glutathione *S*-transferase were constructed. Recombinant vWF truncates were tested for binding to GST-Spa. Single Spa domains bound to the vWF D'-D3 and A1 domains, but not to the A3 domain. Binding to the A1 domain of vWF was shown to be the dominant interaction, with estimated half-maximal binding at 100 nM of ligand. Furthermore, single domain Spa variants with substitutions known to disrupt Fcγ or V_H3-Fab binding were constructed. Residues involved in Fcγ binding were demonstrated to also be of importance in vWF binding. In contrast, substitutions affecting binding of V_H3-Fab did not affect the vWF interaction. vWF may act as a bridge between platelets and bacterial cells under the shear conditions present in flowing blood and have importance in the pathogenesis of staphylococcal endocarditis.

ABSTRACT 45

Identification, Cloning and Expression of two human Metapneumovirus (hMPV) Genes.

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Viral respiratory diseases are a major health problem. In 2001, a new paramyxovirus, human Metapneumovirus (hMPV) was identified in the Netherlands. The virus, like human Respiratory Syncytial Virus (hRSV), appears to be ubiquitous and is an important cause of respiratory diseases in diverse subpopulations. Symptoms include upper respiratory tract infections to severe bronchiolitis and pneumonia. HMPV is most prevalent in the December to April periods. The hMPV genome encodes key genes corresponding to the Matrix (M), Phosphoprotein (P), Glycoprotein (G), Fusion (F) and nucleoprotein (N) viral proteins. In this study, the M and P genes were individually cloned and expressed in *E. coli*. Both proteins were purified to homogeneity by His_6 affinity chromatography and MALDI-ToF mass spectrometry used to confirm recombinant protein identity (M protein: 36% sequence coverage). Serological analysis showed low IgG immunoreactivity against the M recombinant protein in normal human sera by ELISA. This is possibly a result of minimal, or loss of, antibody response to the M protein or the absence of conformational epitopes in the M protein.

ABSTRACT 46

Role of Cortactin in pedestal formation by EPEC

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Escherichia coli enterophatogenic (EPEC) is able to adhere to epithelial cells through the formation of an actin pedestal. Some of the signals that control pedestal formation are well established. Thus, phosphorylation of Tir, a bacterial protein, allows the recruitment of the cellular adaptor Nck which in turns brings and possibly activates N-WASP. Then N-WASP would activate the Arp2/3 complex initiating actin synthesis. N-WASP K.O. cells are impaired in pedestal formation what confirms the essential role of this protein.

Cortactin is another actin nucleator due to its capacity to directly activate the Arp2/3 complex. Even more, as we reported, cortactin can indirectly activate the Arp2/3 complex through the secondary activation of N-WASP. We proposed a switch on/off model of regulation of cortactin activation of N-WASP by its respective erk and src mediated phosphorylation (Martinez-Quiles et al., Mol Cell Biol 2004, 24(12): 5269).

Although cortactin is recruited to the pedestal its function on pedestal formation has not been well characterised. The aim of this work is to dissect the role of cortactin in pedestal formation focusing in our proposed model of regulation. For that purpose we have used various cortactin mutants fused to GFP and analyzed their effect on pedestal formation. Some of these mutants mimick or impair erk or src phosphorylation, others impair Arp2/3 complex activation or N-WASP binding/activation. To demonstrate that cortactin, similarly to N-WASP, is essential for pedestal formation we have Knock-out cortactin by siRNA.

Our results indicate that suppression of Cortactin by siRNA inhibits pedestal formation and that both erk and src phosphorylation of cortactin are required for pedestal formation. Our findings will be discussed in detail

ABSTRACT 47

Meningococcal secreted proteins modulate apoptosis in human cells.

