

Supplementary Information For:

Characterization of a Rifampin Inactivating Glycosyltransferase from a Screen of Environmental Actinomycetes

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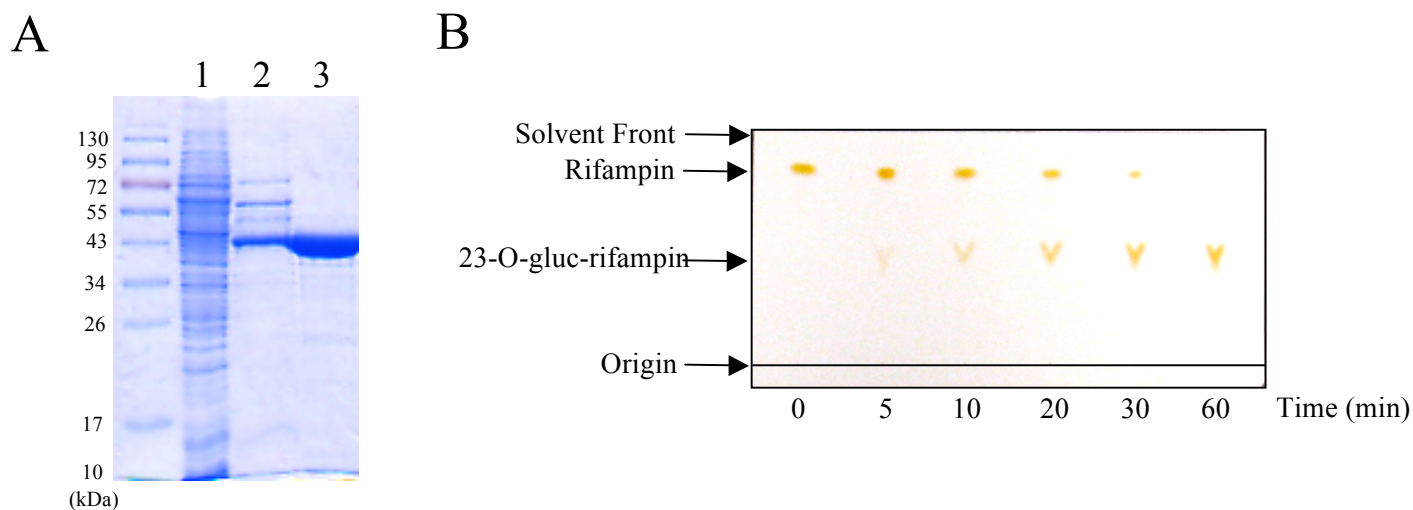


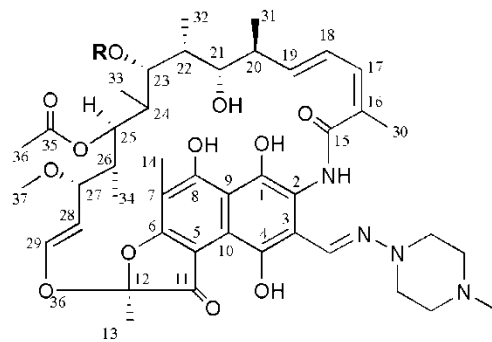
Figure S1. Purification and *in vitro* assay of recombinant Rgt1438. (A) SDS-PAGE analysis of purified Rgt1438. 1, Crude soluble protein fraction; 2, Nickel-affinity chromatography; 3, MonoQ chromatography. (B) Purified Rgt1438 was assayed as described in material and methods over 60 minutes. At specified time intervals, aliquots of the reaction were quenched with an equal volume of MeOH and product formation was assessed using TLC using conditions previously described (1).

Table S1. Oligonucleotide primers used in this study.

Primer	Sequence	Reference
F27	5'-AGAGTTTGATC(A/C)TGGCTCAG-3'	2
R1432	5'-TACGG(C/T)TACCTTGTTACGACTT-3'	2
rgtPartF	5'-ATGCATGAATTCCGAGGCGGCCGAGGGTTGT-3'	This study
rgtPartR	5'-ATGCATAAGCTTCCGCGAGCGAGGACCACACG-3'	This study
rgtF	5'-ATCGATCATATGCGCATGCTGCTGACCAC-3'	This study
rgtR	5'-ATGCATAAGCTTCCGACGGGGGCTGGACTC-3'	This study
rgtR-pIJK	5'-ATGCATGGATCCCCGACGGGGGCTGGACTC-3'	This study

Table S2. ^1H Chemical shifts of Rifampin and 23-O-gluc-rifampin

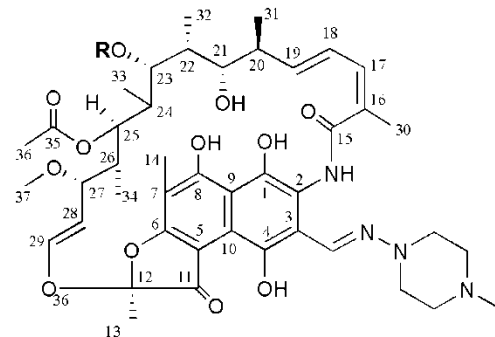
Proton	Rifampin	23-O-gluc-rifampin
17	6.24	6.17
18	7.11	7.24
19	5.91	5.95
20	2.19	2.10
21	3.76	3.72
22	1.59	1.63
23	2.83	3.33
24	1.33	1.23
25	5.07	5.07
26	1.03	0.97
27	3.23	3.23
28	4.93	5.05
29	6.19	6.13
30-CH ₃	1.90	1.90
31-CH ₃	0.84	0.82
32-CH ₃	0.89	0.88
33-CH ₃	0.43	0.51
34-CH ₃	-0.26	0.06
36-CH ₃	1.97	1.94
37-CH ₃	2.89	2.89
13-CH ₃	1.63	1.64
14-CH ₃	1.90	1.87
Pip-N-CH ₃	2.80	2.84
1'	-	4.19
2'	-	2.85
3'	-	3.07
4'	-	2.97
5'	-	3.02
6'	-	3.38



