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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

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Marc Alex Bailie

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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 \textit{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

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For your technical expertise, and advice.

To all my colleagues and friends at X-Labs
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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<sub>GlcNAc</sub> The GlcNAc specific PTS with all domains also known as the PTS18CBA
Ert  Erythromycin
FDA  Food and Drug Administration
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<th>Symbol</th>
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<tr>
<td>g</td>
<td>Gram</td>
<td></td>
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<tr>
<td>GAS</td>
<td>Group A Streptococcal</td>
<td></td>
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<tr>
<td>gDNA</td>
<td>Genomic DNA</td>
<td></td>
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<tr>
<td>GccF</td>
<td>Glycocin F</td>
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<tr>
<td>GlcNAc</td>
<td>N-Acetylglucosamine</td>
<td></td>
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<tr>
<td>HP</td>
<td>Hairpins</td>
<td></td>
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<tr>
<td>HPr</td>
<td>Histidine-Phosphorylation protein</td>
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<tr>
<td>HPrK/P</td>
<td>HPr kinase/Phosphatase</td>
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<tr>
<td>kbp</td>
<td>Kilobase pair</td>
<td></td>
</tr>
<tr>
<td>kDa</td>
<td>Kilodalton</td>
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</tr>
<tr>
<td>kPa</td>
<td>Kilopascal</td>
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<tr>
<td>L</td>
<td>Litre</td>
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</tr>
<tr>
<td>LAB</td>
<td>Lactic acid bacteria</td>
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<tr>
<td>Lac</td>
<td>Lactose</td>
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<tr>
<td>MDR</td>
<td>multi-drug resistant</td>
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<td>multi-drug resistant organisms</td>
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<tr>
<td>MIC</td>
<td>Minimum inhibitory concentration</td>
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<td>NaCl</td>
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<tr>
<td>NCBI</td>
<td>National Center for Biotechnology Information</td>
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<tr>
<td>NGS</td>
<td>Next generation sequencing</td>
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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
\times g  Multiple of earth’s gravitational force
°C  Degree Celsius