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Neisseria meningitidis is capable of secreting soluble proteins (MSPs) into the extracellular environment. MSPs include the iron-regulated proteins FrpC and FrpC2, which are homologous to a number of repeat-in-toxin (RTX) toxins, and are secreted via the specialised type I secretion pathway, several autotransporter proteins including IgA protease and App, as well as a large number of uncharacterised proteins.

The biological effects of MSPs were investigated *in vitro* using cell lines of both epithelial and endothelial origin. MSPs were purified from wild-type *N. meningitidis* (wt MSPs) and from a *N. meningitidis* mutant not expressing either FrpC or FrpC2 (DKO MSPs). Cells were pre-incubated for 48 hours, prior to harvest with wt / DKO MSPs (12µg/ml), and challenged 18 hours prior to harvest with a sub-lethal dose of an apoptosis-inducing agent, staurosporine (STS). Once harvested, the host cells were stained with rhodamine 123, which stains polarised, healthy mitochondria, and the cells were then analysed using flow cytometry.

Both the wt and DKO MSPs resulted in an enhancement of apparent apoptosis against cells of a human bronchial epithelial cell line (BEAS-2B) challenged with a sub-lethal dose of STS. This synergistic activity was not observed with the other cell lines tested. Caspases are responsible for mediating the apoptotic response in damaged or stressed cells. Addition of caspase inhibitors attenuated the apoptotic response observed in cells treated with MSPs and STS.

The results demonstrate that unidentified MSPs may play a role in the modulation of apoptosis of certain host cells during meningococcal disease but that other factors are also likely to play a role.

ABSTRACT 48

Investigation of host cell response after infection of *N. meningitidis* with cerebrovascular endothelial and respiratory epithelial cell lines.

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Meningococcal cellular association and invasion has the ability to potentiate bacterial pathogenesis through the use of specialised surface-exposed adhesins, such as type IV pili and Class 5 opacity proteins. Physiologically relevant cells derived from the cerebrovascular and respiratory systems would provide a suitable model of potential contact sites of *Neisseria meningitidis* during infection.

Using functional *in vitro* assays, including confocal microscopy and transmission electron microscopy, meningococcal cells have been show to effectively associate and invade physiologically relevant cell lines derived from the cerebrovascular region (HBMEC and meningothelial), as well as cells derived from the respiratory epithelium (BEAS-2B).

After meningococcal infection (8hrs), RNA was extracted from these host cells and hybridised to 30K-gene human microarray slides. The profile of gene expression after meningococcal infection was investigated and compared across the cell lines used, for example in terms of origin specificity, as well as significantly expressed genes. Preliminary analysis has already identified generic as well as cell line-specific differential expression of key genes involved in inflammation and apoptosis. The possible significance of cell lineage-specific differential gene expression will be discussed.

ABSTRACT 49

Adhesion and cytokine induction profiles of two *Helicobacter pylori* motility mutants

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Helicobacter pylori colonises the gastric mucosa of humans and contributes to the development of a spectrum of diseases including gastritis and gastric cancer. *H. pylori* motility is driven by polar flagella, and non-motile strains cannot successfully colonise, at least in animal model studies. In the present study we used two motility-deficient mutant strains to investigate if motility was important for adhesion to, and cytokine induction by, gastric epithelial cells.

Mutations in the *H. pylori fliK* gene, and that of a potential flagellar chaperone were generated by insertion of a chloramphenicol resistance marker into strain CCUG17874. Bacterial motility was measured using a Bactracker instrument. Adhesion to AGS human gastric epithelial cells was assessed using a previously described *in vitro* plate count method, and induction of interleukin-8 (IL-8) was measured using ELISA.

