The Bacteriostatic Spectrum and Inhibitory Mechanism of Glycocin F, a Bacteriocin from *Lactobacillus plantarum* KW30

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Abstract

Bacteriocins have been deemed the "microbial weapon of choice". The ability to ribosomally synthesise these toxins means that their peptide scaffolds can be rapidly adapted to optimise stability, potency and specificity, allowing producers to outgrow closely related strains and become dominant. In some cases, a bacteriocin may inhibit a broader spectrum of microbes than just its species/genus of origin. Recently, the bacteriocin glycocin F (GccF), produced by *Lactobacillus plantarum* KW30, was biochemically and structurally characterised. GccF is unique, as it has two covalently linked N-acetylglucosamine (GlcNAc) moieties, one *O*-linked and one *S*-linked, that are critical for the inhibition of target cell growth.

How GccF causes bacteriostasis in sensitive *Lactobacillus* cells was unknown. Experiments were developed and conducted to probe the antimicrobial spectrum of GccF and how this spectrum is affected by free GlcNAc. It was found that a variety of species and strains, not just those closely related to *L. plantarum* KW30, were inhibited by the addition of GccF to cultures in solid or liquid media. Susceptible strains were identified in the genera *Streptococcus*, *Enterococcus*, and *Bacillus*. Interestingly, assays indicated that free GlcNAc plays a more dynamic role in modulating GccF activity than previously thought. The protective effect of high concentrations of GlcNAc, including the reversal of GccF-induced bacteriostasis, was confirmed for susceptible *L. plantarum* strains, but surprisingly addition of relatively low concentrations of GlcNAc prior to GccF led to a concentration-dependent increase in bacteriostasis for some other species including *Enterococcus faecalis*. GccF's mechanism of action was found to be different to the bactericidal membrane-permeabilising effect of the lantibiotic nisin, as *L. plantarum* cells treated with GccF did not die, and there was no substantial release of ATP from cells upon GccF-induced bacteriostasis.

It was also found that for Gram-negative bacteria, which are generally resistant to GccF, growth inhibition was greatly enhanced if the integrity of the outer membrane was compromised by treatment with polymyxin, or by expression of a 'leaky' mutant of the outer membrane secretin PulD. Thus GccF-mediated inhibition of growth is limited to Gram-positive bacteria mainly because of the barrier function of the Gram-negative outer membrane.

Experiments to identify changes in *E. faecalis* V583 gene expression or the levels of specific proteins in response to free GlcNAc were inconclusive due to time constraints. Further research is needed to determine GccF's exact mechanism of action.

The results of experiments with GccF, with and without added GlcNAc, on a range of bacterial species led to a hypothetical model for the mechanism of action of GccF, specifically that GccF may be ‘hijacking’ GlcNAc-specific phosphotransferase system signalling pathways. This could disrupt normal GlcNAc metabolism, perhaps resulting in UDP-GlcNAc becoming limiting for peptidoglycan synthesis, thus preventing cell wall expansion, and normal cell growth and division.

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List of Abbreviations

ABC	ATP binding cassette
Abs	Absorbance (values)
ACN	Acetonitrile
ATCC	American Type Culture Collection
ATP	Adenosine triphosphate
BLiS	Bacteriocin-like substance
BP (bp)	Base pairs
cDNA	Copied DNA
Cfu	Colony forming units
Da	Dalton
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic acid
ESR	Institute of Environmental Science and Research
EtBr	Ethidium bromide
G/Gram –ve	Gram-negative
G/Gram +ve	Gram-positive
GccF	Glycocin F
GcnA	N-acetyl-β-D-glucosaminidase
GlcN	Glucosamine
GlcNAc	N-acetylglucosamine
GlcNAc-1/6-P	N-acetlyglucosamine-1/6-phosphate
GRAS	Generally regarded as safe
IM	Inner membrane
kDa	kilodalton
KEGG	Kyoto Encyclopaedia of Genes and Genomes
LAB	Lactic acid bacteria
LB	Luria broth

man-PTS	Mannose-phosphotransferase system
mRNA	Messenger RNA
MRS	de Man, Rogosa and Sharpe medium
MRSA	Methicillin resistant *Staphylococcus aureus*
MS	Mass Spectrometry
NCBI	National Centre of Biotechnology Information
NZRM	New Zealand Reference Culture Collection, Medical Section
OD	Optical density
OM	Outer membrane
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PEP	Phosphoenolpyruvate
PTM	Post-translational modification
PTS	Phosphotransferase system
qPCR	Quantitative polymerase chain reaction
RNA	Ribonucleic acid
RT	Room Temperature
RT-PCR	Real-time polymerase chain reaction
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
T(x)	Time (value in minutes)
TBE	Tris/Borate/EDTA buffer
TEMED	*N,N,N′,N′*-Tetramethylethylene diamine
TSB	Tryptone soya broth
TSBgly	Tryptone soya broth with 1.2% (w/v) glycine
UDP	Uridine diphosphate
UV	Ultraviolet
VRE	Vancomycin resistant *enterococci*
w/v	Weight per volume