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Investigating the Bacteriocin Library of *Lactobacillus plantarum* A-1

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Investigating the Bacteriocin Library *Lactobacillus plantarum* A-1

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Abstract

Bacteriocins are a highly diverse group of ribosomally synthesised, antimicrobial polypeptides produced by nearly all bacterial and archaeal species. Individual bacteriocins typically exhibit a narrow phylogenetic range of activity, but collectively inhibit a wide range of species through a variety of mechanisms. Glycocins are uncommon bacteriocins with rare, *S*-linked glycosidic bond;, currently there are only four characterised glycocins.

Preliminary characterisation of the bacteriocin ASM1 from *Lactobacillus plantarum* A-1 was reported by Hata *et al.* in 2010. ASM1 is structurally similar to GccF, a glycocin produced by *L. plantarum* KW30. Like GccF, ASM1 has two covalently linked N-acetylglucosamine moieties, one of which is attached through a rare *S*-glycosidic bond.

Due to its structural similarity, it was hypothesised that ASM1 would have similar inhibitory activity to GccF. Experiments showed that the two bacteriocins have almost identical inhibitory activity and both glycocins rely on their GlcNAc moieties to inhibit target cells.

The range of species inhibited by ASM1 was shown to be wider than previously thought. The inhibitory activity, however, varied considerably even between strains in a species.

The ASM1 gene cluster was established by sequencing and Southern hybridisation to be located on a 11,905 bp plasmid pA1_ASM1. An *in silico* analysis of the ASM1 gene cluster showed it to have the same operonic organisation similar as the GccF cluster, and overall DNA sequence identity of 75%.

A second active bacteriocin gene cluster was detected in the *L. plantarum* A-1 genome. A synthetic peptide, named ASM2, corresponding to this bacteriocin was partially characterised. ASM2 has 82% amino acid sequence identity to the recently identified bactofencin A produced by *L. salivarius* DPC6502.

A brief *in silico* analysis of proteins from the A-1 bacteriocin library and their orthologues provided some evolutionary context for the glycocins of *Lactobacillus* and gave hints as to the evolutionary history of the four characterised glycocins.

ASM1 is one of four characterised glycocins and this work has increased the overall knowledge of glycocins. Identification of a novel secondary bacteriocin in *L. plantarum* A-1 shows the complexity of bacteriocin systems and provides many avenues for future research regarding bacteria that produce multiple bacteriocins.

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List of Abbreviations

aa	Amino Acid
ABC	ATP Binding Cassette
ATCC	American Type Culture Collection
ATP	Adenosine Triphosphate
bac^-	Bacteriocin minus (mutants lacking expected bacteriocin production)
BLAST	Basic Local Alignment Search Tool
bp	Base Pairs
cd	Conserved Domain
Da	Dalton
dH_2O	Distilled Water
DNA	Deoxyribonucleic Acid
dNTP	Deoxyribose nucleotide triphosphate
DSM	Deutsche Sammlung von Mikroorganismen (German Collection of Microorganisms)
DUF	Domain of Unknown Function
et al.	*et alteri* (and others)
EtOH	Ethanol
g	standard gravity (9.81 ms^{-2})
GccF	Glycocin F
gDNA	Genomic DNA
GlcNAc	N-Acetyl Glucosamine
GRAS	Generally Regarded As Safe
GTase	Glycosyltransferase
HPK	Histidine Protein Kinase
HTH	Helix-Turn-Helix
IC_{50}	Concentration required to inhibit 50% of the population
LAB	Lactic Acid Bacteria
LB	Luria Broth
M	Molar (moles per litre)
min	minute
MUSCLE	Multiple Sequence Comparison by Log-Expectation
MRS	de Man, Rogosa and Sharpe medium
MRSA	Methicillin Resistant *Staphylococcus aureus*
NCBI	National Centre of Biotechnology Information
NZRM	New Zealand Reference Culture Collection, Medical Section
OD_{600}	Optical Density at 600 nm wavelength
ORF	Open Reading Frame

PCR	Polymerase Chain Reaction
pH	Negative $\log_{10}$ of proton concentration
RP-HPLC	Reverse-Phase High Pressure Chromatography
rpm	revolutions per minute
RT	Room Temperature
SDS-PAGE	Sodium Dodecyl Disulfate Polyacrylamide Gel Electrophoresis
T-COFFEE	Tree-based Consistency Objective Function For alignment Evaluation
TE	Tris-EDTA
TRX	Thioredoxin
TSB	Tryptone soya broth
UV	Ultraviolet
VRE	Vancomycin resistant *Enterococci*
w/v	Weight per volume