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Insusceptibility of Members of the Class *Mollicutes* to Rifampin: Studies of the *Spiroplasma citri* RNA Polymerase β-Subunit Gene

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In order to study the mechanism of insusceptibility of *Spiroplasma citri* to rifampin, we have cloned and sequenced its *rpoB* gene, which encodes the β subunit of RNA polymerase. By comparison of the deduced amino acid sequence with sequences of β subunits from susceptible and resistant bacteria, it was possible to identify several differences in the so-called Rif region (encompassing *rpoB* codons 500 to 575 in the *Escherichia coli* sequence). We constructed a chimeric *rpoB* gene made of the *E. coli rpoB* gene in which the Rif region was replaced by the equivalent region from *S. citri*. *E. coli* cells harboring this chimeric gene were resistant to rifampin. Subsequent experiments involving site-directed mutagenesis demonstrated that a single amino acid substitution (asparagine at position 526) was able to provide high-level rifampin resistance in *E. coli*.

Organisms belonging to the class *Mollicutes* are wall-less prokaryotes characterized by a small genome with a low guanine-plus-cytosine (G+C) content. They are phylogenetically related to gram-positive bacteria with low G+C contents (45, 46). All *Mollicutes* species tested so far, as well as some of their walled relatives (*Clostridium innocuum*, *Clostridium ramosum*, and *Clostridium acidiurici*), are insusceptible to rifampin (14, 31, 38), an RNA polymerase inhibitor (15, 43). It has been demonstrated that purified RNA polymerases of two spiroplasmas (mollicutes with helical morphology and motility), *Spiroplasma melliferum* and *Spiroplasma apis*, were insusceptible to rifampin in vitro (14).

Rifampin resistance in *Escherichia coli* results from mutations (Rif^r) in the *rpoB* gene encoding the 1,342-amino-acid β subunit of RNA polymerase (47), with most of the mutations occurring in the so-called Rif region, encompassing amino acids 500 to 575 (19, 30, 36). Two additional strong Rif^r point mutations were found outside of the Rif region, at positions 146 and 687 (19, 25), and several weak Rif^r mutations were also isolated around position 146 (37). In *Mycobacterium tuberculosis*, *Mycobacterium leprae*, *Mycobacterium smegmatis*, and *Neisseria meningitidis*, rifampin resistance was also associated with mutations within *rpoB*, and most of these mutations were found in the Rif region (3, 8, 10, 16, 17, 22, 24, 41).

In order to investigate further the mechanism of insusceptibility of mollicutes to rifampin, we cloned and sequenced a 6.6-kb DNA fragment from the mollicute *Spiroplasma citri* harboring the entire *rpoB* gene (23). Here we report the construction of a chimeric *rpoB* gene and the use of site-directed mutagenesis to demonstrate which amino acids may be involved in rifampin insusceptibility.

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MATERIALS AND METHODS

Bacteria, plasmids, and antibiotics. *E. coli* XL1-Blue (Stratagene, La Jolla, Calif.) was used for plasmid propagation, vector constructions, and expression experiments. XL1-Blue cells were grown in LB medium or on LB plates containing 12.5 μg of tetracycline (Sigma Chemical Co., St. Louis, Mo.) per ml and 50 μg of ampicillin (Sigma) per ml when they were transformed by a plasmid vector.

Plasmids pMKSe2 and pS531Y were kindly provided by K. Severinov (Institute of Molecular Genetics, Russian Academy of Sciences, Moscow, Russia). Plasmid pMKSe2 is an inducible β-subunit expression plasmid with the wild-type *rpoB* gene of *E. coli* driven by the *lac* promoter of pUC19 (36). Plasmid pS531Y is a derivative of pMKSe2 harboring a point mutation in the *rpoB* gene, which results in the substitution of a serine at position 531 (S531) by a tyrosine (Y). This plasmid provides a high level of rifampin resistance in *E. coli* cells (36). MICs were determined in liquid medium as recommended by the National Committee for Clinical Laboratory Standards (28) by using a microplate with twofold dilutions of rifampin, from 500 to 0.5 μg/ml, in LB medium containing 12.5 μg of tetracycline per ml and 50 μg of ampicillin per ml, with or without 1 mM isopropyl-β-D-thiogalactopyranoside (IPTG). The medium was inoculated with 10^6 cells per ml.

