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Natural Rifampin Resistance in *Treponema* spp. Correlates with Presence of N531 in RpoB Rif Cluster I

Rifampin is an antibiotic that interacts specifically with the β-subunit of DNA-dependent RNA polymerase (RpoB) encoded by the *rpoB* gene (2). Rifampin resistance (Rif^r) is usually due to changes in the amino acid sequence of the target site resulting in reduced affinity of RpoB for rifampin. The majority of *Escherichia coli* Rif^r mutations are located in two highly conserved regions of RpoB encompassing amino acid residues 507 to 533 (Rif cluster I) and 563 to 572 (Rif cluster II) (2). Substitutions in these clusters, particularly at "hot spots" such as the residue cognate to *E. coli* S531, are responsible for acquired Rif^r in several species of bacteria. Less common mechanisms of Rif^r include mutations in RpoB outside Rif clusters I and II, decreased membrane permeability, efflux, and enzymatic modification of the antibiotic.

Several species of free-living and host-associated spirochetes of the genera *Spirochaeta*, *Leptospira*, and *Treponema* that were isolated without rifampin are resistant to relatively high concentrations of this antibiotic (MICs, 50 to >200 μg/ml) (5). Rif^r is widespread among spirochetes, and rifampin sensitive strains have not been isolated (5, 10). Studies conducted by Leschine and Canale-Parola (5) with purified *Spirochaeta aurantia* RpoB suggested that spirochetal Rif^r may be due to a low affinity of RpoB for rifampin. Alekshun et al. (1) proposed that an N at the RpoB residue cognate to *E. coli* S531 is the primary molecular determinant of naturally occurring Rif^r in *Borrelia burgdorferi* and possibly in other spirochetes. Furthermore, Lee et al. (4) observed an N531 in the RpoB of 22 *Borrelia* reference strains. Our analysis of the complete amino acid sequences of *Treponema pallidum* strain Nichols (AE001205) and *Leptospira biflexa* (AF150880) RpoB confirmed the presence of an N531 in both organisms. Additional amino acid substitutions associated with Rif^r were not present inside or outside Rif clusters I and II. These observations prompted us to examine the Rif clusters of several Rif^r host-associated *Treponema* spp.

Treponema pallidum Street strain 14, an erythromycin-resistant clinical isolate, was grown by testicular cultivation in rabbits as previously described (8). *T. denticola* (ATCC 35405) and *T. phagedenis* (Reiter) were grown as previously described (7). *T. socranskii* subsp. *socranskii* (ATCC 35536) and *T. medium* G7201 were grown in NOS medium as previously described (6). Four clonal isolates of bovine papillomatous digital dermatitis (PDD)-associated *Treponema* were grown as previously described (9). Genomic DNA was extracted from each of the *Treponema* spp. as previously described (8). The complete *rpoB* of *T. pallidum* Street strain 14 was PCR amplified using primers (forward, 5′-CGGCGTCTCCCCTGTGTG-3′; reverse, 5′-ATTGTCTCAGGCTTTTTCAC-3′) based on Nichols strain nucleotide sequences flanking *rpoB* (AE001205). An approximately 2.3-kb internal fragment of *rpoB* was amplified from each of the cultivable *Treponema* spp. using PCR primers (forward, 5′-CGTTCGCCTGGTGTTATC-3′; reverse, 5′-AGACCCTTGTTTCCGTGG-3′) based on the preliminary sequence of *T. denticola rpoB* (The Institute for Genomic Research, http://www.tigr.org). Gel-purified PCR amplicons were cloned and both DNA strands were sequenced as previously described (8).

A deduced amino acid sequence alignment representing the RpoB region containing Rif clusters I and II for each of the *Treponema* spp. is presented in Fig. 1. The corresponding sequences of rifampin-sensitive *E. coli* and *Staphylococcus aureus* and Rif^r *L. biflexa* and *B. burgdorferi* are shown for comparison. Although substitutions in Rif cluster I residues 508, 518, and

