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Detection of Point Mutations in *rpoB* Gene of Rifampin-Resistant *Rickettsia typhi*

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The *rpoB* gene of rifampin-resistant *Rickettsia typhi* (Rif mutant) and wild-type *R. typhi* were sequenced and compared. The Rif mutant *rpoB* had three nucleotide substitutions, which resulted in amino acid changes at residues 151, 201, and 271 and may be the basis for the rifampin resistance.

Rickettsia typhi, the causative agent of murine typhus, is transmitted to humans by fleas. Rickettsiae infect the host's endothelial cells and cause symptoms including rashes, severe headaches, fever, chills, hepatic and renal dysfunction, central nervous system abnormalities, and pulmonary compromise (2). Most cases are clinically mild, but severe and even fatal cases have been reported (1, 2). The recommended antibiotic regimen includes administration of doxycyline, tetracycline, or chloramphenicol (9). Here, we report the detection of a rifampin-resistant laboratory strain of *R. typhi* (Ethiopian) during the development of a transformation protocol for *Rickettsia*. The basis of resistance was investigated by sequencing and mapping point mutations in the *rpoB* gene of the mutant.

Renografin-purified *R. typhi* (Ethiopian, 10^8 PFU/ml) was subjected to electroporation in 100 μl of electroporation buffer (272 mM sucrose, 15% glycerol, which was filter sterilized [0.2-μm-pore-size filter; Millipore]) with a 0.1-cm gap cuvette (BTX Inc., San Diego, Calif.). The samples were electroporated at 2.5 kV, 200 Ω, and 25 μF, with a time constant of approximately 5 ms (nonelectroporated controls remained on ice). Immediately, 400 μl of cold SPG buffer (0.218 M sucrose, 0.0038 M KH_2PO_4, 0.0072 M K_2HPO_4, and 0.0049 M L-glutamate [pH 7.2]) was added to the cuvette. The electroporated *R. typhi* PFU were then grown in Vero cells in the presence of rifampin, chloramphenicol, doxycycline, or erythromycin (1, 2, 4, 8, or 100 μg/ml) (Sigma Chemical Co., St. Louis, Mo.) in Dulbecco modified Eagle medium and 1% fetal bovine serum at 34°C. After 8 days, samples were assayed for detection of *Rickettsia* by indirect fluorescent-antibody assay (7) with monoclonal antibody T62-3-A6 (mouse anti-*R. typhi* lipopolysaccharide, kindly provided by D. H. Walker, University of Texas Medical Branch at Galveston, Tex.) and by dye uptake assay (8) for antibiotic susceptibility.

Rifampin-resistant *R. typhi* (Rif mutant) was detected in the electroporated sample. The mutant, grown in the presence of 100 μg of rifampin per ml, infected 75% of the Vero cells by day 8 postinfection (approximately 10^9 bacteria/ml of culture). In contrast, 1.0 μg of rifampin per ml inhibited growth of wild-type *R. typhi* (Ethiopian) in the control groups (*R. typhi* with no electroporation). Rickettsial growth was not detected by indirect fluorescent-antibody assay on day 8 in these samples (data not shown). All *R. typhi* (with or without electroporation) incubated without rifampin infected approximately 75% of the Vero cells on day 8. The rifampin-resistant *R. typhi* isolate remained susceptible to chloramphenicol (MIC = 1 μg/ml), doxycycline (MIC = 1 μg/ml), and erythromycin (MIC = 2 μg/ml); these values are comparable to that for the susceptibility of wild-type *R. typhi*; therefore, electroporation did not affect the *Rickettsia* susceptibilities to these antibiotics.

Both the wild-type *R. typhi* (Ethiopian) *rpoB* gene and the Rif mutant (Ethiopian) *rpoB* gene were amplified by PCR, cloned, and sequenced. Primers (Table 1) were selected on the basis of the *rpoB* gene sequence of *Rickettsia prowazekii* (GenBank accession no. Z82356). The PCR included 10 μM each primer, 0.5 μg of *R. typhi* genomic DNA, 47 μl of PCR Supermix (Gibco), and an overlay of 50 μl of mineral oil. All reactions consisted of 25 cycles each, with an initial denaturing temperature of 94°C for 1 min, the specified annealing temperature (Table 1) for 90 s, and an extension temperature of 72°C for 2 min on a DNA thermocycler (Perkin-Elmer Cetus, Norwalk, Conn.). PCR products were cloned with the TA cloning kit (Invitrogen) and sequenced by the dye terminator

TABLE 1. Oligonucleotide primers used for PCR amplification of the *R. typhi rpoB* gene

Primer name	5′ position	Sequence (5′–3′)	Annealing temp[a] (°C)
1fwd	5–18	GGTTTCATTAAGGG	45
1rev	520–502	GATAAAAGAATACACCTG	45
2fwd	466–488	CGGAAAGAGTAGTTGTATCACA	37
2rev	673–656	TGCTGCCTCACGGTCCT	37
3fwd	1139–1156	AGGACCGTGAGGCAGCA	37
3rev	1708–1688	CGACCGTAATGAGTAGGATG	37
4fwd	1654–1671	AGACTTTCAGCCCTTGG	50
4rev	2089–2072	CGGTTCGCATCATCATT	50
5fwd	2083–2098	TTAGAAAATGATGAT	46
5rev	2594–2577	AGGACCAAGGCGTGTAT	46
6fwd	2592–2609	AGATACACGCCTTGGTC	46
6rev	3122–3105	TTCATTCATTACATTTG	46
7fwd	3122–3139	CAAATGTAATGAATGAA	45
7rev	3889–3872	CCACCAAAATGAGACTT	45
8fwd	3839–3856	ATTCACTCTCGTTCTAT	45
8rev	4105–4088	AGCTTTACGTTGAGACA	45

[a] Annealing temperature used during PCR amplification.

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method with an automated sequencer (model 373; Applied Biosystems, Foster City, Calif.). All gene segments were sequenced a minimum of two times (forward and reverse each time) to allow detection and elimination of errors in the sequencing process.

The sequences of wild-type and Rif mutant *R. typhi rpoB* genes were compared. The *rpoB* sequence was 4,127 bp, and identity between *R. typhi* and the *R. typhi* Rif mutant was approximately 99.8%. A total of eight nucleotide substitutions occurred, three of which resulted in amino acid substitutions in the Rif mutant RpoB: leucine for phenylalanine at residue 151, phenylalanine for leucine at residue 201, and valine for isoleucine at residue 271.

The mutations described in our study (residues 151, 201, and 271) do not correspond exactly to amino acid substitutions seen in rifampin-resistant *Escherichia coli* (4, 5, 10) or rifampin-resistant *Mycobacterium* spp. (3, 6, 11). However, identity of the *rpoB* gene between *Rickettsia* and *rpoB* of *E. coli* or *Mycobacterium* is less than 40%, so a direct correlation is not expected. Many of the amino acid substitutions in the RpoB of rifampin-resistant *E. coli* occur between residues 500 and 600. However, one rifampin-resistant *E. coli* isolate was characterized with an amino acid substitution at residue 146 of RpoB (5). This substitution is located closer to the *R. typhi* amino acid substitutions (residues 151, 201, and 271). At present, it is unclear if one or all three of the mutations are responsible for the rifampin resistance in *R. typhi*. In future research we will address this issue by mutating the *rpoB* genes in these specific areas and subsequently assessing rifampin sensitivity.

Nucleotide sequence accession number. The GenBank accession number for the wild-type *R. typhi rpoB* gene sequence is U73742.

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