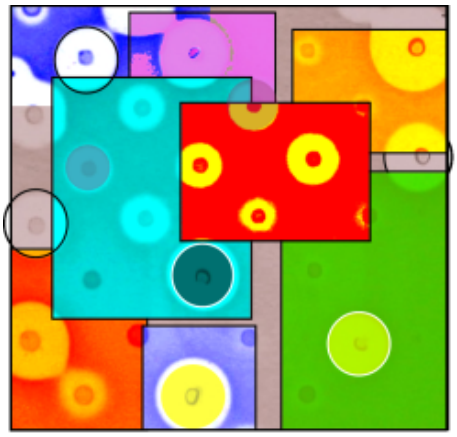
**Project number:** 5367
**Funding source:** DAFF (04/RD/C/232)

**Date:** March, 2012
**Project dates:** Jul 2005 – Jun 2009

# BIOCONTROL: bioactive ingredients for the control of undesirable bacteria in ready-to-eat foods.



## Key external stakeholders:

Food manufacturers and processors

## Practical implications for stakeholders:

In 2003, the US Food and Drug Administration issued a Final Rule which explicitly states that post-processing technologies must be included to limit the growth of *Listeria* in ready-to-eat products.
The Biocontrol project has resulted in the generation of a suite of food grade antimicrobials on which future novel anti-*Listeria* biopreservative products could be based.

- The identification of nisin derivatives with enhanced activity against Gram positive pathogens, including *Listeria*, is a major breakthrough. The fact that single amino acid changes can have such dramatic impacts is particularly noteworthy. From a commercial perspective it is significant that nisin is the only bacteriocin which has been approved as a food additive and nisin derivatives may be more likely to be approved by authorities than completely new compounds. In addition, nisin has been shown to have a number of other applications in animal and human health. Thus enhanced forms of nisin have the potential to impact on food safety, health and agriculture.
- A *Lactobacillus salivarius* strain producing an ABP118-like bacteriocin, which we designated salivaricin P, was identified. The fact that bacteriocins are produced by potentially probiotic strains is relevant to industry and consumers, since such strains could potentially be employed to control pathogens in the gut or to alter the overall gut microbial composition in a beneficial way.

## Main results:

- Novel anti-Listeria agents were identified and developed
- Food trials to demonstrate effectiveness were performed
- Patented IP resulted

## Opportunity / Benefit:

A patent relating to the novel nisin derivatives was filed: Publication number: WO2011076903

## Collaborating Institutions:

University College Cork



Contact Paul Cotter Email: paul.cotter@teagasc.ie.

**Teagasc project team:** Paul Ross
Paul Cotter
Eileen O'Shea
**External collaborators:** Colin Hill, University College Cork

## 1. Project background:

This project focused on the "biological mining" of natural food sources for food grade biopreservatives, with particular emphasis on the isolation of novel anti-listerial agents with a view to their exploitation in ready-to-eat foods. This project was designed against an increasingly difficult legislative background in an attempt to meet the conflicting demands for higher food safety standards in a consumer-driven market which is increasingly hostile to chemical preservatives and overly processed foods. These potentially opposing demands for better microbiological control in the absence of traditional chemical additives and processing steps (salting, acidification, heat, freezing) create a need for novel approaches using natural food ingredients and/or biopreservatives to achieve the higher levels of controls necessary to guarantee safe foods. For example, anti-*Listeria* bacteriocins produced by food grade bacteria can be a very important tool in controlling or suppressing the growth of this dangerous pathogen in ready-to-eat foods – thus meeting the legislative requirements in the USA for such technologies (the US Food and Drug Administration in 2003 issued a Final Rule which explicitly states that post-processing technologies must be included to limit the growth of *Listeria* in ready-to-eat products). However, such strategies are not limited to anti-*Listeria* bacteriocins, but can also be extended to naturally occurring food constituents that inhibit listerial growth. It was anticipated that this project would not only assist food manufacturers in meeting the stipulations of the Final Rule by providing bioactive anti-*Listeria* ingredients, but would also provide a platform from which a variety of functional ingredients would be identified with the potential to suppress other undesirable organisms and give additional benefits such as extended shelf life through the exploitation of viable alternatives to traditional additives.

## 2. Questions addressed by the project:

- Can we identify novel microbially produced anti-Listerials?
- Can we demonstrate effectiveness in food systems?
- Can we improve the existing Nisin to generate a more effective nisin based biopreservative?

