Alimentary Pharmabiotic Centre:
Microbe/microbe interactions in the gastrointestinal tract

Key external stakeholders:
Food manufacturers, pharmaceutical industry, gastroenterologists, wider research community

Practical implications for stakeholders:
- It is widely recognised that the gut microbiota plays an important role in human health and this is currently one of the most dynamic, complex and exciting areas of research in both the food and pharmaceutical arenas. The mining of the gastrointestinal tract (GIT) has revealed that the gut microbiota represents a repository of potential therapeutic molecules for food and pharmaceutical applications.

Main results:
- The antimicrobial Thuricin CD has been patented and is licensed to Alimentary Health for the treatment or prevention of Clostridium difficile infection.
- Bacteriophages MR299-2 and NH-4 can eliminate Pseudomonas aeruginosa in a murine model of Cystic Fibrosis and this combined with other results has led to the establishment of Phageworks™ - a one-stop development and IP licensing company bringing phage based products to market for customers.
- The novel antimicrobial Bactofencin LS1 is effective for the control of both Listeria monocytogenes and Staphylococcus aureus. Bactofencin LS1 has been patented and is currently undergoing a programme of investigation with the Irish SME Sigmoid Pharma.

Opportunity / Benefit:
A bank of novel antimicrobials produced by GI microbiota is available for development.

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1. Project background:
This project aimed to understand the role of antimicrobials produced by the gut microbiota, and the conditions governing their activity in the gastro-intestinal tract (GIT). Characterisation of a range of novel isolates was undertaken and their activity investigated both in vitro and in vivo in order to improve our understanding of the role of antimicrobials produced by the gut microbiota, and the conditions governing their activity in the GIT. To gain regulatory approval for gut microbes for use in food or pharma industries, applications have to be underpinned by high quality scientific data. Therefore, studies were undertaken to gain a thorough understanding of the safety, structure/function relationships and mode of action of isolates and the bioactive compounds they produce. In addition literature reports have suggested that probiotic drinks demonstrate a protective effect against CDI (Clostridium difficile infection) in patients undergoing antibiotic treatment. C. difficile is now the most common cause of hospital acquired diarrhea and the incidence of hypervirulent strains is increasing worldwide. Therefore reducing C. difficile carriage and outgrowth by novel probiotics was a primary target of research.

2. Questions addressed by the project:
- Can we attempt to understand the role of microbe-microbe interactions in determining the overall composition and flux of the human intestinal microflora?
- Can we control Clostridium difficile overgrowth using novel antimicrobials?
- Can we isolate novel antimicrobials and apply these to control undesirable microbes?
- Can we confirm a role for bacteriocins as colonising and anti-infective mechanisms in probiotics?
- Can we isolate a probiotic strain with the capability to reduce C. difficile carriage?

3. The experimental studies:
We performed substantial high throughput screening of the microbiota and isolated and characterized a variety of antimicrobial producing strains.

We undertook a number of studies in parallel relating to the prevalence and carriage of Clostridium difficile in a range of population demographics and found that various increased rates of carriage is associated with poor gut health – with healthy subjects having a carriage rate of 1-2% compared to IBS (4-5%), IBD (11%), institutional aged (20%) and cystic fibrosis (50%). A major outcome was the isolation and characterisation of Thuricin CD, a post-translationally modified bacteriocin with activity against Clostridium difficile. In two consecutive publications in the Proceedings of the National Academy of Science this bacteriocin was shown to have a very narrow spectrum of activity such that it killed C. difficile without the associated collateral damage of gut microbiota associated with the antibiotics normally used to treat CDI namely vancomycin and metronidazole. In further experiments rectal delivery of Thuricin CD resulted in a 100 fold reduction of Clostridium difficile carriage in vivo in mice. As a result of these data, Thuricin CD is licensed to Alimentary Health for treatment or prevention of Clostridium difficile infection.

After intensive screening of the DPC and APC culture collections three lactobacillus strains with potential to reduce C. difficile carriage were identified. These strains are currently being evaluated for their IP/licencing potential.

A further specific goal was to characterise novel antimicrobials produced by gut microbiota. Within this goal we had significant success at mining the gut for bacteriophage with the ability to kill Pseudomonas aeruginosa, an opportunistic superbug that infects the lungs of patients suffering from Cystic Fibrosis. In collaboration with visiting Professor James G. Martin of McGill University, we demonstrated that bacteriophages MR299-2 and NH-4 can eliminate Pseudomonas aeruginosa in a murine model. This finding, combined with other bacteriophage research performed within the APC, has led to the establishment
of Phageworks™ - a one-stop development and IP licensing company bringing phage based products to market for customers.

The intensive screening for antimicrobials also uncovered a completely novel bacteriocin produced by *Lactobacillus salivarius* DPC 6502. We identified Bactofensin LS1, a new type of cationic bacteriocin as part of the specific goal devoted to novel antimicrobials produced by the gut. Bactofensin LS1, produced by a porcine intestinal isolate, is highly positively charged which is very unusual in a prokaryotic peptide. Indeed in this respect it demonstrates closer similarities to eukaryotic defensins and plant derived antimicrobial peptides, than those of bacterial origin. It has been demonstrated to be effective for the control of both *Listeria monocytogenes* and *Staphylococcus aureus*. Bactofensin LS1 has been patented and is currently undergoing a programme of research with the Irish SME Sigmoid Pharma.

4. Main results:
   - Thuricin CD, an antimicrobial with activity against *Clostridium difficile* was isolated, identified and characterized at the molecular, structural and mechanism of action level. This antimicrobial has applications in the food and pharma sectors.
   - Bacteriophage, isolated from the gastrointestinal tract, were found to be effective for the elimination of *Pseudomonas aeruginosa* in an animal model, indicating its potential as a treatment for individuals suffering from Cystic Fibrosis. These bacteriophages have potential for applications in the pharma sector.
   - The antimicrobial, Bactofensin LS1, a gut isolate has been demonstrated to have the ability to control *Listeria monocytogenes* and *Staphylococcus aureus*, two food pathogens. This antimicrobial has applications in the food industry.

5. Opportunity/Benefit:
Outputs from this research are of significance in a number of areas including food, medical devices, pharmaceutical and veterinary industries. Microbial isolates and metabolites mined from the gut environment have potential applications for the improvement of food safety and human health.

6. Dissemination:
Main publications:


Patents:


7. Compiled by: Dr. Sheila Morgan and Dr. Mary Rea