Plasmid pSCRPOB contains a 6.0-kb *Hin*dIII fragment of *S. citri* R8A2 (ATCC 27556) cloned into pBS+ vector (Stratagene). This DNA fragment harbors the *rpoB* gene of *S. citri* and has been totally sequenced (23).

Site-specific mutagenesis and plasmid derivative constructions. For plasmid transformation, preparation of *E. coli* XL1-Blue competent cells and electroporations were performed as described previously in a Gene Pulser (Bio-Rad Laboratories, Hercules, Calif.) (11).

A unique *Apa*I site between positions 533 and 535 (amino acids numbering) of *rpoB* was engineered in pMKSe2 by oligonucleotide-directed mutagenesis without modification of the protein sequence. A 32-mer oligonucleotide (5′-CGTT CACGGGTCAGACCGCCTGGGCCCCAGTGC-3′), corresponding to amino acids 532 to 542 of the *rpoB* gene, was used as a mutagenic primer to alter one base (boldface) and to create a unique *Apa*I site (underlined). It was used in combination with an upstream primer (5′-GTCATCGATATCCGTAACGG-3′), corresponding to amino acids 430 to 438 and containing the unique natural *Cla*I site of *rpoB* (underlined), in a PCR amplification with 1 ng of pMKSe2 in a 40-μl reaction mixture containing 20 mM Tris (pH 8.5), 10 mM $(NH_4)_2SO_4$, 2 mM $MgCl_2$, 100 μg of bovine serum albumin per ml, 0.1% Triton X-100, 200 μM (each) deoxynucleoside triphosphate, 0.5 μM (each) primer, and 2.5 U of *Pfu* DNA polymerase (Stratagene), to increase the fidelity of amplification. Thirty cycles were performed by using three steps, each of 1 min, at 92, 55, and 72°C, in a Thermojet thermocycler (Eurogentec, Seraing, Belgium). The resulting 314-bp DNA fragment carrying the mutation was used as a primer in a second PCR in combination with a downstream primer (5′-GACTTCGTTAACTTTG-3′), corresponding to amino acids 730 to 736 and containing the unique natural *Hpa*I site of *rpoB* (underlined). The 30 cycles were performed in three steps, each of 2 min, at 92, 45, and 72°C. The resulting DNA fragment of 910 bp was cleaved with *Cla*I and *Hpa*I (MBI Fermentas, Vilnius, Lithuania) and was cloned into pMKSe2, which was previously treated with the same restriction enzymes. The

resulting plasmid, pMKSe2Apa, possesses an unique *Apa*I site and has a functional *rpoB* gene, as verified by sequence analysis. It was used for the generation of a chimeric *rpoB* gene and site-directed mutagenesis.

The chimeric *rpoB* gene was constructed by replacing the region containing cluster I of the *E. coli rpoB* gene (amino acids 435 to 534) by the corresponding region from *S. citri rpoB* gene (amino acids 470 to 569 in the *S. citri* sequence). Two primers were used in a PCR amplification by using 1 ng of pSCRPOB as the template, and 30 cycles were performed in three steps, each of 1 min, at 92, 45, and 72°C. The first primer (5′-TA**ATCGAT**TTAACAGATGGAATTGGGG-3′), corresponding to amino acids 467 to 477 in *S. citri*, contains three mismatches (boldface), allowing the creation of a *Cla*I site (underlined). The second primer (5′-CCT**GGGCCC**AGGGCTGTTAATCG-3′), corresponding to amino acids 563 to 571 in *S. citri*, contains two mismatches (boldface), allowing the creation of an *Apa*I site. The 313-bp amplified DNA fragment was cleaved with *Cla*I and *Apa*I (MBI Fermentas) and was cloned into pMKSe2Apa, which was cleaved by the same restriction enzymes. The resulting plasmid was named pMKCG, and it was verified by sequence analysis that the chimeric *rpoB* gene was functional.

