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Obesibiotics

Key external stakeholders:

Food supplement companies producing probiotics or food companies interested in probiotic-related food products. Food production companies working in the field of food preservation Nutritional and functional ingredient manufacturer/suppliers.

Practical implications for stakeholders:

The utilization of novel antimicrobials or antimicrobial-producing bacteria isolated from the gut microbiota and probiotic foods that target specific obesity-associated populations can be applied to the treatment/prevention of obesity and related disorders. Also, the utilization of novel antimicrobials in foods presents a viable and attractive alternative to the use of chemical food biopreseratives. Through this research:

- Food supplement companies will be able to offer probiotic strains and/or antimicrobials for the prevention of obesity and related disorders
- Food companies will be able to offer a healthy range of probiotic food products
- Food preservatives suppliers will be able to offer other products (novel bacteriocins and/or bacteriocin-producing strains) to increase the safety of food products

Main results:

The characterization of lead bacteriocin-producing probiotics with antimicrobial activity against obesity-associated microorganisms as well as non-obesity associated food and gut pathogens.
The bacteriocin bactofencin A has a relatively subtle influence on intestinal communities with a potentially positive impact on anaerobic populations such as *Bacteroides*, *Clostridium* and *Bifidibacterium* spp., as demonstrated using *ex vivo* trials
A bacteriocin-producing strain of *Streptococcus saliviarius* can have a positive impact on intestinal communities as determined using *ex vivo* trials
The ability of traditional kefir to potentially regulate obesity and obesity-related gut microbiota has been demonstrated using *in vivo* trials

Opportunity / Benefit:

Food supplements industry: bacteriocin-producing probiotics for the treatment/prevention of obesity and related disorders
Food Industry: assure and improve the microbial quality and safety status of Irish food
Food Industry Development: To provide Technology Development support for food industries, SMEs and start up food businesses in the Transfer of Research Knowledge Transfer Technologies

Collaborating Institutions:

Teagasc and UCC

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Teagasc project team: Dr Catriona Guinane, Dr. Clare Piper, Elaine Lawton, James Hegarty, Calum Walsh, Peter Skuse, Siobhan Clarke, Enriqueta García-Gutiérrez, Ben Bourrie, Aisling Heaney, Dr. Sara Arbulu, Prof Paul Cotter

External collaborators: Prof Colin Hill, Prof. Paul O'Toole (UCC)

1. Project background:

The microbes present in the gut are an underutilized source of valuable antimicrobial producers. Such antimicrobial producers could be employed to alter the overall composition of the gut microbial population in a beneficial way. Studies of the gut microbiota have already highlighted the importance of gut microbes with respect to obesity and related disorders. The overall objectives of this research programme were to harness the antimicrobial-producing capacity of the gut, and utilise this resource to identify 'obesibiotics', i.e., antimicrobial-producing gut microbes that can target specific obesity-associated populations with an ultimate view to the treatment/prevention of obesity and related disorders.

2. Questions addressed by the project:

- Can the gut microbiota be a source of novel antimicrobial peptides to treat and/or prevent obesity and related disorders?
- Can we use genetic tools to modify existing microbial producers to be more effective against obesity-related microorganisms?
- What are the environmental factors that trigger the production of gut bacteriocins?
- What is the impact of the production of one bacteriocin on the gut microbiota and consequently on the weight gain?

3. The experimental studies:

Antimicrobial activity tests on bacteriocin producing gut-microbes isolated from foods and lean donors

Mining bacteriocin gene clusters identified in genome sequenced microbes

Heterologous production of new bacteriocins in *Lactococcus lactis* NZ9000

Creation of isogenic, non bacteriocin-producing derivatives of selected strains

Purification and characterisation of the bacteriocin nisin P

Determination of the probiotic properties of novel strains of *Lactobacillus gasseri*, Lactobacillus *amylovorus*, *Lactobacillus crispatus* and *Staphylococcus epidermidis*.

Ex vivo trials using a simulated distal colon model to determine the effect of bacteriocin isolates and bacteriocin-producing bacteria on gut microbiota

In vivo trials using a diet-induced obesity (DIO) mouse model to study the effect of bacteriocin-producing probiotic strains on weight gain and metabolic health

Mouse trials to examine the effect of commercial versus traditional kefir isolates to regulate obesity and obesity gut microbiota

4. Main results:

The characterization of lead bacteriocin-producing probiotics with antimicrobial activity against obesity-associated microorganisms as well as non-obesity associated food and gut pathogens. These bacteriocin-isolates were then tested to specific *ex vivo* and *in vivo* trials to determine the full impact these antimicrobial producers have on the gut microbial composition, weight gain and on overall metabolic health.
Ex vivo trials through use of a simulated distal colon model was used to determine the effect of the bacteriocin producers on the gut microbiota. The results revealed that bactofencin A has a relatively subtle influence on intestinal communities, with a potentially positive impact on anaerobic populations such as *Bacteroides*, *Fusobacterium*, *Clostridium* and *Bifidobacterium* spp., which may indicate that the bacteriocin is impacting positively on gut health.

