Project number: 6585
Funding source: DAFM

Date: Nov 2018
Project dates: March 2014 – Oct 2018

PeptideProtectants

Key external stakeholders:

Food production companies and all industries working in the field of food preservation. Nutritional and functional ingredient manufacturer/suppliers. Policy makers.

Practical implications for stakeholders:

The utilization of novel bacteriocins, combinations with other antimicrobials or the direct application of the bacteriocin-producing strains in foods, are viable solutions to the development of natural food biopreseratives to replace the current market leader Nisin A, by providing better activity against food spoilage and pathogenic bacteria. Through this research

- Food production companies can enhance the food safety and increase the shelf-life of the final products while protecting the health of the consumers
- Food preservatives suppliers will be able to offer other products (novel bacteriocins and bacteriocin-producing strains) to increase the safety of food products
- Policy makers can consider the use of other bacteriocins other than Nisin A in order to properly regulate their use in foods. The data generated within the project can contribute to this process

Main results:

New approaches for the quantification of bacteriocins (by ELISA and/or HPLC) in different materials.
In order to target food related pathogens, a range of effective concentrations of bacteriocins with other food grade antimicrobial/food preservatives have been established
A food grade approach has been used to obtain a collection of novel nisin-variant producer strains, with very effective activity against spoilage and disease-causing bacteria, which can be used as starters. By using this food grade approach, the strains are not regarded as being genetically modified.
An affordable media using milk-based substrates for the production of bacteriocins at a semi-industrial scale has been developed
Food trials with different bacteriocins and bacteriocin-producing strains have been completed

Opportunity / Benefit:

Food: 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, UCC and TCD

Teagasc project team: Dr Mary Rea, Dr. Beatriz Gomez Sala, Gwynneth Halley, Dr Sheila Morgan, Dr André Brodkorb, Dr Paula O'Connor, Prof Paul Ross, Prof Paul Cotter

External collaborators: Prof Colin Hill, Dr Des Field, Dr. Kevin Egan (UCC, Microbiology Department), Prof. Elke Arendt (UCC, Nutritional Sciences Department) and Prof. Ursula Bond (TCD)

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1. Project background:

Food processors are facing an extraordinary dilemma as they try to address consumer demands for healthy, minimally-processed foods, while, paradoxically, being required to meet ever-increasing microbial safety standards. Antimicrobial peptides (AMPs), such as novel bacteriocins and defensins, offer viable solutions to the development of natural food biopreservatives to replace the current market leader Nisin A, by providing better activity against food spoilage and pathogenic bacteria. The participants of this research project have patented a number of novel peptides including variants of Nisin A, Bactofencin LS1, and human beta-defensin 3 (HBD3) which display potent activity against several Gram positive and negative bacteria, including *Listeria* and *Staphylococcus*, *Lactobacillus* and *Pediococcus* species. This research expands on the findings that combinations of different antimicrobials or their *in situ* production can address major microbe-related food safety issues and aims to characterize the available AMPs through combination-based approaches and to identify additional antimicrobial peptides. Furthermore, tasks focused on larger food-based studies, scaling up of peptide production and the formation of a steering group to advise with respect to regulatory issues have been developed to facilitate the possible commercialization of these novel AMPs.

2. Questions addressed by the project:

- How can nisin and other bacteriocins be accurately quantified?
- Can novel antimicrobial combinations be developed that are better than Nisin A with respect to controlling food pathogens and spoilage microorganisms associated with foods?
- What are the opportunities and challenges associated with upscaling the food grade production of antimicrobials?
- What is the regulatory status of these novel antimicrobials?

3. The experimental studies:

Obtaining polyclonal antibodies against the novel nisin variant, nisin V, and a new bacteriocin, bactofencin LS1, and the use of these to optimize an ELISA method for the quantification of bacteriocins

Identification of the most efficacious combination of nisin and other antimicrobials/food preservatives for laboratory scale food trials

Use of a food grade approach to develop nisin variant producers

Development and optimization fermentation and filtration facilities to obtain bacteriocins at a semi industrial scale to facilitate subsequent further upscaling

Food trials to assess the efficacy of bacteriocins and bacteriocin-producing strains to inhibit the growth of food-borne pathogens such as *Listeria monocytogenes* and *Salmonella*