	Rif I						Rif II
	507	517	527 ↓	537	547	557	567
Eco	GSSQLSQFMD	QNNPLSEITH	KRRISALGPG	GLTRERAGFE	VRDVHPTHYG	RVCPIETPEG	PNIGLI
Sau	GSSQLSQFMD	QANPLAELTH	KRRLSALGPG	GLTRERAQME	VRDVHYSHYG	RMCPIETPEG	PNIGLI
Lbi	GSSQLSQFMD	QTNPLAELTH	KRRLNALGPG	GLSRDRAGFE	VRDVHYSHYG	RMCPIETPEG	PNIGLI
Bbu	ATSQLSQFMD	QVNPLAELTH	KRRLNALGPG	GLSRDRAGFE	VRDVHYTHYG	RMCPIETPEG	PNIGLI
Tpa	GASQLSQFMD	QVNPLAELTH	KRRLNALGPG	GLSRERAGFE	VRDVHYTHYG	RMCPIETPEG	PNIGLI
Tde	GASQLSQFMD	QVNPLAELTH	KRRLNALGPG	GLSRDRAGFE	VREVHYTHYG	RMCPIETPEG	PNIGLI
Tme	GASQLSQFMD	QVNPLAELTH	KRRLNALGPG	GLSRDRAGFE	VRDVHYTHYG	RMCPIETPEG	PNIGLI
Tso	GASQLSQFMD	QVNPLAELTH	KRRLNALGPG	GLSRDRAGFE	VREVHYTHYG	RMCPIETPEG	PNIGLI
Tph	GGSQLSQFMD	QVNPLAELTH	KRRLNALGPG	GLSRDRAGFE	VRDVHYTHYG	RMCPIETPEG	PNIGLI
PDD1	GASQLSQFMD	QVNPLAELTH	KRRLNALGPG	GLSRDRAGFE	VREVHYTHYG	RMCPIETPEG	PNIGLI
PDD2	GGSQLSQFMD	QVNPLAELTH	KRRLNALGPG	GLSRDRAGFE	VRDVHYTHYG	RMCPIETPEG	PNIGLI
PDD3	GASQLSQFMD	QVNPLAELTH	KRRLNALGPG	GLSRDRAGFE	VRDVHYTHYG	RMCPIETPEG	PNIGLI
PDD4	GGSQLSQFMD	QVNPLAELTH	KRRLNALGPG	GLSRDRAGFE	VRDVHYTHYG	RMCPIETPEG	PNIGLI

FIG. 1. Deduced amino acid sequence alignment of *Treponema* spp. RpoB region containing Rif clusters I and II with the corresponding sequences of wild-type rifampin-sensitive *E. coli* (AE000472) and *S. aureus* (X64172) and naturally Rif^r *L. biflexa* (AF150880) and *B. burgdorferi* (L48488). Numbering is based on the *E. coli* RpoB sequence. Rif clusters I and II are indicated. Residues where amino acid substitutions are known to correlate with Rif^r are boldfaced. Arrow, N531 substitution that is present in all *Treponema* spp. and correlates with Rif^r. Abbreviations: *Eco*, *E. coli*; *Sau*, *S. aureus*; *Lbi*, *L. biflexa*; *Bbu*, *B. burgdorferi*; *Tpa*, *T. pallidum* Street strain 14; *Tde*, *T. denticola* 35405; *Tme*, *T. medium* G7201; *Tso*, *T. socranskii* subsp. *socranskii*; *Tph*, *T. phagedenis* Reiter; PDD1, PDD-associated *Treponema* isolate 1-9185MED; PDD2, isolate 9-3379; PDD3, isolate 7-2009; PDD4, isolate 2-1498. The amino acid sequence of *T. pallidum* Street strain 14 is identical to that of *T. pallidum* strain Nichols (AE001205) in the RpoB region shown.

531 are present in all of the *Treponema* spp., only the N531 substitution correlates with Rif^r. While not commonly observed in Rif^r bacteria, an N531 substitution is associated with high-level resistance in *Mycobacterium celatum*, an organism that is naturally Rif^r (3). Substitutions in Rif cluster II are not present in any of the *Treponema* spp.

Our results support the hypothesis of Alekshun et al. (1) that N531 is primarily responsible for spirochetal Rif^r. However, some variations in the level of Rif^r among cultivable spirochetes, including *Treponema* spp., have been reported (5, 10). It is possible that additional mechanisms such as membrane permeability or mutations occurring in RpoB outside Rif clusters I and II are responsible for such observations. Further studies are required to elucidate this. Finally, our results also support the use of rifampin as a selective agent for the isolation of *Treponema* spp. from human and animal specimens (5).

The sequences of the 198-bp region of *rpoB* from the *Treponema* spp. have been deposited in GenBank under accession numbers AF389072 to AF389080.

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