Two additional plasmids were derived from pMKSe2Apa. One, pH526N, harbors the *E. coli rpoB* gene, in which codon 526 specifying a histidine (H526) has been mutated to code for an asparagine (N). In the second plasmid, pS531T, codon 531, specifying a serine (S531), has been mutated to code for a threonine (T). They were obtained by using two mutagenic primers, 5′-CCT**GGGCCC**AG TGCGGAGATACGACGTTTGT**T**CG-3′ (for pH526N) and 5′-CCT**GGGCCC** AGTGCGG**T**GATACG-3′ (for pS531T), corresponding to amino acids 525 to 536 and 529 to 536, respectively, and carrying an *Apa*I site (underlined) and one mismatch (boldface), allowing the introduction of the mutation. They were used in combination with an upstream primer containing the natural *Cla*I site in a PCR experiment by using 30 cycles of three steps, each of 1 min, at 92, 55, and 72°C, and 1 ng of pMKSe2Apa as the template. The amplified fragments of 310 bp were cleaved with *Cla*I and *Apa*I and were cloned into pMKSe2Apa, which was treated with the same restriction enzymes. The constructions were verified by sequence analysis.

Sequence analysis. DNA sequencing was performed with the T7 sequencing kit (Pharmacia, Uppsala, Sweden), [^{35}S]dATP, and appropriate primers. Alignments were done with the GAP and PILEUP modules of the GCG package (9), and searches for similar sequences in GenBank were performed by using the BLAST program (2).

Nucleotide sequence accession number. The nucleotide sequence has been deposited in GenBank/EMBL under accession number U25815.

RESULTS

Identification of the amino acids of *S. citri rpoB* gene potentially involved in rifampin insusceptibility. The amino acid sequence deduced from the *S. citri rpoB* gene was aligned with those of *E. coli* (29) and *M. tuberculosis* (26) (Fig. 1). It is 1,302 amino acids long and has a predicted molecular mass of 146 kDa. It shows 51.2% identity and 71.1% similarity to the *E. coli* sequence. The alignment reveals several strongly conserved motifs interspersed with less conserved regions, as already described for RNA polymerases from various sources (40). The majority of the differences between the sequences were found in the NH_2 and COOH termini and in two regions around amino acids 300 and 1000 (in the *E. coli* sequence), which have been shown to be nonessential for RNA polymerase function (6, 33, 34). It is unlikely that these regions are involved in the insusceptibility of *S. citri* RNA polymerase to rifampin. In contrast, the regions where Rif^r mutations have been found are fairly conserved: the V at position 146 as well as the amino acids around position 146, cluster I (amino acids 507 to 534), cluster II (amino acids 563 to 574), and the R at position 687 (19, 25, 30, 36). For convenience, amino acid positions will be given according to the *E. coli rpoB* numbering. In cluster I, eight amino acids are different between *E. coli* and *S. citri* (amino acids 507, 508, 518, 522, 524, 526, 530, and 531). Two of these differences (L524 and L530) are also found in *M. tuberculosis*, and two other differences (T518 and A522) are shared by *Chlamydia trachomatis*, *Bacillus subtilis*, and *Staphylococcus aureus rpoB* genes (1, 4, 12). Thus, these four amino acids are unlikely to be involved in rifampin insusceptibility. Positions 507 and 508 in *E. coli rpoB* have been found to be associated with the Rif^r phenotype only if they are deleted together with positions 509 to 511 and replaced by a V (19). Consequently, it is unlikely that these two substitutions contribute insusceptibility to rifampin. The N at position 526 and the T at position 531 have not been found in any *rpoB* amino acid sequences of rifampin-susceptible bacteria. Mutations leading to the substitution of H526 by an N have been described in Rif^r *N. meningitidis* strains, but their role in resistance was not clearly established since strains having the same mutation displayed different levels of resistance (8). Because mutations at positions 526 and 531 have been associated with numerous Rif^r phenotypes in several bacterial species, they are good candidates for explaining *S. citri* insusceptibility to rifampin.

In cluster II, only one amino acid was different between *S. citri* and *E. coli*. In *S. citri*, S574 is replaced by an N, which corresponds to the replacement of a tiny polar amino acid by a small polar amino acid. A similar substitution (T) was found in the sequence of the rifampin-susceptible bacterium *Campylobacter jejuni* (7). Thus, this position is unlikely to play a role in *S. citri* insusceptibility to rifampin.

