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

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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 *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.*

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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 |
| $\mu$L | Microlitre |
| $\mu$M | 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 |