

UREKA 2011

List of Projects on Offer for UREKA Summer 2011

Principal Investigator: Prof. Douwe van Sinderen

Project Title: Exploring Bifidobacterial Carbohydratases

Description: The gastrointestinal tract is the natural habitat for a large and diverse microbial ecosystem. Many species have evolved and adapted to live in the human intestine and are important factors in both human health and disease. Bifidobacteria represent an important probiotic component of this ecosystem where they play a beneficial role in human health by providing metabolic products useful to the host or a crucial line of defence against colonisation by potentially harmful microorganisms (reviewed by Turrone *et al.*, 2009).

Bifidobacteria have been identified as saccharolytic microorganisms and several studies have demonstrated that bifidobacteria dedicate a significant portion of their coding capacity to the metabolism of a wide variety of carbohydrates. To date over fifty different bifidobacterial carbohydratases have been described in the literature. We have used *B. breve* UCC2003 as a model to study bifidobacterial carbohydrate metabolism and in the last number of years have characterised an operon encoding a β -fructofuranosidase (Ryan *et al.*, 2005), an extracellular amylopullulanase which hydrolyses α -1,4 and α -1,6 glucosidic linkages in starch and related polysaccharides (Ryan *et al.*, 2006; O'Connell-Motherway *et al.*, 2008), two novel α -glucosidases exhibiting hydrolytic activities towards panose, isomaltose, isomaltotriose and trehalose (Pokusaeva *et al.*, 2009), and a gene cluster dedicated to ribose metabolism (Pokusaeva *et al.*, 2010). In addition, a PEP-PTS system involved in fructose metabolism was identified and studied in this bacterium (Maze *et al.*, 2007).

This UREKA project will expand our understanding of bifidobacterial carbohydrate metabolism in the gastrointestinal tract. The student will exploit available bifidobacterial genome sequences to identify gene clusters involved in prebiotic metabolism and will avail of expertise in functional genomics and advanced molecular biology techniques to decipher the precise mechanisms.

Principal Investigator: Dr Ken Nally

Project Title: Inhibition of the Toll-like Receptor Triggered Inflammatory Response in Macrophages by Nuclear Receptors

Project Description: Inflammatory Bowel Disease (IBD) constitutes a group of inflammatory conditions of the small & large intestine. At present the exact causes of IBD are not completely understood but it is thought that intestinal bacteria drive an inappropriate intestinal immune response & inflammation in genetically susceptible hosts. Toll-like receptors (TLRs) are key receptors of the innate immune system that recognise viruses and bacteria and provide an early warning sign to the presence of infectious microorganisms. However, inappropriate activation of TLRs, such as may be the case in IBD can contribute to the chronic nature of inflammatory conditions. Nuclear receptors (NRs) (of which there are 49 known receptors) are a superfamily of structurally conserved, ligand-dependent transcription factors and have been shown to repress pro-inflammatory gene expression triggered by TLR activation. Recently, NRs (PPAR α & LXR α) have been identified as possible therapeutic targets in IBD. In an effort to identify additional NRs which might possess an anti-inflammatory function we conducted an RNAi screen for all known human NRs (49 NRs) in the human monocytic THP-1 cell line. The aim of this UREKA research project will be to functionally validate one of the NRs identified from this screen using RNAi, qRT-PCR, western blotting and cytokine analysis.

Principal Investigator: Prof Rosemary O'Connor

Project Title: Essential function of UTP carrier PNC1 in maintaining mitochondria

Project description: The Pyrimidine Nucleotide Carrier PNC1 is a highly conserved mitochondrial outer membrane protein that transports UTP into mitochondria. We initially discovered PNC1 in cancer cells where it promotes cell growth and proliferation (1). PNC1 is the mammalian orthologue of a yeast protein RIM2, which is also a UTP transporter and is essential for yeast cell growth (2). Our recent findings in cancer cells have shown that PNC1 is required for mitochondrial DNA replication and transcription of mitochondrial-encoded components of the respiratory chain (3), possibly by acting as a UTP cofactor for the mitochondrial DNA helicase TWINKLE (4). Cells with decreased expression of PNC1 leak Reactive Oxygen Species (ROS). However, we do not know whether PNC1 is essential for TWINKLE activity and whether it could protect cells from hypoxia and stress.