4. Main results:

In this project a methodology for the purification and concentration of nisin and nisin derivatives from commercially produced products and from bacterial fermentates has been developed. Additionally, an ELISA method and a HPLC method have been successfully developed for quantification of nisin and bactofencin LS1. Based in this methodology, a range of effective concentrations of nisin peptide with other food grade antimicrobial/food preservatives have been established to target food related pathogens including *L. monocytogenes* and *E. coli*. Also, a collection of strains converted to nisin variant producers using food grade approaches were generated. Nisin variants that effectively inhibit organisms possessing nisin resistance proteins (including food-related and pathogenic microbes e.g. *Enterococcus*, *Streptococcus*, *Staphylococcus*) have been generated. An affordable media using milk-based substrates, mainly whey permeate, for the upscale production of bacteriocins has been developed. Also, bacteriocin-containing powders have been generated using different drying techniques and different carriers. These powders have been used to carry out several food trials with different bacteriocins in cottage cheese, a chicken juice model and fresh salmon against several common food-borne pathogenic bacteria. Each food system tested gave different results, but in general it can be concluded that bacteriocins and/or bacteriocin-producing strains can be used to increase the safety, food security as well as the economic value of food systems tested. Finally, the legal status of the antimicrobial peptides used in this project has been established and further steps towards commercialization have been made clear.

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5. Opportunity/Benefit:

A quantification method is now available as a tool for the detection of nisin and bactofensin in different media. Semi-industrial facilities for the fermentation and filtration of bacteriocin-containing material are in place at Teagasc and can be used for fermentation optimization prior to upscaling. A collection of novel strains that produce new and improved forms of nisin or the novel bacteriocin, bactofensin, are available for use in food systems.

6. Dissemination:

Main publications:

1. Field D, Daly K, O'Connor PM, Cotter PD, Hill C, Ross RP. 2015. Efficacies of nisin A and nisin V semi-purified preparations alone and in combination with plant essential oils for controlling *Listeria monocytogenes*. Appl Environ Microbiol 81:2762–2769.
2. Egan, K., Field, D., Rea, M.C., Ross, R.P., Hill, C., and Cotter, P.D. (2016). Bacteriocins: novel solutions to age old spore-related problems? Frontiers in Microbiology 7.
3. Alicia Campion, Ruth Morrissey, Des Field, Paul D. Cotter, Colin Hill and R. Paul Ross. (2017) Use of enhanced nisin derivatives in combination with food-grade oils or citric acid to control *Cronobacter sakazakii* and *Escherichia coli* O157:H7. Food Microbiology 65: 245-263.
4. Gough, R., Gómez-Sala, B., Rea, M.C., Miao, S., Hill, C. and Brodkorb, A. (2017) A simple method for the purification of nisin. Probiotics and Antimicrobial Proteins, 9:363-369.
5. Smith, M., Draper, L., Hazelhoff, P.J., Cotter, PD., Ross, R.P., Hill, C. (2016) A bioengineered derivative, M21A, in combination with food grade additives eradicates biofilms of *Listeria monocytogenes*. Front. Microbiol. 7: 1939
6. Kevin Egan, Paul Ross and Colin Hill (2017) Bacteriocins: antibiotics in the age of the microbiome. (2017). Emerging topics in Life Science, 1: 55-63.
7. Egan K, Kelleher P, Field D, Rea MC, Ross RP, Cotter PD, Hill C (2017) Genome sequence of *Geobacillus stearothermophilus* DSM 458, an antimicrobial-producing thermophilic bacterium, isolated from a sugar beet factory. Genome Announcements 2017, 5(43).
8. Field D, Baghou I, Rea M, Gardiner G, Ross R, Hill C (2017) Nisin in combination with cinnamaldehyde and EDTA to control growth of *Escherichia coli* strains of swine origin. Antibiotics 2017, 6(4):35.
9. Suda S, Field D, Barron N (2017) Antimicrobial peptide production and purification. Methods in molecular biology (Clifton, NJ) 2017, 1485:401-410.
10. Mathur H, Field D, Rea MC, Cotter PD, Hill C, Ross RP (2017) Bacteriocin-antimicrobial synergy: a medical and food perspective. Frontiers in microbiology 2017, 8:1205.
11. Campion A, Morrissey R, Field D, Cotter PD, Hill C, Ross RP (2017) Use of enhanced nisin derivatives in combination with food-grade oils or citric acid to control *Cronobacter sakazakii* and *Escherichia coli* O157:H7. Food microbiology 2017, 65:254-263.
12. Aspri M, O'Connor PM, Field D, Cotter PD, Ross P, Hill C, Papademas P (2017) Application of bacteriocin-producing *Enterococcus faecium* isolated from donkey milk, in the bio-control of *Listeria monocytogenes* in fresh whey cheese. International Dairy Journal 2017, 73:1-9.
13. Mathur H, Field D, Rea MC, Cotter PD, Hill C, Ross RP (2018) Fighting biofilms with lantibiotics and other groups of bacteriocins. npj Biofilms and Microbiomes 2018, 4(1):9.
14. Field D, Ross RP, Hill C (2018) Developing bacteriocins of lactic acid bacteria into next generation biopreservatives. Current Opinion in Food Science 2018, 20:1-6.

7. Compiled by: Beatriz Gómez Sala and Paul Cotter

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