The Bacteriostatic Diglycosylated Bacteriocin Glycocin F Targets a Sugar-Specific Transporter

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Dedicated to Nana and Pop

Abstract

The increasing prevalence of antibiotic-resistance bacteria is threatening to end the antibiotic era established following Alexander Fleming's discovery of penicillin in 1928. Over-prescription and misuse of broad-spectrum antibiotics has hastened the development and spread of antibiotic resistance. This, combined with a lack of research and development (R&D) of new antibiotics by major pharmaceutical companies, may lead to a widespread recurrence of 'incurable' bacterial diseases. However while commercial R&D of antibiotics has waned, much research has been carried out to characterise bacteriocins, ribosomally-synthesised antimicrobial polypeptides thought to be produced by virtually all prokaryotes. Although hundreds of bacteriocins have been identified and characterised, only a handful of their cognate receptors on susceptible cells have been identified. Glycocin F is a bacteriostatic diglycosylated 43-amino acid bacteriocin produced by the Gram-positive bacterium *Lactobacillus plantarum* KW30 that inhibits the growth of a broad range of bacteria. The mechanism of action of glycocin F is unknown, however evidence suggested that glycocin F binds to cells via a N-acetylglucosamine (GlcNAc) specific phosphoenolpyruvate:carbohydrate-phosphotransferase system (PTS) transporter, as had been shown for lactococcin A, lactococcin B and microcin E492 that target a mannose specific PTS transporter. These other bacteriocins are, however, bactericidal suggesting that glycocin F uses a different mechanism of action to stop cell growth.

To test the hypothesis that one of the putative GlcNAc-specific PTS transporters identified in glycocin F-sensitive *L. plantarum* strains is the primary membrane receptor for glycocin F, a GlcNAc-specific PTS transporter gene knockout mutant was generated and analysed for glycocin F sensitivity. The GlcNAc-specific PTS transporter, *pts18CBA*, was successfully knocked out in *L. plantarum* NC8 which conferred the resulting *L. plantarum* NC8 Δ*pts18CBA* a degree of resistance to glycocin F confirming the GlcNAc-specific PTS transporter is a receptor of glycocin F. Additionally the genomes of wild-type (glycocin F sensitive) *L. plantarum* ATCC 8014, *L. plantarum* subsp. *plantarum* ATCC 14917, and multiple glycocin F-resistant mutants of these two strains were sequenced, assembled and comparatively analysed to identify changes consistent with increased resistance to glycocin F. Mutations, mapped to *pts18CBA* in all sequenced mutants, appeared to be deleterious to both the structure and function of PTS18CBA. A correlation of glycocin F resistance to the degree of mutation in the transmembrane domain of the *pts18CBA* gene was established confirming that glycocin F targets the EIIC transmembrane domain of PTS18CBA.

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List of Abbreviations

3D	Three-dimensional
Å	Ångström (0.1 nm)
ABC	ATP-binding cassette
ADP	Adenosine diphosphate
ATP	Adenosine triphosphate
bp	Base Pair
CCA	Carbon catabolite activation
CcpA	Carbon catabolite protein A
CCR	Carbon catabolite repression
CDS	Coding DNA sequence
cm	Centimetre
CRE	Catabolite responsive element
ChbC	N,N'-diacetylchitobiose-specific PTS from *B. cereus*
da	Dalton
DNA	Deoxyribonucleic acid
dNTP	Deoxyribonucleotide triphosphate
E-06	Micro
EDTA	Ethylenediaminetetraacetic acid
EII	Enzyme I
EII	Enzyme II
EIIA	Enzyme IIA
EIIB	Enzyme IIB
EIIC	Enzyme IIC
EIID	Enzyme IID
FBP	Fructose-1,6-bisphosphate
g	Gram
gDNA	Genomic DNA
GlcNAc	N-acetylglucosamine
GlpK	Glycerol kinase
His_6	Hexa-Histidine
Hpr	Histidine-phosphorylation protein
HPrK/P	HPr kinase/phosphatase
IPTG	Isopropyl β-D-1-thiogalactopyranoside
ITC	Isothermal titration calorimetry
kbp	Kilobasepair
kDa	Kilodalton
kPa	Kilopascal
L	Litre
LAB	Lactic acid bacteria
Lac	Lactose
LB	Luria-Bertani medium
LB agar	Luria-Bertani medium agar

M	Molar
MccE492	Microcin E492
MIC	Minimum inhibition concentration
MCS	Multiple cloning site
mg	Milligram
MGS	Massey genome service
ms	Millisecond
nL	Nanolitre
NCBI	National Center for Biotechnology Information
µL	Microlitre
µM	Micromolar
mL	Millilitre
mM	Millimolar
MOA	Mechanism of action
MLST	Multilocus sequence typing
MRS	De Man, Rogosa and Sharpe medium
MscL	Large-conductance mechanosensitive channel
MW	Molecular weight
NaCl	Sodium chloride
NGS	Next generation sequencing
°C	Degrees Celsius
OD_{600nm}	Optical density at 600 nm
PCR	Polymerase chain reaction
PDB	Protein data bank
PEG	Polyethylene glycol
PEP	Phosphoenolpyruvate
PMF	Proton motive force
PRD	PTS regulatory domain
PTM	Post-translational modification
PTS	Phosphoenolpyruvate:carbohydrate-phosphotransferase system
RBS	Ribosome binding site
RMSD	Root mean square deviation
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
TBE	Tris-Boric Acid-EDTA
TCBD	Transporter classification database
TEMED	*N,N,N′,N′*-tetramethylethylenediamine
Tm	Melting temperature
TMH	Transmembrane helices
UV	Ultra violet
V	Volts
v/v	Volume/volume
w/v	Weight/volume
WT	Wild-type
x g	Multiple of earth's gravitational force

Amino Acid and Nucleotide Abbreviations

Amino Acids

Full Name	**Three letter name**	**One letter name**
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartic acid	Asp	D
Cysteine	Cys	C
Glutamine	Gln	Q
Glutamic acid	Glu	E
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V

Nucleotides

Adenine	A
Thymine	T
Cytosine	C
Guanine	G
Uracil	U

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