Both mutants had decreased motility in terms of curvilinear velocity and run-stop time compared to wild-type. Western blot revealed the *fliK* mutant had truncated flagella which contained mostly hook protein and very little flagellin while the potential chaperone mutant appeared to have wild-type levels of flagellin protein. Both mutants had a statistically significant reduction in adhesion to AGS cells of approximately 50% ($p<0.005$). There was no difference in the amount of IL-8 produced by AGS cells infected with the wild-type or *fliK* mutant strains. The potential chaperone gene mutant induced 20.1% ($p<0.02$) less IL-8 than the wild-type

Motility-deficient *H. pylori* mutants adhered less well to AGS cells. This maybe because these mutants are unable to swim through the culture media and have less contact with AGS cells, or because aberrant flagellar expression has also altered expression of an adhesion. The observation that a 50% reduction in adhesion had little or no effect on cytokine induction suggests cell-cell interaction is not the main mechanism involved in inducing IL-8 during *H. pylori* infection.

ABSTRACT 50

Comparison of Biochemical and PCR assays to distinguish *Ralstonia pickettii* and *Ralstonia insidiosa*

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Ralstonia pickettii is an emerging serious nosocomial pathogenic organism. It has been implicated in many serious infections including bacteremia and respiratory infections and has also been detected in the lungs of Cystic Fibrosis sufferers [1]. *Ralstonia insidiosa* which very similar to *R. pickettii* but of which very little is known is also emerging as a pathogen. This bacterium is being misidentified as *R. pickettii*.

To date no commercially available biochemical kits have been updated to differentiate between *R. pickettii* and *R. insidiosa*. A PCR assay for identification of *R. pickettii* and *R. insidiosa* targeting the 16S rRNA gene has recently been described [2].

We undertook a study on 58 strains identified as *R. pickettii* through biochemical means (Vitek NFC and API 20 NE) for comparison of traditional and molecular identification systems. Biochemical identification kits (API 20 NE and RapID NF plus) were also used in conjugation with other biochemical reactions including susceptibility to desferrioxamine to determine if patterns could be distinguished that might be used to differentiate *R. pickettii* and *R. insidiosa*.

14 strains identified biochemically as *R. pickettii* were identified as *R. insidiosa* using the PCR assay. Strains previously verified susceptibility of *R. pickettii* to desferrioxamine could not be used to differentiate the two bacteria [3], as 33% of the *R. pickettii* strains (identities confirmed by PCR) were resistant to the chemical. All *R. insidiosa* (identities confirmed by PCR) strains were found to be resistant to desferrioxamine as previous verified.

[1] Ryan, M.P., Pembroke J.T., and Adley C.C. (2006) *Ralstonia pickettii*: a persistant gram-negative nosocomial infectious organism. *J Hosp Infect* **62**, 278-284

[2] Coenye, T., Goris, J., De Vos, P., Vandamme, P. & LiPuma, J. J. (2003) Classification of *Ralstonia pickettii*-like isolates from the environment and clinical samples as *Ralstonia insidiosa* sp. nov. *Int J Syst Evol Microbiol* **53**, 1075–1080.

[3] Vaneechoutte M, Kampfer P, De Baere T, Falsen E, Verschraegen G. (2004) *Wautersia* gen. nov, a novel genus accommodating the phylogenetic lineage including Ralstonia eutropha and related species, and proposal of *Ralstonia* [*Pseudomonas*] *syzygii* (Roberts *et al*. 1990) comb. nov. *Int J Syst Evol Microbiol*; **54**: 317-327.

