The role of the N-acetylglucosamine phosphoenolpyruvate phosphotransferase system from *Lactobacillus plantarum* 8014 in the mechanism of action of glycocin F

A thesis presented in partial fulfilment of the requirements for the degree of

Master of Science
in
Biochemistry

at Massey University, Manawatū, New Zealand.

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2017

Abstract

The rise in antibiotic-resistant bacteria is becoming a severe public health problem because of the shortage of new antibiotics to combat existing resistant bacterial pathogens. Should this trend of increasing bacterial drug resistance continue, the previously treatable conditions may once again become fatal. Using broad-spectrum antibiotics causes collateral damage to the commensal microbiota of the host leading to complications and a greater susceptibility to opportunistic pathogenic infection. As a result, narrow spectrum antibacterials effective against specific pathogens, are becoming increasingly sought after. Among the many alternative classes of narrow-spectrum antibiotics, is a diverse group of ribosomally-synthesised antimicrobial peptides known as bacteriocins. Glycocin F (GccF), a rare and uniquely diglycosylated bacteriocin produced by *Lactobacillus plantarum* KW80, appears to target a specific N-acetylglucosamine (GlcNAc) phosphotransferase system (PTS) and causes almost instant bacteriostasis by an as yet unknown mechanism. This thesis demonstrates how the GlcNAc-PTS is involved in the GccF mechanism of action and that the *gccH* gene provides immunity to GccF. Using transgenic and gene editing techniques, regions of the GlcNAc-PTS were either removed or altered to prevent normal function before being tested *in vivo*. The results demonstrated that only the EIIC domain of the GlcNAc-PTS is required in the GccF mechanism of action and that it acts like a "lure" that attracts the bacteriocin to the main target that is as yet unknown. Furthermore, the immunity gene was discovered, and using PTS knockout cell lines the immunity mechanism was shown to act independently of the GlcNAc-PTS. This work will form the foundation for the work needed to unravel the bacteriostatic mechanism of action of GccF, which may lead to the development a novel antimicrobial agent.

My loving wife

A guiding light in the darkness, and a place of solitude and shelter through the tempest of life. The go-to authority on me and my work.

Mom and Dad

A pair of rare, irreplaceable models of excellence and support, who made all this possible.

In memory of

Aunty Hester Wallace

You gave me an addiction to stationery that would lead to literacy and ultimately science. There is no greater gift than intelligence, and with every caressing pen stroke, you are missed.

1957 - 2015

"dubito, ergo cogito, ergo sum"
Antoine Léonard Thomas, praise of Descartes, 1765.

Acknowledgements

Associate Professor Gill Norris and Dr Mark Patchett.
For your patience, guidance and supervision.

Mr Trevor Loo
For your technical expertise, and advice.

To all my colleagues and friends at X-Labs
A dear thank-you for being a good distraction, a source of debate, and allowing me to bounce ideas off you.

Institute of Fundamental Sciences
For housing and funding my research.

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List of Abbreviations

Å	Angstrom
ADP	Adenosine diphosphate
Amp	Ampicillin
ATP	Adenosine triphosphate
bp	Base pair
cm	Centimeter
Chl	Chloramphenicol
Da	Dalton
DNA	Deoxyribonucleic acid
dNTP	Deoxyribonucleotide triphosphate
EAT	Empirical antibiotic therapy
EDTA	Ethylenediaminetetraacetic acid
EI	Enzyme I
EII	Enzyme II
EIIA	Enzyme IIA
EIIB	Enzyme IIB
EIIC	Enzyme IIC
EIID	Enzyme IID
$EIICBA^{GlcNAc}$	The GlcNAc specific PTS with all domains also known as the PTS18CBA
Ert	Erythromycin
FDA	Food and Drug Administration

g	Gram
GAS	Group A Streptococcal
gDNA	Genomic DNA
GccF	Glycocin F
GlcNAc	N-Acetylglucosamine
HP	Hairpins
HPr	Histidine-Phosphorylation protein
HPrK/P	HPr kinase/Phosphatase
kbp	Kilobase pair
kDa	Kilodalton
kPa	Kilopascal
L	Litre
LAB	Lactic acid bacteria
Lac	Lactose
MDR	multi-drug resistant
MDRO	multi-drug resistant organisms
M	Molar
MIC	Minimum inhibitory concentration
MCS	Multiple cloning site
mg	Milligram
ms	Millisecond
μL	Microlitre
μM	Micromolar
mL	Millilitre
mM	Millimolar
MRS	De Man, Rogosa and Sharpe medium
MW	Molecular weight
NaCl	Sodium chloride
NCBI	National Center for Biotechnology Information
NGS	Next generation sequencing

nL	Nanolitre
OD_{600}	Optical density at 600 nm
OF	Outward facing
PCR	Polymerase chain reaction
PDB	Protein data bank
PEG	Polyethylene glycol
PEP	Phosphoenopyruvate
PH	periplasmic helices
PMF	Proton motive force
PRD	PTS regulatory domain
PTM	Post translational modification
PTS	Phosphoenopyruvate phosphotransferase system
RBS	Ribosome binding site
SDS	Sodium dodecyl sulfate
SDS-PAGE	SDS-polyacrylamide gel electrophoresis
SLS	Streptolysin S
TBE	Tris-Boric Acid-EDTA
TEMED	N,N,N′,N′-Tetramethylethane-1,2-diamine
Tm	Melting temperature
TH	Transmembrane helix
V	Volts
v/v	Volume/Volume
w/v	Weight/Volume
WT	Wild-type
× g	Multiple of earth's gravitational force
°C	Degree Celsius