Abstract

Rifampicin is a chemotherapeutic agent used to combat mycobacterial and nocardial infections. Four enzymatic inactivation mechanisms have been identified which are partially responsible for the increasing number of rifampicin resistant strains. These are ADP-ribosylation, phosphorylation, decomposition and glucosylation. The gene encoding the latter, *rgt*, has been cloned and characterized from the opportunistic pathogen *Nocardia brasiliensis.* However, as of yet nothing is known of these inactivation enzymes. Thus in order to study the properties of the mechanism it is necessary to observe structure-function relationships through the characterization of mutants.

Furthermore, the *rgt* gene confers a small yet reproducible increase to the vancomycin MIC. This has indicated that there may be other enzymatic mechanisms which are involved in the inactivation of vancomycin. Vancomycin is an important antibiotic as it is used to treat gram-positive infections by multi-drug resistant strains. Hitherto, no mechanisms of enzymatic inactivation have been identified for vancomycin. Thus in order to identify regions of DNA which may play a role in the high level resistance to vancomycin as observed in *N. brasiliensis* it was necessary to screen a genomic library of this organism. This was performed in a gram-positive background. No clones were identified in this study that had an increased resistance to vancomycin, indicating that the DNA involved in the phenotype is greater than that of the average insert size of the library, 1.9 kb.

Future work will thus involve the generation of a genomic library with larger fragments and the subsequent screening of this. Additionally, performing a mutational analysis on the *rgt* gene may provide further insight into the specifics of the inactivation enzymes and thus will contribute to combating infection by opportunistic and other pathogens.

Declaration

I, Alison Saxe Baker, declare that this is my own unaided work. It is being submitted in partial fulfilment for the Degree of Master of Science through the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination through any other University.

_____

_____ day of _____ 2005

Dedication

I dedicate this work to my parents, Beth and Peter, and also to my beloved siblings, Carrie, Will and Rob. Your unconditional love and support have always given me the strength to continue.

Acknowledgements

I would like to acknowledge the continuous support I have received from my family during my studies. Thank you for always believing me and encouraging me at the times I needed it most.

Thank you to Eric and the team of GH700, I appreciate the knowledge and assistance you provided. I would further like to acknowledge the financial assistance I received from the NRF and the Postgraduate Merit Award.

Finally, huge thanks to Chrissie, Margaret, Louise and Jean for the encouragement they continually provided. And last, but certainly not least, to Azola, Vetja and Rozida for providing me with the opportunity to become friends with the future leaders of South African biotechnology☺.

Table of Contents

5.0 Appendices

List of Figures

List of Tables

Frequently used Abbreviations

Amp	ampicillin
ATP	adenosine triphosphate
bp	base pair
$CaCl_2$	calcium chloride
Cm	chloramphenicol
CsCl	cesium chloride
dH_2O	distilled water
DNA	deoxyribonucleic acid
dNTP’s	deoxynucelotide triphosphates
dsDNA	double stranded DNA
EDTA	ethylenediaminetetraacetic acid
EtBr	ethidium bromide
EtOH	ethanol
g	gram
GC	guanine and cytosine
HCl	hydrogen chloride
hr	hour
Kan	kanamycin
kb	kilobase
kV	kilovolt
LA	Luria agar

LB	Luria-Bertani medium
LBSG	Luria-Bertani with sucrose and glucose
M	molar
mA	milliampere
mg	milligram
MIC	minimal inhibitory concentration
min	minute
ml	milliliter
mM	millimolar
M_w	molecular weight
NaCl	sodium chloride
NaOH	sodium hydroxide
NMR	nuclear magnetic resonance
no.	number
OD	optical density
ORF	open reading frame
p	plasmid
PEG	polyethlylene glycol
R	resistant
rgt	rifampicin glycosyl-transferase
Rif	rifampicin
RNase	ribonuclease
rpm	revolutions per minute

rRNA	ribosomal ribonucleic acid
sec	second
sdH_2O	sterile distilled water
SDS	sodium dodecyl sulphate
Tc	tetracycline
TBE	Tris-boric Acid-EDTA
TE	Tris-EDTA
TES	*N*-tris(hydroxymethyl)-methyl-2-amino-ethanesulphonic acid
Tris	Tris(hydroxymethyl)-aminomethane
UDP	uridine diphosphate glucose
UV	ultraviolet
V	volt
vol/s	volume/s
λ	lambda
μF	microfarad
μg	microgram
μl	microlitre
Ω	Ohm
~	approximately