Project number: 5271
Funding source: Science Foundation Ireland
Date: November, 2014
Project dates: Sept 03 – Sept 2013

Alimentary Pharmabiotic Centre:

Microbe/microbe interactions in the gastrointestinal tract

Alimentary Pharmabiotic Centre
Interfacing Food & Medicine

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.

Collaborating Institutions:

University College Cork
University of Alberta Canada

Contact Mary Rea Email: Mary.Rea@teagasc.ie

Teagasc project team: Prof. Paul Ross (PI)
Dr. Mary Rea
Dr. Paul Cotter
Dr. Debebe Alemayehu
Dr. Eileen O'Shea
Paula O'Connor
Dr. Daniel Burke
Dr. Alan Marsh

External collaborators: Prof. Fergus Shanahan
Prof Colin Hill and the APC team
Prof John Vederas

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

Contact Mary Rea Email: Mary.Rea@teagasc.ie

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:

- Rea, M.E., E. Clayton, P. O'Connor, F. Shanahan, B. Kiely, R.P. Ross and C. Hill. 2007. Antimicrobial activity of lacticin 3147 against clinical *Clostridium difficile* strains. J. Med. Microbiol. 56:940-946.
- Gardiner, G.E., MC. Rea, B. O'Riordan, PM. O'Connor, S. Morgan, PG. Lawlor, PB. Lynch, MC. Cronin, RP. Ross and C. Hill 2007. Fate of the two component lantibiotic lacticin 3147 in the gastrointestinal tract. Applied and Environmental Microbiology. 73: 7103-7109
- Clayton, E.M., M.C. Rea, F. Shanahan, E.M. Quigley, B.E. Kiely, C. Hill and R.P. Ross. 2009. The vexed relationship between *Clostridium difficile* and Inflammatory Bowel Disease - an assessment of carriage in an outpatient setting among patients in remission. American Journal of Gastroenterology 104:1162-9.
- O'Shea, E.F., G.E. Gardiner, P.M. O'Connor, S. Mills, R.P. Ross and C. Hill. 2009. Characterization of enterocin and salivaricin producing lactic acid bacteria from the mammalian gastrointestinal tract. FEMS Microbiology Letters 2009 29:24-34.
- Rea, M., C.S. Sit, E. Clayton, P.M. O'Connor, R.M. Whittal, J. Zheng, J.C. Vederas, R.P. Ross and C. Hill. 2010. Thuricin CD, a post-translationally modified bacteriocin with a narrow spectrum of activity
- against *Clostridium difficile*. PNAS (Proc Natl Acad Sci USA) 107:9352-7.
- O'Shea, E.F., P.M. O'Connor, P.D. Cotter, R.P. Ross and C. Hill. 2010. Synthesis of Trypsin-Resistant Variants of the Listeria-Active Bacteriocin Salivaricin P. Applied and Environmental Microbiology 76:5356-5362.
- Marsh, A.J., O. Sullivan, R.P. Ross, P.D. Cotter and C. Hill. 2010. In silico analysis highlights the frequency and diversity of type 1 lantibiotic gene clusters in genome sequenced bacteria. BMC Genomics 11:679.
- Rea, M.C., A. Dobson, O. O'Sullivan, F. Crispie, F. Fouhy, P.D. Cotter, F. Shanahan, B. Kiely, C. Hill and R.P. Ross. 2011. Microbes and Health Sackler Colloquium: Effect of broad- and narrow-spectrum antimicrobials on *Clostridium difficile* and microbial diversity in a model of the distal colon. Proc. Natl Acad Sci USA 108 Suppl 1:4639-44
- Clayton, E.M., C. Hill, P.D. Cotter and R.P. Ross. 2011. Real-time PCR assay to differentiate Listeriolysin S-positive and –negative strains of *Listeria monocytogenes*. Applied and Environmental Microbiology 77:163-71.
- O'Shea, E.F., P.M. O'Connor, E.J. Raftis, P.W. O'Toole, C. Stanton, P.D. Cotter, R.P. Ross and C. Hill. 2011. Production of multiple bacteriocins from a single locus by gastrointestinal strains of *Lactobacillus salivarius*. J. Bacteriology 193:6973-82.
- Clayton, E.M., M.C. Rea, F. Shanahan, E.M.M. Quigley, B. Kiely, R.P. Ross and C. Hill. 2012. Carriage of *Clostridium difficile* in outpatients with irritable bowel syndrome. Journal of Medical Microbiology 61, 1290-1294.
- Alemayehu, D., P.G. Casey, O. McAuliffe, C.M. Guinane, J.G. Martin, F. Shanahan, A. Coffey, R.P. Ross and C. Hill. 2012. Bacteriophages MR299-2 and NH-4 can eliminate *Pseudomonas aeruginosa* in the murine lung and on cystic fibrosis lung airway cells. MBio. 2012 Mar 6;3(2);e00029-12

