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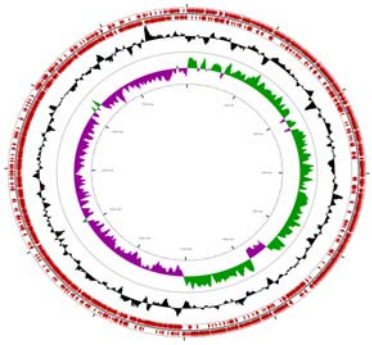
FUNLAC: Lacticin-based ingredients for biopreservative and functional food applications



Key external stakeholders:

Food producers

Practical implications for stakeholders:

- A genome sequence of the lacticin producing strain was completed, which allows identification of genes relevant to industrial and food safety applications. This genetic blueprint can additionally be used to identify and exploit other interesting traits (both fundamental and commercial) associated with the strain
- A *Lactococcus lactis* strain identified as producing elevated antimicrobial activity was investigated. This is of relevance to the food industry given that the use of this strain results in elevated lacticin 3147 activity at no additional cost thereby improving commercial value and impacting on the use of the antimicrobial lacticin 3147 in food industry applications.
- When assessed *in vivo*, lacticin 3147 was found to be degraded within the gastrointestinal tract by the enzyme α-chymotrypsin. Thus, lacticin 3147 was deemed safe for ingestion, given that it would not impact negatively on commensal gut flora. Additionally, the fact that lacticin 3147 is effective in the oral cavity provides the opportunity to influence dental health through the development of oral food applications
- Lacticin 3147 has been demonstrated to be a robust antimicrobial with the ability to control food spoilage and pathogenic bacteria in non dairy foods. It was found to be particularly effective for the control of *Bacillus cereus* on beansprouts, with results indicating that is more effective than the conventional hypochloride solutions, currently used.

Main results:

- The genome sequence of the lacticin 3147 producing strain was completed
- In one of the first reports of its kind, where a lantibiotic was assessed *in vivo*, lacticin 3147 was found to be degraded within the gastrointestinal tract by the enzyme α-chymotrypsin. Thus, lacticin 3147 was deemed safe for ingestion.
- Lacticin 3147 was demonstrated to be a robust antimicrobial with the ability to control food spoilage and pathogenic bacteria in non dairy foods. Lacticin was demonstrated to be particularly effective for the control of *Bacillus cereus* on beansprouts, with results indicating that is more effective than the conventional hypochloride solutions, currently used.

Opportunity / Benefit:

The antimicrobial lacticin 3147 has both food and biomedical applications and has been demonstrated to be effective against all Gram positive bacteria tested to date. It has a demonstrated safe history of use and therefore is free from additive status. It is a natural antimicrobial that could be the solution to a broad range of microbial problems. Equally the genome sequence of the strain is available for exploitation.

Collaborating Institutions:

UCC

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1. Project background:

Food biopreservation is an area of immediate concern to all food producers. The production of foods which are safe for consumers requires continuous attention to safeguard against spoilage and pathogenic microorganisms. Increasingly consumers are selecting foods with natural rather than chemical preservatives and as such lacticin is well positioned to offer the food industry the potential to reduce the incidence of morbidity and mortality associated with certain high-risk foods, to extend the shelf life of perishable foods through control of spoilage organisms, and to confer functionality and added value on commodity items. The development of lacticin as a bioactive ingredient for commercial application requires that fundamental information on some of the key properties of lacticin be determined, in addition to developing a commercially scaled optimised fermentation systems for production, determination of its fate during gastric transit, determining resistance development in target organisms and investigating its effectiveness in non dairy foods.

2. Questions addressed by the project:

This project addressed the following questions:

- Can we design better antimicrobial producing strains?
- Can we develop a lacticin production system to generate lacticin powders for commercial applications
- When lacticin is ingested, what is the subsequent effect on gut flora?
- Is there an issue with lacticin 3147 resistance development?
- At a structure/function level how does lacticin differ from another 2 component antimicrobial, staphyloccin C55?
- Lacticin has been shown to control pathogens in dairy based foods, will it also work in non dairy foods?

