DEVELOPMENT OF THE BACTERIOCIN, LACTICIN 3147 FROM *LACTOCOCCUS LACTIS*, INTO AN ANTIBIOTIC

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Background: Due to an alarming increase in antimicrobial resistance worldwide, alternatives to traditional antibiotics, such as antimicrobial peptides are being investigated. Lacticin 3147 is a two-component antimicrobial peptide (LtnA1 and LtnA2), produced by *Lactococcus lactis* DPC6577. It is active against many antimicrobial-resistant bacteria at a nanomolar scale, e.g. *Clostridium difficile* and methicillin-resistant *S. aureus*. The use of antimicrobial peptides, such as lacticin 3147, is limited due to their susceptibility to enzymatic cleavage, their propensity to unfold and aggregate and their instability *in vivo*. This project involves (i) the production and purification of lacticin 3147 (ii) the study of its stability and solubility and (iii) the design of a formulation strategy to optimise its activity upon oral administration.

Methods: Lacticin 3147 was produced and purified following a protocol developed by Rea *et al*. Lacticin 3147 production was confirmed by MALDI-TOF spectroscopy using sinnapinic acid as the matrix. The effect of the gastrointestinal enzymes on lacticin 3147s and activity and structure was investigated. Known concentrations of pepsin, trypsin and α-chymotrypsin were added to a 400 nM lacticin solution in a 96 well plate and left for 3 hours. 1 ul of each was removed and analysed by MALDI-TOF. 150 ul of *L. monocytogenes* ATCC1916 in TSB (OD=0.1) was added to the remaining solutions. The 96 well plate was incubated in a Biotek ELx808 Ultra microplate reader at 37 °C. Readings were taken every 30 mins for 24 hrs at a wavelength of 590 nm. To investigate lacticin 3147's solubility and activity in a variety of media, solutions of the individual lacticin 3147 peptides (0.5 mg/ml) were made up in PBS buffer (pH 7.4), HCl/KCl buffer (pH 2), FaSSGF (pH 1.6) and FaSSIF (pH6.5). At 0 hrs, 5 hrs and 24 hrs, aliquots of each sample were taken, filtered and analysed by RP-HPLC. 25 µl of the filtered aliquots were added in triplicate to a 96 well plate. The wells were then filled to 200µL with bacterial cell culture and analysed for bacterial growth.

Results: Lacticin 3147 was rendered inactive after incubation with trypsin and α-chymotrypsin indicting that both enzymes degrade it. Pepsin did not have any effect on lacticin 3147's activity despite the presence of pepsin's target amino acids in LtnA1. The lacticin 3147 peptides work synergistically to kill bacteria therefore the structures of the peptides after incubation with trypsin and α-chymotrypsin were examined by MALDI-TOF spectroscopy. The absence of the LtnA1 and LtnA2 peaks in the resulting spectra showed that both peptides were degraded by trypsin and α-chymotrypsin. Both lacticin 3147 peptides showed low solubility in pH 2.2 and pH 7.4 aqueous buffers. Both peptides' solubility increased in FaSSGF and FASSIF but LtnA1 was seen to be unstable over time. As the concentration of lecithin and NaTc is above the critical micelle concentration in FaSSIF the encapsulation of LtnA1 and LtnA2 in micelles may account for the increase in solubility. Despite lacticin 3147's poor solubility in PBS (only 0.34% and 0.55% of the 0.5 mg of LtnA1 and LtnA2 added dissolved), it was still found to be active. This is because <1 ug/ml of LtnA1 and LtnA2 is active against *L. monocytogenes*.

Conclusions Both lacticin 3147 peptides exhibit some aqueous solubility but it is low and can be unstable in biorelevant media. This coupled with its susceptibility to degradation by enzymes found *in vivo* necessitates the development of a controlled release system for this dual-acting antimicrobial peptide. From these results, two approaches are currently being studied – polymer conjugation and lipid-based delivery systems.