The aim of this project is to investigate the function of PNC1 in cells that are exposed to low levels of oxygen (hypoxia) compared with normal oxygen levels (normoxia). Mitochondrial function will be assessed as well as markers of ROS release and downstream signaling responses. We will also investigate whether PNC1 may rescue cells from effects of hypoxia and if this is connected with TWINKLE activity. The results of this project will help us to understand how mitochondrial function facilitates cancer progression.

References:

1. Floyd S, Favre C, Lasorsa FM, Leahy M, Trigiant G, Stroebel P, Marx A, Loughran G, O'Callaghan K, Marobbio CM, Slotboom DJ, Kunji ER, Palmieri F, O'Connor R (2007): The Insulin-like Growth Factor-I mTOR Signaling Pathway Induces the Mitochondrial Pyrimidine Nucleotide Carrier to Promote Cell Growth. **Mol Biol Cell** 18:3545-3555.
2. Van Dyck E, Jank B, Ragnini A, Schweyen RJ, Duyckaerts C, Sluse F, Foury F (1995): Overexpression of a novel member of the mitochondrial carrier family rescues defects in both DNA and RNA metabolism in yeast mitochondria. **Mol Gen Genet** 246:426-36.
3. Favre, C. Zhadnov, A., Papkovsky, D, O'Connor, R (2010) The mitochondrial Pyrimidine Nucleotide Carrier (PNC1) regulates mitochondrial biogenesis and the invasive phenotype of cancer cells. **Oncogene** 29:3964-3976. Epub 2010 May 10.
4. Korhonen JA, Gaspari M, Falkenberg M (2003): TWINKLE Has 5' - 3' DNA helicase activity and is specifically stimulated by mitochondrial single-stranded DNA-binding protein. **J Biol Chem** 278:48627-32.

Principal Investigator: Dr David Clarke

Project Title: Molecular analysis of virulence in *Escherichia coli* associated with Inflammatory Bowel Disease (IBD).

Project description: Crohn's Disease (CD) is a chronic inflammatory bowel disease, the cause of which is still unknown. However, recent work has shown that a new pathotype of *Escherichia coli*, Adherent and Invasive *E. coli* (AIEC) may be associated with CD. AIEC has been shown to adhere to and invade epithelial cells and to replicate within macrophages without inducing apoptosis. In this study we will screen an AIEC mutant library to identify genes that are involved in the ability of this bacterium to replicate in macrophages. Mutants identified through these screens will be characterized by DNA sequencing and analysed by further phenotypic profiling and bioassays. Replication in macrophages is a key virulence factor in AIEC and therefore these studies will facilitate a description of the molecular mechanisms underpinning the pathogenicity of this new bacterial pathotype.

Principal Investigator: Dr Cormac Gahan

Project Title: Is *Listeria innocua* pathogenic for insects? Potential role of the Panton-Valentine leukocidin toxin

Project description: *Listeria innocua* has evolved from its pathogenic ancestor *L. monocytogenes* to become non-pathogenic for humans and other mammals. However there is good evidence that *L. innocua* is pathogenic for insects (including *Galleria mellonella* and *Drosophila melanogaster*). We have previously used a fosmid library screen to identify possible mediators of pathogenicity in *L. innocua*. One fosmid clone capable of killing insects contains genes encoding a Panton-Valentine leukocidin that is not present in *L. monocytogenes*. In order to determine whether this PVL toxin mediates insect toxicity we will carry out bioinformatic analysis of the PVL locus in *L. innocua* to compare this genomic region with that of other *Listeria* species. We will also use SOEing mutagenesis to delete a portion of the *pvl* locus in *L. innocua* CLIP strain and will then compare the wildtype and mutant in the *Galleria mellonella* larval infection model. We will also attempt to clone the entire *pvl* operon into *E. coli* – using PCR with long-template Taq – followed by standard cloning procedures and will test this *E. coli* clone for toxicity in the insect host. Overall the work will help to determine the major differences between pathogenic and supposedly non-pathogenic *Listeria* and will shed light upon the evolution and niche adaptation of *Listeria* species using an alternative model host.

