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**The Molecular and Cellular Characterisation of the First
Glycocin: Plantaricin KW30**

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2009

**The Molecular and Cellular Characterisation of the First
Glycocin: Plantaricin KW30**

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

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Judith Stepper

2009

“What we know is a drop. What we don’t know is an ocean.”

Isaac Newton (1643 - 1727)

ABSTRACT

Bacteriocins, typically secreted by Gram-positive and -negative bacteria, are ribosomally-synthesised antimicrobial peptides which inhibit the growth of competing bacteria. We have purified a 43 amino acid bacteriocin, plantaricin KW30 (PlnKW30) produced by *Lactobacillus plantarum* KW30, that has little amino acid sequence similarity to any other characterised bacteriocin.

The gene encoding *plnKW30* is in a cluster with the genes required for maturation and export of, and immunity to, the bacteriocin. This arrangement of genes is similar to the genomic context of bacteriocin genes in other lactic acid bacteria. The *plnKW30* gene cluster comprises six genes encoding a glycosyltransferase, a proteolytic ABC-transporter, two putative thioredoxins, a response regulator and PlnKW30 itself.

PlnKW30 was found to possess two unusual post-translational modifications: an *O*-glycosylated serine and an unprecedented *S*-glycosylation of the C-terminal cysteine. The modified serine is located on an eight residue loop that is tethered by a disulfide bridge. Both modifications have been identified as *N*-acetylglucosamines (GlcNAc), making PlnKW30 the first described class IV bacteriocin. A post-translational modification with *S*-linked GlcNAc is unprecedented in bacteriocins as well as in all genera. The antimicrobial activity of PlnKW30 on *L. plantarum* ATCC 8014 was analysed using enzymatic dissection coupled with bioassays. It was found to be concentration dependent and both the N- and C-terminal fragments are necessary for activity. Furthermore, reduction of the disulfide bonds results in abolishment of antimicrobial activity and it appears that deglycosylation of the serine 18 decreases the antimicrobial activity by about two thirds. These results show that all post-translational modifications contribute to the antimicrobial activity of PlnKW30. The addition of *N*-acetylglucosamine to cultures of the indicator strain *L. plantarum* ATCC 8014 protects it from the antimicrobial effect of the added PlnKW30. PlnKW30 probably targets an *N*-acetylglucosamine transporter in the target cell membrane, similar to the mannose phosphotransferase system targeted by lactococcin A.

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Abbreviations

Å	Ångström (10^{-10}m)
aa	Amino acid
ABC-transporter	ATP-binding cassette transporter
ACN	acetonitrile
AHL	acyl-homoserine lactone
Amp	Ampicillin
APS	Ammonium persulfate
ATCC	American Type Culture Collection
ATP	Adenosine-5'-triphosphate
BLAST	Basic Local Alignment Search Tool
bp	Base pairs
BSA	Bovine serum albumin
°C	Degrees Celsius
C	Carbon
ca.	Circa
CAZy	Carbohydrate-Active enZymes
CBM	Carbohydrate-binding module
Cds	Conserved domains in sequences
Cm	Chloramphenicol
CTP	Cytidine-5'-triphosphate
ddH ₂ O	Double distilled water
Dha	Dehydroalanine
Dhb	Dehydrobutyrine
DMSO	Dimethyl sulphoxide
DNA	Deoxyribonucleic acid
DNase	Deoxyribonuclease
dNTP	Deoxyribose nucleotide triphosphate
DTT	Dithiothreitol
EDTA	Ethylene diamine tetraacetic acid

Abbreviations

Em	Erythromycin
<i>et al.</i>	<i>et alteri</i> (and others)
ER	Endoplasmic reticulum
EtBr	Ethidium bromide
ETD	Electron Transfer Dissociation
EtOH	Ethanol
ExPASy	Expert Protein Analysis System
FLP	FNR-like regulatory proteins
FNR	fumarate-nitrate reduction
FPLC	Fast Protein Liquid Chromatography
FTase	Farnesyltransferase
g	Gramm; standard gravity (9.81 m/s ²)
GalNAc α 1-Ser/Thr	<i>N</i> -acetyl-D-galactosamine and L-serine or L-threonine
gDNA	Genomic DNA
GDP	Guanidine-5'-diphosphate
G+C %	Percentage of guanine and cytosine
Gal	Galactose
GalNAc	<i>N</i> -acetylgalactosamine
GlcNAc	<i>N</i> -acetylglucosamine
GlcNAc β 1-Asn	<i>N</i> -acetylglucosaminyl-asparagine
G protein	GTP binding protein
GT	Glycosyltransferase
GTP	Guanosine-5'-triphosphate
hrs	Hours
HEPES	n-(2-hydroxyethyl)piperazine-n-(2-ethanesulfonic acid)
HPK	Histidine Protein Kinase
HPLC	High Pressure Liquid Chromatography
HTH	Helix-turn-helix
IEX	Ion Exchange Chromatography
IM	Inner membrane
IMAC	Immobilised Metal Affinity Chromatography