Replacement of *E. coli* cluster I by *S. citri* cluster I. In order to test the possible role of *S. citri* cluster I in rifampin insusceptibility, we constructed a chimeric *rpoB* gene in which cluster I has been replaced by the equivalent region of the *S. citri rpoB* gene. For this purpose, we used a plasmid, pMKSe2, carrying the wild-type *E. coli rpoB* gene under the control of the *lac* promoter and inducible by IPTG. This plasmid was modified by the introduction of a unique *Apa*I site which does not affect the coding capacity of the *rpoB* gene. It was named pMKSe2Apa. Cells carrying pMKSe2 or pMKSe2Apa had the same high level of susceptibility to rifampin (Table 1), indicating that these plasmids had no significant effect on the natural susceptibility of *E. coli* cells to the antibiotic.

The region corresponding to amino acids 433 to 533 and containing cluster I in the *E. coli rpoB* gene harbored by pMKSe2Apa was replaced by the equivalent region of *S. citri*. This was possible because the DNA fragment from *S. citri* does not possess any UGA codons, which specify tryptophan (W) in *S. citri* but which are stop codons in *E. coli* (32). The resulting plasmid, harboring a chimeric *rpoB* gene, was named pMKCG and was used to transform *E. coli* XL1-Blue host cells.

The level of in vivo resistance to rifampin was evaluated by MIC determinations in liquid medium (Table 1) and on rifampin gradient plates (data not shown). Plasmid pMKCG was able to induce a moderate, but significant (40 to 62.5 μg/ml) level of rifampin resistance in *E. coli*. It was noticed that in the presence of IPTG and in the absence of rifampin, the growth of *E. coli* cells carrying pMKCG was at least two times slower than that of cells harboring pMKSe2Apa (data not shown). In the absence of IPTG, cells carrying pMKCG had normal growth. Thus, IPTG-induced overexpression of the chimeric *rpoB* gene altered the growth properties of host cells.

The level of resistance induced by the chimeric gene was three to four times higher than that induced by the wild-type *rpoB* gene (pMKSe2 or pMKSe2Apa), suggesting that the presence of the cluster I from the *S. citri rpoB* gene may be responsible for that phenomenon.

Site-directed mutagenesis in cluster I of the *E. coli rpoB* gene. As mentioned before, two amino acids found in cluster I of *S. citri*, N526 and T531, may be involved in rifampin insusceptibility. These substitutions have never been observed or tested in *E. coli*. We engineered these substitutions in cluster I of the *E. coli rpoB* gene using the unique *Apa*I site of pMKSe2Apa and appropriate PCR primers. H526 was replaced by an N and S531 was replaced by a T, leading to plasmids pH526N and pS531T, respectively. The in vivo levels of resistance induced by these plasmids were measured (Table 1). *E. coli* cells transformed with pS531T were not resistant to