## 3. The experimental studies:

A novel strategy for screening for bacteriocins, which relied on a robotics based approach, was developed and implemented. PCR was employed to differentiate between producers of known and potentially novel bacteriocins. A method for assessing the anti-listerial activity of multiple strains in milk, which relied on the use of light emitting *Listeria*, was also developed and implemented.
Comparative genome hybridisation was successfully employed for the first time to identify novel salivaricins. These required the development of bacteriocin purification technologies as well as procedures to generate synthetic forms thereof to facilitate further investigation.
Random, site specific and site saturation mutagenesis was employed to make large collections of lantibiotic derivatives. DNA sequencing was employed to uncover the nature of the changes made. Subsequent purification, specific activity studies and food trials revealed the potency and value of these peptides.

## 4. Main results:

- A delivery system was generated to facilitate the expression of pediocin PA-1 (an anti-Listeria bacteriocin) by the probiotic strain *Lactobacillus paracasei* 338.
- Co-cultures of *Listeria* with the pediocin PA-1-producing probiotic resulted in a two log decrease of *Listeria* compared to the control.
- 169 antimicrobial isolates (including cocci and bacilli) were screened from food and plant samples. Those with the most potent anti-*Listeria* activity were found to be enterococci, many producing enterocins.
- Enterocin A was heterologously expressed and produced in a strain of *Lactococcus lactis*.
- The enterocin A-producing *Lactococcus* was used as an adjunct culture to make cottage cheese and was found to successfully control *Listeria monocytogenes* in this food.
- A further Enterococcal isolate was found to control *L. monocytogenes* in milk over a 17 hour period. This strain was found to be the producer of mundticin.
- A further 18 bacteriocin producers of note were isolated from human or animal sources. A *Lactobacillus*



Contact Paul Cotter Email: paul.cotter@teagasc.ie.

*salivarius* strain producing an ABP118-like bacteriocin, which we designated salivaricin P, was identified.

- The activity of the natural salivaricin P peptides were investigated and their potency demonstrated. Synthetic peptides were generated and a number of derivatives were created to investigate structure/function relationships.
- The use of array Comparative Genomic Hybridisation (aCGH), mass spectrometry and, ultimately, peptide purification technologies uncovered two novel bacteriocins produced by an intestinal Lb. salivarius isolates of porcine origin.
- A bank of derivatives of nisin were screened to identify peptides with enhanced anti-Listeria activity. 51 nisin variants with enhanced anti-*Listeria* activity were ultimately identified. Of these, a strain in which a M21V amino acid substitution occurs was notable by virtue of its activity.
- The potency of M21V was confirmed through MIC assays with multiple strains, co-culture studies as well as through food trials with reconstituted skim milk and hot dog meat.
- Food-grade producers of M21V and of other nisin derivatives were generated.

## 5. Opportunity/Benefit:

It is anticipated that novel salivaricins and nisin derivatives can be exploited by industry. Bacterial strains producing novel salivaricins have the potential to be employed as probiotics. Nisin derivatives are of relevance to the food, health and agriculture-associated industries since they have been demonstrated to have applications in food preservation, animal health and human health

The benefits of the Biocontrol project include the generation of novel bioactive peptides for pathogen control which will result in safer food for human consumption.

A patent was filed as a result of this research: Publication number: WO2011076903, based on the novel nisin derivatives.

## 6. Dissemination:

The findings of the Biocontrol project were disseminated widely through peer reviewed publications, national and international oral presentations and poster presentations.

### Main publications:


- Liu, L., P.M. O'Connor, P.D. Cotter, C. Hill and R.P. Ross. (2008). Controlling Listeria monocytogenes in Cottage cheese through heterologous production of enterocin A by Lactococcus lactis. *Journal of Applied Microbiology* 104:1059
- O'Shea, E.F., Gardiner, G.E., O'Connor, P.M., Mills, S., Ross, R.P. and C. Hill. (2009) Characterization of enterocin- and salivaricin-producing lactic acid bacteria from the mammalian gastrointestinal tract. *FEMS Microbiology Letters* 29:24
- Field, D., L. Quigley, P.M. O'Connor, M.C. Rea, K. Daly, P.D. Cotter, C. Hill and R.P. Ross. (2010). Studies with bioengineered Nisin peptides highlight the broad-spectrum potency of Nisin V. *Microbial Biotechnology* 3:473


### Oral presentations:

- Paul Ross - "New bacterial functionality in dairy products" - Invited speaker at IDF World Dairy Summit Mexico City, November 2008.
- Colin Hill - Oral presentation entitled "Applications of lantibiotics" presentations at LAB 9, Egmond aan Zee, Netherlands, September 2008
- Paul Cotter - Invited oral presentation at the SGM Spring Conference, Harrogate, UK. April 2009. 'Lantibiotics - mechanisms of killing and novel applications'

## 7. Compiled by: Paul Ross, Paul Cotter, Sheila Morgan



Contact Paul Cotter Email: paul.cotter@teagasc.ie.