Contact Mary Rea Email: Mary.Rea@teagasc.ie

- Rea, M.C., O. O'Sullivan, F. Shanahan, P.W. O'Toole, C. Stanton, R.P. Ross and C. Hill. 2012. *Clostridium difficile* carriage in elderly subjects and associated changes in the intestinal microbiota. J. Clinical Microbiology 867-875
- O'Shea, E.F., P.D. Cotter, C. Stanton, R.P. Ross and C. Hill. 2012. Production of bioactive substances by intestinal bacteria as a basis for explaining probiotic mechanisms: Bacteriocins and conjugated linoleic acid. International Journal of Food Microbiology 152:189-205
- Rea, M.C., D. Alemayehu, R.P. Ross and C. Hill. 2013. Gut Solutions to a Gut Problem: bacteriocins, probiotics and bacteriophage for control of *Clostridium difficile* infection. J Medical Microbiology 62:1369-78
- Marsh, A.J., O. O'Sullivan, C. Hill, R.P. Ross and P.D. Cotter. 2013. Sequencing-based analysis of the bacterial and fungal composition of kefir grains and milks from multiple sources. PLos One19;8(7):e69371.
- Marsh, A.J., O. O'Sullivan, C. Hill, R.P. Ross and P.D. Cotter. 2013. Sequence-based analysis of the microbial composition of water kefir from multiple sources. FEMS Microbiology Letters:348:79-85
- O'Shea, E.F., P.D. Cotter, R.P. Ross and C. Hill. 2013. Strategies to improve the bacteriocin protection provided by lactic acid bacteria. Current Opinion in Biotechnology 24: 130-134
- O'Shea, EF, PM O'Connor, O. O'Sullivan, PD Cotter, R.P. Ross and C. Hill. 2013. Bactofencin a, a new type of cationic bacteriocin with unusual immunity. MBio. 2013 Oct 29;4(6). Doi:pii:e00498-12.
- Rea MC, D. Alemayehu, P.G. Casey, P.M. O'Connor, P.G. Lawlor, M. Walsh, F. Shanahan, B. Kiely, R.P. Ross and C. Hill. 2014. Bioavailability of the anti-Clostridial Bacteriocin Thuricin CD in Gastrointestinal Tract. Microbiology 160:439-45.
- Marsh, A.J., O. O'Sullivan, C. Hill, R.P. Ross and P.D. Cotter. 2014. Sequence-based analysis of the bacterial and fungal compositions of multiple kombucha (tea fungue) samples. Food Microbiology. 2014 38:171-8.

Patents:

- Ross, R.P., Stanton, C., Hill, C. and Fitzgerald, G.F. (2009) Antimicrobial Peptides and Bacterial Strains that Produce them. Publication No.: US2009214498
- Hill, C., Rea, M. and Ross, R.P. (2010) Thuricin CD an Antimicrobial for specifically targeting *Clostridium difficile.* Publication No: MX2010005908
- Ross, R.P., et al (2010) *Pseudomonas aeruginose* Bacteriophage(s) and uses thereof. Patent filed December 2010. EU Patent Application No: 10195995.

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

Contact Mary Rea Email: Mary.Rea@teagasc.ie