Principal Investigator: Dr John Cryan

Project Title: The Microbiome-Gut-Brain Axis: Impact of Germ Free Status on Gene Expression in the Central Nervous System

Project description: The ability of gut microbiota to communicate with the brain and thus modulate behaviour is emerging as an exciting concept in health and disease. The enteric microbiota interacts with the host to form essential relationships that govern homeostasis. Despite the unique enteric bacterial fingerprint of each individual, there appears to be a certain balance that confers health benefits. It is, therefore, reasonable to note that a decrease in the desirable gastrointestinal bacteria will lead to deterioration in gastrointestinal, neuroendocrine or immune relationships and ultimately disease. Therefore, studies focusing on the impact of enteric microbiota on the host and in particular on the central nervous system are essential to our understanding of the influence of this system. Recent studies demonstrate that germ free mice display alterations in stress-responsivity, central neurochemistry and behaviour indicative of a reduction in anxiety in comparison to conventional mice. Such data offer the enticing proposition that specific modulation of the enteric microbiota may be a useful strategy for stress-related disorders and for modulating the co-morbid aspects of gastrointestinal disorders such irritable bowel syndrome and inflammatory bowel disease. This project will use RT-PCR and in situ hybridisation to examine the impact of microbiota on the expression of genes for neurotransmitters relevant for emotional responding. Briefly, different brain regions will be harvested from mice that have grown up either under conventional conditions, under germ free conditions and those that have grown up germ free and were recolonised in adulthood . Correlations will be made between behavioural, cytokine and gene expression analysis. The hope is to gain novel insight into immune regulation of brain-gut-microbe axis which has direct implications for functional gastrointestinal disorders.

Principal Investigator: Dr Niall Hyland

Project Title: The contribution of mast cell-derived chymase in the pathophysiology of irritable bowel syndrome

Project description: Recent studies from our laboratory have demonstrated that connective tissue-type mast cells (CTMC) are increased in number and potentially contribute to visceral hypersensitivity in a pre-clinical disease model of irritable bowel syndrome (IBS). We hypothesise that chymase, derived from CTMC, may influence colonic epithelial barrier integrity and thereby contribute to the pathogenesis of IBS. Elevated chymase has been associated with overt GI inflammatory disorders, such as inflammatory bowel disease; however its association with IBS remains unproven.

Therefore, we will examine the effects of exogenous chymase on epithelial permeability and assess chymase content in tissues obtained from pre-clinical IBS models. Furthermore, we will examine the relationship between chymase-induced tryptase release. Using chamber electrophysiology and immunology based techniques will be used to conduct these studies.

Principal Investigator: Dr John Morrissey

Project Title: Virulence models for *Candida*

Project description: Pathogenic yeasts, especially in the genus *Candida*, have become an increasing problem in recent years, primarily due to an increased number of susceptible immunocompromised patients. We are interested in the virulence of two species, *C. albicans* and *C. glabrata*. Our work involves study of signaling pathways, transcription factors and virulence factors. We have developed an amoeba model to investigate the importance of virulence genes and mutants and the student will use this model. This project will integrate with our ongoing research and will involve molecular work (PCR, cloning), some microbiology (culturing, phenotypic tests) and some cell culture.

Principal Investigator: Prof John Atkins

Project Title: Nascent peptide effects of a viral sequence

Project description: In the decoding of certain diverse genes, a specific residue or segment of the growing nascent polypeptide chain within the ribosomal exit tunnel influences translation of a downstream portion of its encoding mRNA. Such a nascent peptide signal is important for half the ribosomes decoding phage T4 topoisomerase subunit gene 60 being able to bypass a block of 50 non-coding nucleotides present in mRNA between codons 46 and 47 to synthesize a single protein from two disjointed ORFs.

We have searched virus genomes for sequences that are likely to have such nascent peptide effects during decoding. This project will test one of these candidate sequences. Technically, there will be some cloning followed by transfection into HEK-293T cells and then dual luciferase assays to monitor the possible effect of the nascent peptide.

Principal Investigator: Dr Eoin Fleming

Project Title: *Helicobacter pylori* regulation of histamine biosynthesis

Project description: *Helicobacter pylori* infection in humans is associated with gastritis, it has also been identified as a contributory factor in the development of gastric cancer. Infection is characterized by an immune response that involves the release of histamine from mast cells and histamine mediated increases in gastric acid secretion. Knockout mice unable to produce histamine show decreased immune response during *Helicobacter pylori* infection, and when mice infected with *Helicobacter felis* are simultaneously treated with histamine receptor antagonists they show decreased development of gastric cancer (*Prog Nucleic Acid Res Mol Biol.* (2006) 81:231-70).