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A second *ex vivo* trial was used to determine the impact of a bacteriocin-producing *Streptococcus salivarius* on obesity-linked species and on overall gut microbial composition and diversity, showing that this strain can positively impact on intestinal communities.
Preliminary findings from a mouse model demonstrated the ability of traditional kefir to potentially regulate obesity and obesity-related gut microbiota. Furthermore, isolates from traditional kefir resulting in decreased weight gain, total plasma cholesterol, plasma non-HDL cholesterol, and liver triglycerides in traditional kefir fed mice along with alterations in gene expression relating to fatty acid metabolism.

5. Opportunity/Benefit:

A collection of isolates with antimicrobial activity against obesity associated targets as well as non-obesity associated food and gut pathogens is now available at Teagasc. From this collection, an in depth characterization of some of the bacteriocin-producing probiotics and study of their impact on gut microbial populations is also available. Additionally, new bioinformatics-based approaches have also been developed to study the distribution of bacteriocin gene clusters. The results from the Obesibiotics project gives important insights into how bacteriocin-producing probiotics may function within the gut and alter the microbiota with respect to obesity and obesity linked disease.

6. Dissemination:

Main publications:

1. Bourrie CTB, Cotter PD, Willing PB. 2018. Traditional kefir reduces weight gain and improves plasma and liver lipid profiles more successfully than a commercial equivalent in a mouse model of obesity. Journal of Functional Foods, 46: 29–37.
2. García-Gutiérrez E, Mayer MJ, Cotter PD, Narbad A. 2018. Gut microbiota as a source of novel antimicrobials. Gut Microbes, 10(1): 1–21.
3. Walsh CJ, Guinane CM, O' Toole PW, Cotter PD. 2017. A Profile Hidden Markov Model to investigate the distribution and frequency of LanB-encoding lantibiotic modification genes in the human oral and gut microbiome. PeerJ. 5: e3254.
4. Hegarty JW, Guinane CM, Ross RP, Hill C, Cotter PD. 2017. Lack of heterogeneity in bacteriocin production across a selection of commercial probiotic products. Probiotics Antimicrob Proteins, 9(4): 459–465.
5. Guinane CM, Lawton EM, O'Connor PM, O'Sullivan Ó, Hill C, Ross RP, Cotter PD. 2016. The bacteriocin bactofencin A subtly modulates gut microbial populations. Anaerobe. 40:41–9.
6. Hegarty JW, Guinane CM, Ross RP, Hill C, Cotter PD. 2016. Bacteriocin production: a relatively unharnessed probiotic trait? F1000Res, 5: 2587.
7. Bourrie BC, Willing BP, Cotter PD. 2016. The microbiota and health promoting characteristics of the fermented beverage kefir. Front Microbiol, 7: 647.
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9. Guinane CM, Kent RM, Norberg S, O'Connor PM, Cotter PD, Hill C, Fitzgerald GF, Stanton C, Ross RP. 2015. Generation of the antimicrobial peptide caseicin A from casein by hydrolysis with thermolysin enzymes. International Dairy Journal, 49:1–7.
10. Guinane CM, Piper C, Draper LA, O'Connor PM, Hill C, Ross RP, Cotter PD. 2015. Impact of environmental factors on bacteriocin promoter activity in gut-derived *Lactobacillus salivarius*. Appl Environ Microbiol, 81(22):7851–9.
11. Clarke SF, Murphy EF, O'Sullivan O, Lucey AJ, Humphreys M, Hogan A, Hayes P, O'Reilly M, Jeffery IB, Wood-Martin R, Kerins DM, Quigley E, Ross RP, O'Toole PW, Molloy MG, Falvey E, Shanahan F, Cotter PD. 2014. Exercise and associated dietary extremes impact on gut microbial diversity. Gut, 63(12):1913–20.
12. Clarke SF, Murphy EF, O'Sullivan O, Ross RP, O'Toole PW, Shanahan F, Cotter PD. 2013. Targeting the microbiota to address diet-induced obesity: a time dependent challenge. PLoS One, 8(6):e65790.
13. Guinane CM, Cotter PD. 2013. Role of the gut microbiota in health and chronic gastrointestinal disease: understanding a hidden metabolic organ. Therap Adv Gastroenterol. 6(4):295-308.
14. Cotter PD, Ross RP, Hill C. 2013. Bacteriocins - a viable alternative to antibiotics? Nat Rev Microbiol, 11(2):95–105.

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