Structure	R
Rifampin	H
23-O-gluc-rifampin	

Table S3. ^{13}C Chemical shifts of Rifampin and 23-O-gluc-rifampin

Carbon	Rifampin	23-O-gluc-rifampin
17	132.1	141.7
18	127.9	126.6
19	137.5	139.0
20	38.0	39.9
21	72.0	71.2
22	33.0	35.5
23	76.14	86.7
24	37.6	29.3
25	73.6	74.1
26	39.9	40.3
27	76.45	76.4
28	117.7	118.5
29	142.7	142.2
30-CH ₃	20.68	20.8
31-CH ₃	17.79	17.62
32-CH ₃	11.35	12.61
33-CH ₃	8.65	9.9
34-CH ₃	9.0	8.9
36-CH ₃	20.4	20.9
37-CH ₃	55.7	55.7
13-CH ₃	21.9	21.5
14-CH ₃	7.6	7.6
Pip-N-CH ₃	42.1	42.1
1'	-	103.0
2'	-	73.8
3'	-	76.6
4'	-	70.1
5'	-	76.8
6'	-	63.1



Structure	R
Rifampin	H
23-O-gluc-rifampin	

Table S4. List of glycosyltransferases used for phylogenetic analysis

Accession Number	Protein Name	Organism
JX028276	Rgt1438	WAC1438 (<i>Streptomyces speibonae</i>)
ZP_08881278		<i>Saccharopolyspora spinosa</i> NRRL 18395
AAK84835	Rgt	<i>Nocardia brasiliensis</i>
YP_004920881		<i>Streptomyces cattleya</i> NRRL 8057
AEV85390		<i>Actinoplanes</i> sp. SE50/110
YP_003340698		<i>Streptosporangium roseum</i> DSM 43021
YP_004961242		<i>Streptomyces bingchenggensis</i> BCW-1
EHI41633		<i>Rhodococcus opacus</i> PD630
YP_703483		<i>Rhodococcus jostii</i> RHA1
ZP_07299174		<i>Streptomyces himastatinicus</i> ATCC 53653
ZP_06912843		<i>Streptomyces pristinaespiralis</i> ATCC 25486
YP_001510066		<i>Frankia</i> sp. EAN1pec
CAE53364	GtfB	<i>Actinoplanes teichomyceticus</i>
AAB49293	GtfB	<i>Amycolatopsis orientalis</i>
AAB49299	GtfE	<i>Amycolatopsis orientalis</i>
CAE53349	GtfA	<i>Actinoplanes teichomyceticus</i>
AAB49294	GtfC	<i>Amycolatopsis orientalis</i>
AAB49292	GtfA	<i>Amycolatopsis orientalis</i>
AAK31352	GtfD	<i>Amycolatopsis orientalis</i>
ADN6848	SorF	<i>Sorangium cellulosum</i>
ZP_08288521		<i>Streptomyces griseoaurantiacus</i> M045
YP_004811856		<i>Streptomyces violaceusniger</i> Tu 4113
YP_001157031		<i>Salinispora tropica</i> CNB-440
YP_004806303		<i>Streptomyces</i> sp. SirexAA-E
ZP_07301298		<i>Streptomyces viridochromogenes</i> DSM 40736
ZP_06581285		<i>Streptomyces ghanaensis</i> ATCC 14672
AAD44209	RtfA	<i>Mycobacterium avium</i>
AAF67506	NovM	<i>Streptomyces caeruleus</i>
AAD12163	TyIN	<i>Streptomyces fradiae</i>
BAC15749	RebG	<i>Lechevalieria aerocolonigenes</i>
AAA26780	Mgt	<i>Streptomyces lividans</i>
AAC12648	OleI	<i>Streptomyces antibioticus</i>
Z22577	OleD	<i>Streptomyces antibioticus</i>
AAC68677	DesVII	<i>Streptomyces venezuelae</i>
BAB96658	MurG	<i>Escherichia coli</i>

References

1. **Yazawa, K., Y. Mikami, A. Maeda, M. Akao, N. Morisaki, and S. Iwasaki.** 1993. Inactivation of rifampin by *Nocardia brasiliensis*. *Antimicrob. Agents Chemother.* **37**:1313-1317.
2. **Heuer, H., M. Krsek, P. Baker, K. Smalla, and E. M. Wellington.** 1997. Analysis of actinomycete communities by specific amplification of genes encoding 16S rRNA and gel-electrophoretic separation in denaturing gradients. *Appl. Environ. Microbiol.* **63**:3233-3241.