THE MOLECULAR BASIS OF GLYCOPEPTIDE RESISTANCE IN TWO CLINICAL ISOLATES: *BACILLUS LENTUS* RSA1208 AND *PAENIBACILLUS THIAMINOLYTICUS* RSA1221

Arshnee Moodley

A dissertation submitted to the Faculty of Health Sciences, University of the Witwatersrand, in fulfilment of the requirements for the Degree of Master of Science in Medicine

Johannesburg, 2005

DECLARATION

I, Arshnee Moodley, declare that this dissertation is my own work. In compiling this dissertation, no form of copyright has been infringed. It is being submitted for the degree of Master of Science in Medicine at the University of the Witwatersrand, Johannesburg, South Africa. It has not been submitted before for any degree or examination at this or any University.

Arshnee Moodley

B. Sc, B. Sc (Hons)

21st day of July, 2005

DEDICATION

To my family and to Krzysztof Blaski, “kocham cie”.

And in special memory of my dearest grandmother.

PRESENTATION/S

The following presentation/s arose from work done towards this dissertation.

Oral presentation

Moodley A. "**The molecular basis of glycopeptide resistance in two clinical isolates: *Bacillus lentus* RSA1208 and *Paenibacillus thiaminolyticus* RSA1221**" presented at the Molecular and Cell Biology Group Annual Symposium, 6th October 2004, Wits Medical School, Johannesburg, South Africa

ABSTRACT

The molecular mechanisms of glycopeptide resistance in two Gram-positive clinical isolates, *Bacillus lentus* RSA1208 and *Paenibacillus thiaminolyticus* RSA1221 were investigated. The glycopeptide resistance genotypes were determined by PCR. If *van* genes were detected, recombinant DNA techniques and sequencing were used to determine the gene sequence. The location of the resistance determinant was investigated using Southern hybridization techniques. To determine the 5' and 3' ends flanking the resistance operon, sub-genomic libraries were constructed. Transmission electron microscopy was used to assess possible structural changes of the *B. lentus* RSA1208 cell wall.

B. lentus RSA1208 exhibits inducible, high-level resistance to both glycopeptides, but does not possess any known *van* resistance genes. Electron micrographs showed a visible increase in cell wall thickness in *B. lentus* RSA1208 grown in vancomycin compared to the isolate grown in vancomycin-free media. However, it remains to be confirmed as to whether this resistance is solely responsible for the high-level resistance phenotype.

P. thiaminolyticus RSA1221 exhibits constitutive, high-level resistance to vancomycin only. It was found to possess a chromosomally-borne, *vanA* gene cassette. The *vanA* gene showed the highest amino acid identity to the *vanA*-like D-ala: D-lac gene found in *P. thiaminolyticus* PT-2B1 and *Enterococcus faecium* BM4147. All five genes of the *vanA* gene cluster (*vanR*, *vanS*, *vanH*, *vanX*, *vanY*) were amplified and sequenced. No *vanZ* gene was detected. The *vanA* operon in *P. thiaminolyticus* RSA1221 was found not to be associated with any known mobile elements. The observed constitutive expression of resistance maybe due to a two amino acid insertion in the $VanSB_{pt1221}$ protein.

ACKNOWLEDGEMENTS

This research was performed in the Division of Clinical Microbiology and Infectious Diseases, School of Pathology at the University of the Witwatersrand, Johannesburg, South Africa.

I would like to thank my supervisors Dr. E Marais and Prof. A.G Duse' for facilitating this project. I would also especially like to take this opportunity to express my sincere gratitude to Dr. Marais for her endless help in the laboratory, invaluable advice and support.

