Isolation and characterisation of an antimicrobial peptide from *Enterococcus* B9510

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ABSTRACT

This work deals with the antimicrobial activity of bacteriocins produced by *Enterococcus* B9510. The greatest importance demonstrated by this work was the range of activity exhibited by this bacterium, across both Gram positive and negative pathogens. This is not typical for bacteriocins, which tend to inhibit only closely related bacteria in similar environments. While attempts to identify the peptide sequence were unsuccessful the overall results of this investigation are enough to suggest that the bacteriocin from *Enterococcus* B9510 was novel and warrants further investigation.

Bacteriocins are small peptides that demonstrate antimicrobial properties and are produced by a number of lactic acid bacteria, including *Enterococcus sp.*. Bacteriocins are important because they are a possible alternative for food preservation. As a natural product they could be used to improve customer perceptions, due to the reduced need for chemical preservatives in products. From a range of lactic acid bacteria which were tested for antimicrobial activity across a selection of pathogens the strain *Enterococcus* B9510, which was a local isolate from bovine rumen, was selected due to its high level of activity. Using 16S ribosomal DNA (rDNA) analysis and sequence alignment with the BLAST network service, B9510 showed the highest homology (97%) to *Enterococcus faecalis* in the genome database.

Fermentation trials were carried out in order to maximise the concentrations of bacteriocins produced. In a controlled pH environment, cellular growth and activity was highest at pH 5.5; however, the effect of low pH showed a higher relative activity at pH 4, possibly due to pH interactions with the bacteriocin. Multi-factor trials were carried out to find the effect of glucose concentration, salt concentration and air saturation on the bacteriocin production. High salt concentrations showed a reduced production of bacteriocins and low cellular growth whereas aeration had little effect on growth but some affect on bacteriocin activity, possibly due to a variation in stress on the bacteria. Glucose concentration effected cellular growth rates, which may be a significant factor in the production of the antimicrobial activity.

Purification of the bacteriocins was carried out using two methods. The pH binding method of attaching the bacteriocins to the cell wall by neutralising the pH of the

fermentation broth was unsuccessful over a range of pH values, pH 6-9. However, the resin binding method was successful in binding the bacteriocins from a cell free broth.

An ion-exchange resin (Macro-Prep CM) was used to purify the active fraction and remove a large pigment component associated with the fermentation media. High Performance Liquid Chromatography provided an effective means to isolate samples for molecular weight determination and N-terminal sequencing.

Antimicrobial activity of the isolated fraction was tested under a range of environmental conditions including temperature, salt concentration and pH. Bacteriocin activity was still present after being held at 100°C for an hour. Optimal activity was observed at pH 3, but activity was shown by the peptide over the range of pH 1.7 - 11. Low levels of salt (<200 mM) increased the activity of the peptides; however, high concentrations (≥500 mM) reduced the effectiveness of the bacteriocin. Enzymes which acted upon peptide bonds were effective at inactivating the bacteriocin, while non-peptidase enzymes were ineffective. Testing against thirteen different bacteria, including *E. coli*, *Listeria* and *Bacillus*, showed that the peptide was active against both Gram positive and Gram negative bacteria.

Mass determination showed that the active peptide was between 1.27 and 1.35 kDa. Attempts to fully sequence the purified peptide were unsuccessful, however, an N-terminal sequence of LMPPYGVIMFF was predicted, which had a molecular weight of 1.314 kDa. This sequence showed no significant homology to any known sequences in the protein databases using the BLAST network service.

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FREQUENTLY USED ABBREVIATIONS

ADP	Adenosine Di-phosphate
AMP	Adenosine Mono-phosphate
ATP	Adenosine Tri-phosphate
au	Arbitrary units
HIC	Hydrophobic Interaction Chromotography
HPLC	High Performance Liquid Chromatography
Da	Daltons
LAB	Lactic Acid Bacteria
LC-MS-MS	Liquid Chromatography Tandem Mass Spectrometry
MIC	Minimum Inhibitory Concentration
M	Molar
PCR	Polymerase Chain Reaction
sp.	Species
rpm	Revolutions per Minute

1 INTRODUCTION

1.1 REASON FOR RESEARCH

This research was carried out to investigate the bacteriocins from *Enterococcus* B9510, and to test the bacteriocin under a range of conditions. Bacteriocins are small peptides produced by bacteria as a form of physiological advantage in their natural environments, in order to kill other bacteria competing for the same resources. Interest in bacteriocins has grown around the desire for a natural preservative in food products and as an alternate to traditional antibiotics in medicine. Investigation of *Enterococcus* B9510 was undertaken after testing showed the antimicrobial activity of this bacterium was the greatest of a range of lactic acid bacteria tested, strength of the bacterial inhibition, against both Gram positive and negative bacteria ere measured. The stability of the activity demonstrated by *Enterococcus* B9510 when exposed to acidic conditions and high temperatures was also higher that the other samples tested.

1.2 PROJECT OBJECTIVES

This project had the following objectives:

- Optimise fermentation conditions for the production of bacteriocins in *Enterococcus faecalis* B9510.
- Compare methods of extraction and purification of the bacteriocins.
- Optimise one method to maximise the yield of bacteriocins.
- Test the spectrum of bactericidal activity of the semi-purified and pure bacteriocins.
- Test the effect of various environmental conditions on the bacteriocin activities.
- Determine molecular mass and amino acid sequence of purified bacteriocins.