ABSTRACT 51

The Role of Dps in Gene Regulation in *Salmonella enterica* serovar Typhimurium

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Salmonella enterica serovar Typhimurium is a Gram-negative facultative intracellular pathogen that causes gastroenteritis in humans and a systemic typhoid fever-like disease in mice. *Salmonella* is acquired through the ingestion of contaminated food or water. Having survived the harsh environment of the stomach it can traverse the intestinal mucus layer and attach to the epithelial cells of the small intestine. Dps (DNA-binding protein from starved cells) is a low molecular mass protein that belongs to the family of nucleoid-associated or histone-like proteins. The level of Dps increases at the stationary phase of growth until it reaches levels of 180,000 molecules per cell, making Dps the most abundant protein in the cell at stationary phase. Dps is required for oxidative stress resistance in *Salmonella typhimurium* and for survival in the host macrophage. 2-D gel electrophoresis and mass spectrometry were used to identify proteins whose expression pattern is affected by the presence and absence of Dps. The results revealed that a wide variety of proteins are differentially affected. A number of proteins involved in metabolic pathways such as glycolysis and the tricarboxylic acid (TCA) cycle are down-regulated more than four-fold in the *dps* mutant. In addition, Dps was revealed to play a role in the regulation of flagellar phase variation. These data suggest that Dps plays an important role in regulation of virulence factors as well as proteins concerned with metabolism.

ABSTRACT 52

Analysis of a Glycosylation locus Containing a Putative Adhesin from *Streptococcus pneumoniae*

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Streptococcus pneumoniae is an important human pathogen causing diseases such as meningitis, pneumonia and septicaemia. The genome of *S.pneumoniae* strain TIGR4 contains a 37kb DNA island which is not present in strain R6. This contains 18 putative genes. Sequence analysis suggests these fall into 3 different categories: (i) Glycosyl-transferases (ii) Secretion machinery (iii) Unknown function. The genes of unknown function include an ORF encoding for a large serine rich repeat protein annotated as SP1772 in the TIGR4 genome. The presence of these repeats suggest that the protein may be heavily glycosylated, probably by the Glycosyl-transferases in this island. Previously SP1772 and 3 further genes from this locus were identified as virulence factors using a signature tagged mutagenesis screen of TIGR4. A homologue of SP1772 in *Streptococcus gordonii* has been shown to be involved in adhesion to a glycoprotein of platelet membranes, Ib_.

The operon structure of the locus in wt TIGR4 has now been assessed using RT-PCR. Results indicate the presence of three different transcripts, each containing between 2 and 11 of the 18 genes. This data has been compared with the GES (Goldman, Engelman and Steitz) hydrophobicity scale for each gene and a correlation between membrane spanning regions and presence on a transcript has been found. Microarray analysis and PCR have been used to determine the distribution and expression pattern of this locus in several clinical isolates of *S. pneumoniae*.

We have constructed several deletion mutants in this region in the TIGR4 strain background. Analysis of a SP1772 null mutant in both systemic and respiratory infection models using MF-1 mice show this putative gene plays no role in pathogenesis by these infection routes. We are investigating whether this locus plays a more subtle role *in vivo* by the use of competitive index methods and models of nasopharyngeal colonisation.

ABSTRACT 53

A novel promoter trap for identifying *in vitro* and *in vivo*-induced genes in *Listeria monocytogenes*

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The Gram positive foodborne pathogen *Listeria monocytogenes* encounters acid environments during growth in low pH foods, during passage through the stomach following consumption and within the macrophage phagosome during systemic infection. In this study, we developed a dual reporter gene plasmid system, which allows us to identify promoters that are induced *in vitro* under stress conditions as well as *in vivo* during murine infection. This dual reporter gene strategy termed the GAD-IVET system is based on a plasmid containing a promoterless copy of the glutamate decarboxylase gene (*gadB)*, which is required for survival at low pH (≤ pH3.5), and the haemolysin gene (*hly*) whose product facilitates escape from the macrophage phagosome *in vivo* and is responsible for zones of haemolysis on blood agar plates *in vitro.* Random fragments of *L. monocytogenes* genomic DNA, were incorporated into the multiple cloning site of this plasmid. To identify clones that expressed GAD upon adaptation to low pH *in vitro*, the bank was subjected to a sublethal acid stress (pH5) and subsequently exposed to pH2.5 (lethal pH) to select clones that were 'on' under pH5 conditions. The resulting clones were then plated on blood agar allowing us to identify clones that are 'off' (haemolysis negative) at pH7. These clones therefore contain fragments (promoters) that are not constitutively expressed but induced specifically in response to acid stress. Similarly we selected *in vivo*-induced clones from the GI tract of mice orally inoculated with the bank by exposing *ex vivo* clones directly to lethal pH (pH2.5).

