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## APC Microbiome Ireland: Microbes to Molecules



### Key external stakeholders:

Food manufacturers, dairy industry, pharmaceutical industry, gastroenterologists, wider research community, policy makers, public health agencies and health professionals.

### Practical implications for stakeholders:

The role of the human gut microbiome in health has been brought to the forefront in recent years, as a result of research performed within APC Microbiome Ireland, and other research institutions worldwide. This research continues to be one of the most dynamic, complex and exciting areas of research in both the food and pharmaceutical arenas. The mining of the human gut and other environments has revealed a repository of potential therapeutic molecules for these industries.

### Main results:

- Antimicrobials in the form of bacteriocins (antimicrobial peptides) and bacteriophage (viruses that infect bacteria) have been identified and investigated to determine their mechanisms of action and their role in the control and manipulation of the human gut environment.
- The demand for novel antimicrobial therapies due to the threat of antimicrobial resistance has resulted in a growing interest in the protective role of human skin bacteria. A number of novel bacteriocins, with the ability to inhibit *Propionibacterium acnes*, *Staphylococcus epidermidis* and methicillin-resistant *Staphylococcus aureus* (MRSA) were isolated from bacteria of skin origin, demonstrating the antimicrobial potential of the human skin microbiota
- The mechanism of action of the anti-*Clostridium difficile* bacteriocin, thuricin CD, was investigated using flow cytometry, and it was determined that the bacteriocin elicits irreversible depolarization of membranes of target bacteria.
- The efficacy of the lacticin 3147 bacteriocin producer, *Lactococcus lactis* DPC3147, as a live bio-therapeutic, was compared to a commercial antibiotic formulation, with a view to treating bovine mastitis. Emulsion-based formulations containing DPC3147 proved equally efficacious as the commercial antibiotic formulation with respect to curing bovine mastitis in a field trial.
- Bacteriophage research has led to the identification of *E. coli* phage and *Streptococcus mutans* phage that have applications in the treatment of infection and dental caries, respectively.

### Opportunity / Benefit:

A repository of potential therapeutic molecules exists, encompassing microbes, antimicrobial peptides, bacteriophage and bacteriophage lysins, all with the ability to modulate the environment to which they are applied.

### Collaborating Institutions:

University College Cork

**Teagasc project team:** Dr. Mary Rea, Dr. Harsh Mathur, Dr. Stephen Stockdale, Dr. Beatriz Mesa Periera, Dr. Sara Arbulu Riaz, Dr. Lisa Quigley, Fergus Collins, Julie O'Sullivan, Catherine O'Reilly, Shone Uniacke Lowe, Kelsey Wiggins Savage, Paula O'Connor.

**External collaborators:** Prof. Paul Ross, Prof. Fergus Shanahan, Prof. Colin Hill and the APC team.

### 1. Project background:

APC Microbiome Ireland, and its research programme, is based on the premise that the gut microbiota plays a significant role in human health and in disease. The Microbes to Molecules theme is built on this hypothesis and is focused on the discovery and characterization of pharmabiotics, in particular bacteriocins, probiotics, prebiotics and bacteriophages, which have been mined from the gut microbiota. Research activities are aimed at the elucidation of the molecular mechanisms underlying probiotic/pharmabiotic action, and the development of guided approaches for the discovery of future pharmabiotics suitable for targeted Food (and Pharma) applications.

### 2. Questions addressed by the project:

- Can new pharmabiotics (microbes and microbial components) with health benefits be identified?
- Can novel anti-infective agents be developed?
- Can bacteriophages be exploited as biological effectors in the gut?

### 3. The experimental studies:

- Screening for novel antimicrobial producing microbes, and novel antimicrobials forms a significant and fruitful part of this research.
- The application of live biotherapeutics (antimicrobial peptides, bacteriophage and phage lysins) to a range of environments continues, including investigation as treatments for bovine mastitis.
- The use of bacteriocin producing strains, such as *L. lactis* DPC3147 which produces lacticin 3147, as live bio-therapeutics and alternatives for conventional antibiotics for treating bovine mastitis in a field trial.

### 4. Main results:

#### Screening studies:

- A number of novel bacteriocins, with the ability to inhibit *Propionibacterium acnes*, *Staphylococcus epidermidis* and methicillin-resistant *Staphylococcus aureus* (MRSA) were isolated from bacteria of skin origin, demonstrating the antimicrobial potential of the human skin microbiota
- A novel two-peptide lantibiotic was isolated and characterized. Formicin was isolated from *Bacillus paralicheniformis*, an antimicrobial-producing strain originally isolated from the intestine of a mackerel.

#### Peptide purification:

- Techniques for the purification of novel antimicrobials were developed and applied to the purification of avicin from *Enterococcus avian* and a defencin like peptide, actifencin, from *Actinomyces ruminicola*.

#### Ongoing research on bacteriocins:

- Research on bacteriocins identified previously continued with bactofencin A and pediocin PA-1 being further characterised in addition to a controlled functional expression system being developed for both.
- Work continues on bioengineered nisin. A nisin A (S29P) derivative was found to be as active as nisin A against a variety of bacterial targets but, crucially, exhibited a 20-fold increase in specific activity against a strain of interest. Another derivative, nisin PV, exhibited similar properties but was much less prone to oxidation. This version of nisin with enhanced resistance to specific resistance mechanisms could prove useful in the fight against antibiotic resistant pathogens.

#### Mode of action studies:

- The mode of action of thuricin CD was elucidated and it was established that the two-peptide sactibiotic elicits depolarization of target membranes and consequent cell lysis.

#### Mastitis studies:

- Emulsion-based formulations containing the live bio-therapeutic, *L. lactis* DPC3147, demonstrated equivalent efficacy to a commercial antibiotic formulation in treating dairy cows with clinical/sub-clinical mastitis.

#### Bacteriophage studies:

- Bacteriophage have been investigated as regulators of the human gut microbiome. A study performed revealed that a CrAssphage ( $\Phi$ CrAss001) isolated from human faecal material infects the human gut symbiont *Bacteroides intestinalis*, confirming predictions of this being the likely host.
- The APC group assembled the metagenomic sequencing reads from 702 human fecal virome/phageome samples and analyzed 99 complete circular crAss-like phage genomes which have been classification into four candidate subfamilies composed of ten candidate genera.
- *Streptococcus mutans* is one of the principal agents for dental caries formation. A phage,  $\phi$ APCM01, with the ability to inhibit *S. mutans* was isolated from human saliva. This phage exhibits promising antimicrobial properties.
- Three new *Escherichia coli* phages from the human gut were isolated and show promising potential for phage therapy applications as biocontrol agents for *E. coli* infections.

### 5. Opportunity/Benefit:

Outputs from this research are of significance in a number of arenas including food, medical devices, pharmaceutical and veterinary industries. Microbial isolates and metabolites mined from the gut and other environments have potential applications for the improvement of food safety in addition to animal and human health.

### 6. Dissemination:

Extensive dissemination of research findings has been undertaken in the form of peer reviewed publications and scientific presentations at conferences, open days, industry fora etc. Furthermore as part of the Education and Public Engagement aspect of Science Foundation Ireland funded research programmes, considerable energy has invested into engaging with school children and the general public.

### Main publications:

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7. **Compiled by:** Sheila Morgan, Harsh Mathur, Mairead Coakley, Paul Cotter