I would like to acknowledge:

- Medical Research Council (MRC), National Health Laboratory Service (NHLS) and the Andrew Mellon Postgraduate Mentoring Programme for their financial assistance
- Respiratory and Meningeal Pathogens MRC Research Unit- Dr. A Smith, Dr. M du Plessis and Mr. G Coulsen for use of their automated Gene sequencer
- Transmission electron microscopy- Mrs. L van der Walt, Electron Microscope Department, Anatomical Pathology, NHLS
- The staff at Infection Control, Wits Medical School, Johannesburg, South Africa

TABLE OF CONTENTS

CHAPTER TWO

AIMS OF THE STUDY

CHAPTER THREE

MATERIALS AND METHODS

LIST OF FIGURES

LIST OF TABLES

ABBREVIATIONS

ADP	adenosine diphosphate
AIDS	acquired immunodeficiency syndrome
Anti-DIG-AP	anti-DIG-alkaline phosphatase
Arg (R)	arginine
Asn (N)	asparagine
Asp (D)	aspartic acid
ATCC	American Type Culture Collection
ATP	adenosine triphosphate
BHI	brain heart infusion
BLAST	Basic Local Alignment Search Tool
bp	base pair
BSA	Bovine Serum Albumin
C	cytosine
C-terminus	carboxy terminus
CAMHB	cation-adjusted Mueller-Hinton broth
cfu/mL	colony forming units per milliliter
CHEF	contour clamp homogenous electric field
CODEHOP	COnsensus DEgenerate Hybrid Oligonucleotide Primer
CSPD	disodium 3-(4-meth-oxyspiro {1,2-dioxetane-3, 2'-(5'-chloro) tricyclo [3.3.1.1^{3,7}] decan}-4-yl) phenyl phosphate
d. H_2O	distilled water
D-ala	D-alanine
D-lac	D-lactate
D-ser	D-serine
D-ala-D-ala	D-alanyl-D-alanine
D-ala-D-lac	D-alanyl-D-lactate
D-ala-D-ser	D-alanyl-D-serine
ddl	D:ala:D:ala ligase
DIG	digoxigenin
DMSO	dimethylsulfoxide
DNA	deoxyribonucleic acid
dNTP	deoxynucleoside triphosphate
EDTA	ethylenediaminetetraacetic acid
g	relative centrifugal force
G	guanine
Glu (E)	glutamic acid

Gly (G)	glycine
GRE	glycopeptide resistant enterococci
GREF	glycopeptide-resistant *E. faecium*
H- bond	hydrogen bond
His (H)	histidine
hr	hour
HPLC	high performance liquid chromatography
IR	inverted repeat
IR_L	inverted repeat left
IR_R	inverted repeat right
kb	kilo base
kDa	kilo Dalton
LB	Luria Bertani
LPS	lipopolysaccharide
Lys (K)	lysine
M	molar
mecA	PBP2a
Met (M)	methionine
MH	Mueller-Hinton
MIC	minimum inhibitory concentration
min	minute
mRNA	messenger ribonucleic acid
MRSA	methicillin-resistant *Staphylococcus aureus*
N-terminus	amino terminus
NAG	N-acetylglucosamine
NAM	N-acetylmuramic acid
NCBI	National Center for Biotechnology Information
NCCLS	National Committee for Clinical Laboratory Standards
NHLS	National Health Laboratory Service
ORF	Open reading frame
P_H	*vanH* promoter region
P_R	*vanR* promoter region
PABA	*p*-aminobenzoic acid
PBP	penicillin-binding protein

PCR	polymerase chain reaction
PEG	polyethylene glycol
PFGE	pulse field gel electrophoresis
PG	peptidoglycan
Phos-VanR	phosphorylated VanR
PMSF	phenylmethylsulfonyl fluoride
Pro (P)	proline
RNA	ribonucleic acid
rpm	revolutions per minute
rRNA	ribosomal RNA
rrs	16S rRNA gene
SDS	sodium dodecyl sulfate
sec	second
spp	species
SSC	saline sodium citrate
T	thymine
TAE	Tris-acetate EDTA buffer
TE	Tris EDTA buffer
TEM	transmission electron microscopy
TP	teicoplanin
tRNA	aminoacyl transfer RNA
U	units
USA	United States of America
UV	ultraviolet
VA	vancomycin
Van^R	vancomycin resistance genes
VanA	D-ala: D-lac ligase
VanH	D-specific α-ketoacid dehydrogenase
VanR	Transcriptional response regulator
VanS	Membrane bound histidine sensor kinase
VanT	Serine racemase
VanX	D, D-dipeptidase
VanY	D, D-carboxypeptidase
VISA	vancomycin intermediate resistant *Staphylococcus aureus*
VRE	vancomycin resistant enterococci
VRSA	vancomycin resistant *Staphylococcus aureus*