We demonstrate that this system can be utilised to identify stress-inducible promoters *in vitro* as well as *in vivo*-inducible promoters expressed *in situ* in the GI tract. The benefits of utilising haemolysin as a reporter for this system will be discussed.

ABSTRACT 54

Expression and Characterisation of a Nonribosomal Peptide Synthetase from *Aspergillus fumigatus*.

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Aspergillus fumigatus is a significant human pathogen. Organismal virulence has been proposed to be multi-factorial and mediated by a range of proteinaceous and non-proteinaceous moieties. Nonribosomal peptide synthesis (NRPS) is responsible for a significant proportion of toxin and siderophore production in the organism. Nonribosomal peptide synthetases are multi-modular enzymes that facilitate NRPS. Furthermore, it has been shown that 4'-phosphopantetheinylation is required for the activation of key enzymes involved in NRPS in both *Aspergillus fumigatus* and other species whereby a coenzyme A (CoA)-derived 4'phosphopantetheine group is transferred to thiolation (T) (or peptidyl carrier protein (PCP) domains) in NRPS enzymes. Here we report the identification of an atypical 25.5 kb NRP synthetase gene (*pes3*) with a non-linear modular arrangement. Phylogenetic analysis indicated that *pes3* shows minimal relatedness to other NRPS. Quantitative RT-PCR analysis has confirmed that *pes3* is differentially expressed in *A. fumigatus*. We have previously cloned and expressed a functional characterisation 4'-phosphopantetheinyl transferase from *A. fumigatus* [1] and now investigate if Pes3 is activated by the 4'-PPTase. Consequently, a 72 kDa fragment of *A. fumigatus pes3*, containing a putative thiolation domain, was cloned and expressed in an *E. coli* expression system and shown to be activated by 4'phosphopantetheinylation using a biotinylated–CoA co-factor analogue only in the presence of the 4'-PPTase. Overall, our data confirm that the NRPS, *pes3*, is expressed in *A. fumigatus* and that the cognate protein is activated, in vitro, by recombinant 4'-PPTase.

Reference:

1. Neville, C., Kavanagh, K., Murphy, A. and Doyle S. (2005) A 4'-phosphopantetheinyl transferase mediates non-ribosomal peptide synthetase activation in *Aspergillus fumigatus*. ChemBiochem 6(4):679-685.

ABSTRACT 55

Characterisation of the *sigB* regulon in *Listeria monocytogenes* by the microarray analysis of a *sigB* mutant.

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The redirection of transcription under stress conditions in Listeria monocytogenes is partially under the control of the alternative sigma factor, sigmaB. To further elucidate the role of sigma^B under stress conditions microarray analysis of a *sigB* mutant was carried out. Cells were grown in Brain Heart Infusion (BHI) under various conditions: exponential and stationary phase and the absence and presence of 0.5M NaCl. RNA was extracted from the wildtype strain *L. monocytogenes* 10403s and from its corresponding *sigB* deletion mutant under the above conditions. Spotted PCR product microarrays were used to directly compare their transcriptomes and differentially expressed genes were identified using the TIGR Tm4 software. Known and novel members of the *sigB* regulon were identified under all conditions. These findings will be discussed in the context of the role of sigma^B in the general stress response.

ABSTRACT 56

Factors influencing the biofilm phenotype of *Staphylococcus epidermidis* isolates causing meningitis in neurosurgical patients.