Histamine is produced by the enzyme L-histidine decarboxylase (HDC). HDC is translated as an inactive and unstable ~74kDa precursor that becomes post-translationally processed into active and stable isoforms (including major ~54kDa and ~63kDa isoforms). Recent studies suggest that these post-translational processing and stabilization events can be regulated by caspase 9 and protein kinase C respectively.

Infection of gastric AGS cells is associated with increased caspase 9 activity and activation of protein kinase C pathways. Accordingly, we will employ a range of molecular and cellular biology approaches to test the hypothesis that increased histamine production in *H.pylori* infected AGS cells is specifically due to post-translational regulation of HDC processing and stabilization by caspase 9 and protein kinase C pathways respectively.

Principal Investigator: Dr Teresa Barbosa

Project Title: Understanding and exploiting marine sponges associated sporeformers

Project description: With the worldwide increase in antibiotic resistance, the scientific community is facing new challenges to develop novel therapeutic approaches for combating the spread of infectious diseases. In the last number of years therefore, there has been an increased focus on unexplored environments as potential sources for novel biologically active molecules. Many of these compounds have been isolated from marine sponges, but accumulating evidence suggests that many actually originate from bacterial symbiontes of these marine invertebrates. *Bacillus* and other endosporeforming bacteria are frequently isolated from different marine sponges, but relatively little is known about their origin, diversity, or the specific properties that might differentiate them from strains isolated from other habitats. Species of the genus *Bacillus* are renowned for the production of a vast array of chemical agents with antimicrobial properties and enzymes with biotechnological interest.

Developing our understanding of the diversity of these bacteria from marine sponges and their associated active products could have important implications in the biotechnological, animal production and clinical fields, and could be exploited to assist in the development of novel prophylactic and therapeutic strategies. In this context the overall aim of this study will be to characterize the biodiversity of the culturable sporeforming population associated with different marine sponges isolated from Irish waters, and to establish their potential as a source for novel bioactives.

Principal Investigator: Prof Fergal O’Gara

Project Title: The identification and characterisation of novel antimicrobial agents and its targets

Project description: The emergence of antibiotic resistant microorganisms is of global concern. This problem is most urgent in nosocomial infections, since more than 70% of the bacteria causing these infections are resistant to first line antibiotics. Patients infected with antibiotic resistant microorganisms are more likely to have longer hospital stays and require treatment with second- and third-choice medicines that may be less effective, more toxic and more expensive. Therefore it is of crucial importance to identify novel antimicrobial agents, with new modes of action against bacteria.

In the BIOMERIT Research Centre (BRC), which was established by Prof. Fergal O’Gara in 1991 as a centre of excellence in the Microbiology Department of the University College Cork, we have a key interest in (1) Microbial-Host Interactions, (2) Functional Genomics and Signalling in Gram-negative bacteria and (3) Environmental Biotechnology (<http://www.ucc.ie/en/biomerit/>). As such we are interested in identifying novel antimicrobial compounds and characterising antibiotic resistance mechanisms with an aim to identify potential therapeutic targets.

Recent evidence from the BRC suggests a novel role for 30 genes from the human opportunistic pathogen *P. aeruginosa* in antibiotic resistance. The student will validate this finding by comparing wildtype and mutants strains using a range of microbiological techniques. Candidate genes will be further characterised in order to elucidate the mechanism involved.

Dependent on the student’s interest, the student could also participate in a project which aims to identify novel antimicrobial compounds. Uncultured micro-organisms are a rich source of novel biologically active metabolites, many of which are structurally different to current antimicrobials. Among the methods designed to gain access to physiology and genetics of uncultured micro-organisms, metagenomics has emerged as a powerful approach. This approach enables the genomic analysis of a population of micro-organisms in an environmental sample, and it opens doors to large scale genomic surveys of uncultured microbial communities. Currently, several genomic surveys are underway in the Microbiology Department using metagenomic libraries derived from marine sponges and soil. Preliminary data from these studies has revealed several interesting metabolites, including lipases, phytases, and polyketide synthases, which are currently being further characterised. The student will screen a 20,000 clone metagenomic library derived from soil with the help of a colony-picker robot for the presence of antimicrobial compounds. Positive clones will be further characterised in order to elucidate the broad spectrum activity and nature of the bioactive compounds.