3. The experimental studies:

As a first step to the development of more effective antimicrobial producing strains the entire genome of the original lacticin producing strain was sequenced. Completion of the genome assembly provides a genetic blueprint of *Lactococcus lactis* DPC3147, a strain with significant commercial potential due to its ability to produce lacticin 3147. Furthermore, a natural isolate of *Lactococcus lactis* was identified as producing elevated lacticin 3147 activity.

A number of pilot plant trials were performed to investigate the development of a suitable system for production of a lacticin powder, for food use. The system involved an overnight pH controlled optimized fermentation, followed by a membrane filtration step, to remove accumulated sodium lactate, prior to evaporation and drying. Powders developed had a lower salt content and were found to be stable during storage at 4^{o}C.

In order to determine the fate of ingested lacticin initial investigations performed involving the feeding two pigs a resuspended lacticin powder preparation for 5 consecutive days. Results of this study indicated that lacticin could not be recovered from the faecal matter of pigs that had ingested it, suggesting that it may become inactivated during gut transit. The digestive enzyme α-chymostrpsin (found in the small intestine) was found to degradation lacticin. To investigate if the oral administration of lacticin would modulates the gut microflora *in vivo* a further study was undertaken, where weaned pigs were fed reconstituted lacticin skim milk for seven days (1 million AU lacticin per day) or simply reconstituted skim milk. Faecal samples were collected and analysed to monitor the impact of lacticin on the established gut microflora. Statistical analysis of the bacterial counts has revealed that there were no differences in faecal counts of Lactobacillus, Enterococcus, coliform, Bacteroides or total anaerobes between lacticin-fed and control pigs, indicating that administration of lacticin does not perturb these dominant groups of Gram-positive or Gram-negative gut microflora, and indicating that lacticin had been degraded in the upper digestive tract.

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As a consequence of the lantibiotic resistance studies performed during the project term, we now have a more detailed understanding of the mechanisms involved in resistance development and we thus expect that results will be of interest to both fundamental and applied scientists. In addition, the identification of a series of novel genes and proteins that are responsible for the innate antimicrobial resistance of pathogens such as *L. monocytogenes* can lead to the development of new and exciting strategies to better control these in foods and other environments. More specifically, while our studies have reassuringly confirmed that bacteria encounter great difficulty when trying to develop resistance to lacticin 3147, it has revealed that targets such as *L. monocytogenes* can possess significant levels of innate resistance to related lantibiotics such as nisin which is mediated via regulators, transporters and proteins which impact on the composition of the cell envelope. Through the targeting of these providers of innate resistance, it may be possible to enhance the efficacy of lantibiotics against many targets.

Two component lantibiotics, such as lacticin 3147 produced by *Lactococcus lactis* DPC3147 and staphylococcin C55 produced by *Staphylococcus aureus* C55, represent an emerging subgroup of bacteriocins. These two bacteriocins are closely related, exhibiting 86% (LtnA1 and C55α) and 55% (LtnA2 and C55β) identity in their component peptides. Structure function relationships between these complex bacteriocins were investigated. Purified peptides of one bacteriocin could be shown to substitute for their counterparts in the other bacteriocin with high specific activities. Indeed, heterologous combinations (LtnA1 with C55β or C55α with LtnA2) were found to have activities in the single nanomolar range, comparing well with the native pairings. In order to examine the specificity of the post-translational/processing machinery, the staphylococcin C55 structural genes were directly substituted for their lacticin counterparts in the ltn operon on the large conjugative lactococcal plasmid pMRC01. The resultant plasmids demonstrated that the lacticin LtnA1 post-translational and processing machinery could produce functionally active C55α, but not C55β. Furthermore, substitution of three residues in LtnA1 to their equivalent residues in C55α demonstrated that LtnA1 is not active when position 21 is changed from Leu to Ala, despite the presence of an Ala in that position in C55α. This is significant given the positioning of this residue in the putative lipid II binding loop.