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It is known that the intercellular adhesin (*ica*) operon of *S. epidermidis* has an important function in adherence and biofilm development. The *icaADBC* locus encodes the enzymes responsible for the synthesis and export of Polysaccharide Intercellular Adhesin (PIA) that facilitates initial attachment to surfaces and development into mature biofilms. Furthermore, two surface proteins, the *S. epidermidis* major autolysin (AtlE) and the accumulation associated protein (AAP), are believed to facilitate initial attachment and cell accumulation on polymeric surfaces respectively. To investigate the role of *icaADBC,* AtlE, and AAP in the pathogenesis of neurosurgical biofilm device-related infections, 11 *S. epidermidis* isolates causing meningitis were collected from patients with an extra ventricular drain (EVD) *in situ*. PCR and Southern blot analysis found 7 (64%) isolates have an *ica*+ genotype. Under standard growth conditions only one isolate had a strong biofilm-positive phenotype, while biofilm growth could be enhanced greatly in a further two isolates by supplementing the media with known inducers. RT-PCR analysis of *icaA* expression was consistent with this. Interestingly, although *ica* transcription was detected in the four remaining *ica*$^+$ isolates, they were not biofilm-positive under any growth conditions. RT-PCR analysis of *aap* showed expression in 6 (50%) of the isolates in each environmental condition tested, while *atlE* was expressed in all. Interestingly, those isolates that lacked the ability to form biofilm, despite having an *ica*$^+$ genotype, were found to be *aap*$^-$ by PCR. The results suggest that both *ica* and *aap* expression may be required for successful development of biofilm in these isolates.

ABSTRACT 57

Functional characterisation of a putative intimin in *Yersinia pseudotuberculosis*.

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Intimin is an outer membrane adhesin expressed by enteropathogenic and enterohaemorrhagic *Escherichia coli*. These *E. coli* strains cause disease through formation of attaching and effacing lesions in the host epithelium. In *E. coli* intimin is involved in mediating intimate attachment of the bacteria to host cells by binding to the bacterial translocated intimin receptor (TIR) which has been injected into the host cells via a type III secretion system. A putative orthologue of intimin was identified in the genome sequences of *Yersinia pestis* and *Yersinia pseudotuberculosis*, but no putative orthologue of TIR could be identified. The putative intimin ortholgue is a pseudogene in all three *Y. pestis* sequenced strains, so is likely to be involved in the enteric lifestyle of *Y. pseudotuberculosis*.

To further understand the function of intimin in *Y. pseudotuberculosis* a C-terminal fragment of the protein was expressed as a MBP fusion in *E. coli*. Using immunofluorescence microscopy and flow cytometry it was discovered that the purified MBP-intimin fusion protein binds to the surface of HEp-2 cells. Knockout mutants and complemented mutants were made in 2 different strains of *Y. pseudotuberculosis* and were used in invasion and adhesion assays for comparison to wild type *Y. pseudotuberculosis*. Even in the absence of a TIR orthologue, *Y. pseudotuberculosis* intimin appears to be involved in both adhesion and invasion of the bacteria into HEp-2 cells.

Further studies are ongoing to gain greater insight into functionality of intimin in *Y. pseudotuberculosis*.

ABSTRACT 58

Determining the Role of VirB in the Regulatory Virulence Cascade of *Shigella flexneri*

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Shigella flexneri is a Gram-negative facultative intracellular pathogen and is the causative agent of bacillary dysentery in humans. The essential steps for *Shigella* virulence are invasion of epithelial cells, intracellular multiplication, and the spread of bacteria into adjacent cells. The invasion ability is dependent on a 230-kb plasmid that includes a 31-kb region essential for virulence. A regulatory cascade involving the products VirF and VirB genes plays a critical role in controlling the transcription of the virulence genes. Here we determine the role played by VirB in the molecular mechanism controlling *icsB* gene expression, a requirement for the post-invasion stage of *Shigella* pathogenicity. DNase 1 footprinting analysis of the *icsB* promoter region with both VirB and H-NS reveals overlapping regions of protection and hypersensitivity upstream of the transcription start site. $KMnO_4$ footprinting shows that the presence of VirB allows the formation of open and initiation complexes. In contrast, H-NS was shown to repress the formation of these complexes. Results also indicate that VirB plays no significant role in the recruitment of RNA polymerase. To investigate further the relationship between VirB and H-NS, *in vitro* transcription experiments were carried out. Results from these experiments show that the repression of the *icsB* gene by H-NS can be reversed upon addition of increasing concentrations of VirB. This suggests the relationship between H-NS and VirB is antagonistic. Moreover, the results presented here show that VirB plays an anti-repressor role, opposing the H-NS repressor protein, in the controlling of gene expression in the virulence regulatory cascade in *Shigella flexneri*.