Abbreviations

IPTG	Isopropyl- β -D-Thiogalactopyranoside
IR	Infrared radiation
IUBMB	International Union of Biochemistry and Molecular Biology
Kan	Kanamycin
Kbp	Kilo basepairs
kDa	kilo Daltons
L	Liter
λ	Wavelength
LAB	Lactic Acid Bacteria
LB	Luria Bertani broth
M	Molar (mol/L)
m	Meter
Man	Mannose
man-PTS	mannose phosphotransferase system
MCS	Multiple Cloning Site
min	Minute
mol	6.023×10^{23} molecules
MRS	De Man, Rogosa and Sharpe broth
MW	Molecular mass
m/z	Mass-to-charge ratio
n/a	Not applicable
NaCl	Sodium chloride
NBD	Nucleotide binding domain
NCBI	National Centre for Biotechnology Information
ND	Not determined
NDP	Nucleotide diphosphate
NEB	New England Biolabs
NH ₂	Amine
(NH ₄) ₂ SO ₄	Ammonium sulphate
NMR	Nuclear magnetic resonance
No.	Number

Abbreviations

O	Oxygen
OD	Optical Density
OM	Outer membrane
o/n	overnight
ORF	Open Reading Frame
p.a.	<i>pro analysis</i>
Pa	Pascal (= 10^{-5} bar = 145.04×10^{-6} psi)
PCR	Polymerase Chain Reaction
pH	Negative decadal logarithm of the proton concentration
PlnKW30	plantaricin KW30
ppm	Parts per million
PTase	Prenyltransferase
PTM	Post-translational modification
PVDF	Polyvinylidene difluorine
QS	Quorum sensing
RBS	Ribosome Binding Sequence
RNA	Ribose nucleic acid
RNase	Ribonuclease
rpm	Revolutions per minute
RT	Room temperature
RP-HPLC	Reverse-Phase High Pressure Liquid Chromatography
RR	Response regulator (originally thought to be FTase)
SAM	S-adenosyl-methionine
s/sec	Second
S/D	Shine-Delgarno-Sequence
SDS-PAGE	Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis
sec pathway	Translocase general secretion pathway
S-layer	surface-layer
Sm	Streptomycin
SOB	Super optimal broth

Abbreviations

SOC	SOB with catabolite repression, indicative of the presence of glucose
Sp.	Species
SSC	Saline sodium citrate buffer
Subsp.	Subspecies
T	Temperature
t	time
TAE	Tris-Acetate-EDTA
TCEP	tris(2-carboxyethyl)phosphine
TCS	Two-Component System
Tc	Tetracycline
TE	Tris-EDTA
TLC	Thin layer chromatography
TMD	Transmembrane domain
TMH	Transmembrane helix
TRIS	Tris (hydroxymethyl) aminomethane
TTP	Thymidine-5'-triphosphate
TRX	Thioredoxin
UDP	Uridine diphosphate
UDP-Glc	Uridine diphosphate- α -D-glucose
USA	United States of America
UV	Ultraviolet light
V	Volts
v/v	Volume per volume
W	Watt
w/v	Weight per volume
w/w	Weight per weight

Abbreviations of Nucleic Acids

One letter code	Base(s) represented
A	Adenine
C	Cytosine
G	Guanine
T	Thymine
U	Uracil
R	GA
Y	TC
K	GT
M	AC
S	GC
W	AT
B	GTC
D	GAT
H	ACT
V	G or C or A
N	ANY

Abbreviations of Amino Acids

Amino acid	3-letter code	1-letter code
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartic acid	Asp	D
Cysteine	Cys	C
Glutamic acid	Glu	E
Glutamine	Gln	Q
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

