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# Mutational analysis of the rifampicin glycosyl-transferase (rgt) inactivation protein from Nocardia brasiliensis and its relationship to the vancomycin resistance of this organism

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# 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.

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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.

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# 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 |