ABSTRACT 59

MLST analysis of Enterotoxigenic *Escherichia coli*

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Enterotoxigenic *Escherichia coli* (ETEC) is the most common cause of *E. coli*-mediated human diarrhoea worldwide, particularly affecting children living in the developing world and visiting travellers and military personnel. ETEC is also major pathogen of cattle and neonatal and post-weaning piglets. Plasmid encoded colonisation factors and enterotoxins are implicated in the major virulence mechanisms of ETEC although more recently chromosomally encoded genes have been associated with increased adherence to and invasion of epithelial cells. MLST analysis based on seven housekeeping genes, *adk, fumC, gyrB, icd, mdh, purA* and *recA* was used to look at the population structure of ETEC. Isolates were identified in the A, B1, B2, D and E groups of the *E. coli* phylogenetic structure, suggesting that no specific genetic background is required for the acquisition of plasmids encoding the colonization factors and toxins. This data was combined with, data on the distribution of the toxins and chromosomally encoded putative virulence factors to reveal the population structure of ETEC.

ABSTRACT 60

Inhibiton of adhesion of *Streptococcus mutans* to hydroxyapatite by enzyme-modified whey products

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Adhesion and colonisation of the tooth surface by *S. mutans* is the first step in the pathogenesis of dental caries. Recent studies have shown that enzyme-modified whey products can inhibit adhesion of this organism. In the present study the effect of a range of enzyme-treated whey products on the adhesion of *S. mutans* to hydroxyapatite was examined, using a fluorescence-based microtitre plate assay.
Neither whey protein isolate nor demineralised whey reduced adhesion at concentrations up to 1mg/ml. Whey protein concentrate (WPC) 35 showed little inhibition at levels up to 0.5mg/ml but inhibited adhesion by 50% at a concentration of 1mg/ml. Acid WPC80 significantly inhibited adhesion at levels > 0.25 mg/ml and gave complete inhibition at a concentration of 1mg/ml. Sweet WPC80 was the most effective inhibitor examined and exhibited substantial inhibition of adhesion at concentrations >0.125mg/ml. Thus, enzyme-modified whey protein products may be useful ingredients in treatments for the prevention of dental caries.

ABSTRACT 61

Structure-function analysis of the unusual extended signal peptide of the autotransporter Pet

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Plasmid encoded toxin (Pet), a prototypical member of the serine protease autotransporters of the *Enterobacteriaceae* family, mediates enterotoxic and cytotoxic activities of Enteroaggregative *Escherichia coli* strain 042. In addition to the typical autotransporter passenger and beta-domain, Pet possesses an extended N-terminal signal sequence; comprised of five regions termed N1, H1, N2, H2 and C domains. N1 and H1 appear conserved with other extended signal peptides. N2, H2 and C show sequence variability typical of Sec-dependent signal peptides. Here we demonstrate the extended signal peptide sequence (ESPR) is present only in proteins secreted via the Type V secretion pathway in the βand γ classes of *Proteobacteria*. *In vitro* approaches demonstrate that the DNA region encoding the ESPR is transcribed and translated. The ability of the extended Pet signal peptide to mediate translocation across the inner membrane was assayed using an alkaline phosphatase reporter system which revealed that translocation efficiency was severely impaired by the ESPR.