Ready-to-eat, fresh cut vegetables have become a commercially important convenience food item in recent years. Food-borne pathogens such as *Listeria monocytogenes* and *Bacillus cereus* are frequently found on the surface of plant materials such as vegetable sprouts and lettuce. Chemical disinfectants are routinely used to inhibit such pathogens; however, consumer concerns about the use of chemical additives in food processing have lead to a requirement to develop acceptable alternatives, to ensure product safety and shelf-life. In this regard, one approach has been to use lactic acid bacteria or their bacteriocins to control the growth and survival of these undesirable micro-organisms. The potential of a resuspended powder preparation of lacticin was investigated for its ability to inhibit *L. monocytogenes* and *B. cereus* on the surface of lettuce. When the lettuce was washed with a lacticin solution, recoveries of *B. cereus* were significantly reduced by 2-3 logs relative to the controls. This reduction occurred even when levels of 10^7 cfu g^{-1} *B. cereus* were inoculated onto the surface of the lettuce. *L. monocytogenes* (10^5 cfu) was also inoculated onto the surface of lettuce and bean sprouts and the vegetables then washed with a lacticin solution. No significant reduction in Listeria numbers was observed. When *Listeria monocytogenes* was inoculated at the lower level of 10^3 cfu g^{-1}, however, a significant reduction in recoveries of *Listeria* following washing with a lacticin solution was observed. As the numbers of pathogenic bacteria required to cause disease *in vivo* are quite low, these studies demonstrate that a wash solution containing lacticin used in the preparation of ready-to-eat vegetables would have potential to protect against the food-borne pathogens *B. cereus* and *L. monocytogenes*.

4. Main results:

- The genome sequence of the lacticin 3147 producing strain was completed
- A production system for the generation of a reduced salt, lacticin 3147 enriched powder has been developed at pilot scale
- Lacticin 3147 powders were found to be stable upon storage at 4ºC
- In one of the first reports of its kind, where a lantibiotic was assessed in vivo, lacticin 3147 was found to be degraded within the gastrointestinal tract by the enzyme α-chymotrypsin. Thus, lacticin 3147 was deemed safe for ingestion.
- A number of novel and previously described loci involved in innate lantibiotic resistance in *L. monocytogenes* were mutated. Mutation of these genes resulted in a significant impact on the sensitivity of the strains to a number of antimicrobials. A selection of mutants were employed to confirm that altered sensitivity to lantibiotics in the laboratory also correlates with an altered behaviour in lantibiotic-containing foods.

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- Lacticin 3147 was found to be very similar to another bacteriocin, C55, produced by *Staphylococcus aureus*, in terms of structure, genetics and functioning.
- Lacticin 3147 has been demonstrated to be a robust antimicrobial with the ability to control food spoilage and pathogenic bacteria in non dairy foods. Lacticin was demonstrated to be particularly effective for the control of *Bacillus cereus* on beansprouts, with results indicating that is more effective than the conventional hypochloride solutions, currently used.

5. Opportunity/Benefit:

Lacticin 3147 can be used for the control of Gram positive bacteria in food systems
Lacticin 3147 can be used to control Gram positive populations in the oral cavity
If delivered in a suitable encapsulated form lacticin 3147 can be used to control Gram positive populations within the mammalian gastrointestinal tract.

6. Dissemination:

Research performed within this project was presented (by the PIs Paul Ross and Colin Hill) to many national and international audiences including:

- The IDF World Dairy Summit, Dublin. October 2007 "Fermentation Processes as a source of Bioactive Metabolites"
- The IDF World Dairy Summit, Mexico City November 2008."New bacterial functionality in dairy products"
- The CNTA Conference on Food and Health, Pamplona, Spain, November 2009. "Bacteriocins; bacterial solutions to bacterial problems".

Main publications:

1. O'Connor, E.B., Cotter, P.D., O'Connor, P., O'Sullivan, O., Tagg. J.R., Ross, R.P., Hill, C. (2007). Relatedness between the two-component lantibiotics lacticin 3147 and staphylococcin C55 based on structure, genetics and biological activity. *BMC Microbiology* 7, 24.
2. Gardiner, E.G., Rea, M.C., O'Riordan, B., O'Connor, P., Morgan, S.M., Lawlor, P.G., Lynch, P.B., Cronin, M., Ross, R.P and Hill, C. (2007). Fate of the two-component lantibiotic lacticin 3147 in the gastrointestinal tract. *Applied and Environmental Microbiology* 73, 7103-9.
3. Collins, B., Joyce, S., Hill, C., Cotter, P.D., Ross, R.P. (2010) TelA contributes to the innate resistance of Listeria monocyctogenes to nisin and other cell wall-acting antibiotics. Antimicrob Agents Chemother. 54(11):4658-63.

7. Compiled by: Sheila Morgan

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