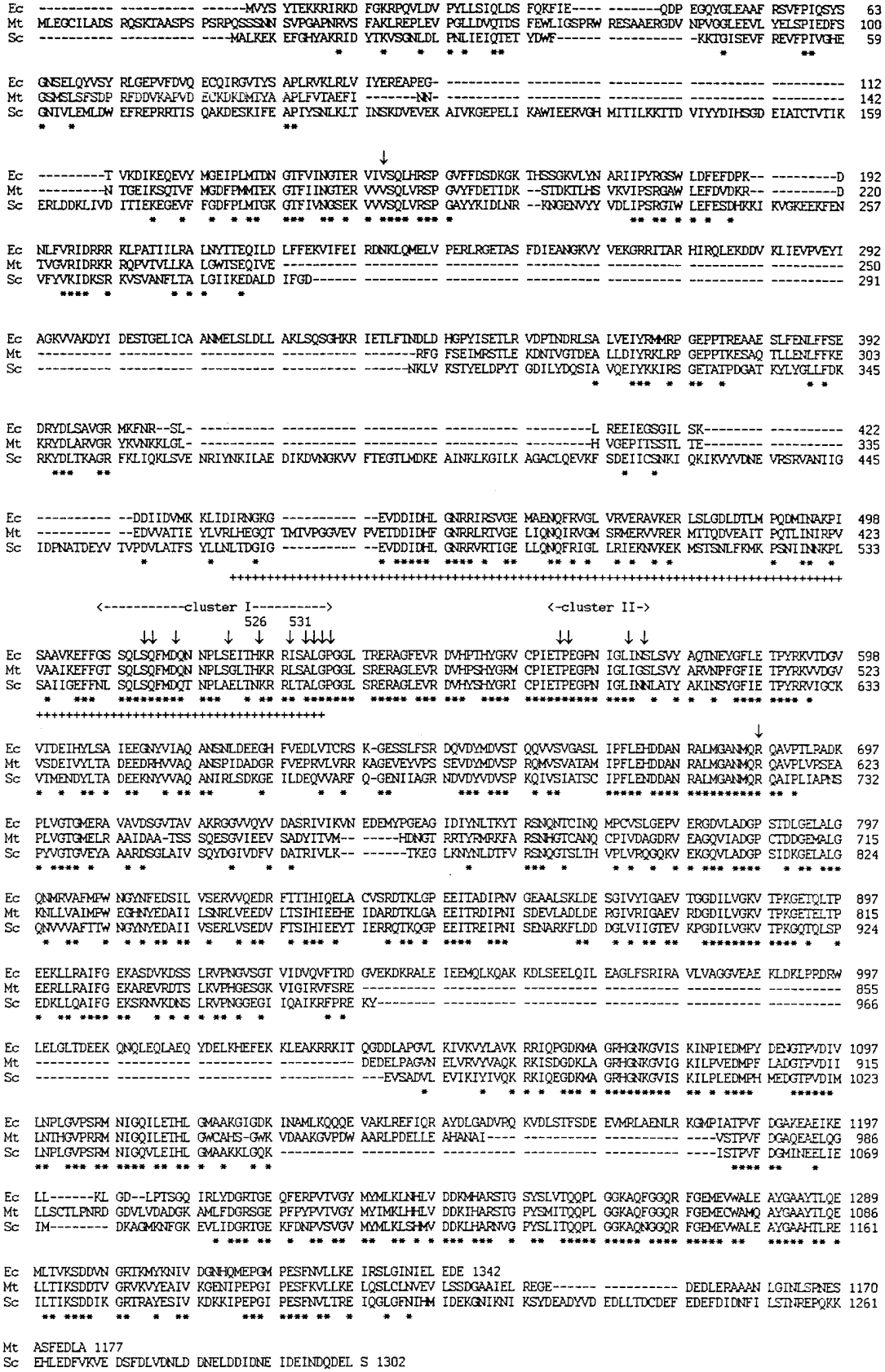

FIG. 1. Alignment of amino acids sequences deduced from the *rpoB* genes of *E. coli* (Ec) (29), *M. tuberculosis* (Mt) (26), and *S. citri* (Sc). Asterisks indicate identical amino acids in all sequences, and gaps are marked by dashed lines. Positions known to be involved in the Rif^r phenotype in *E. coli* are indicated by vertical arrows, and the locations of clusters I and II are shown. The region involved in the construction of the chimeric *rpoB* gene is underlined with plus signs.

TABLE 1. Level of rifampin resistance induced in *E. coli* XL1-Blue cells by different expression plasmids

Plasmid name	*rpoB* gene product	MIC (μg/ml) in liquid medium
pMKSe2	*E. coli* wild-type β subunit	15.6
pMKSe2Apa	*E. coli* wild-type β subunit	15.6
pS531Y	*E. coli* mutant β subunit (S531 replaced by Y)	>500.0
pMKCG	Chimeric β subunit (*E. coli* cluster I replaced by *S. citri* cluster I)	62.5
pS531T	*E. coli* mutant β subunit (S531 replaced by T)	15.6
pH526N	*E. coli* mutant β subunit (H526 replaced by N)	500.0

rifampin, while those harboring pH526N exhibited a high level of resistance (≤500 μg/ml). Thus, the substitution of H526 by N, corresponding to a single point mutation (CAC → AAC), is sufficient to induce a high level of resistance to rifampin in *E. coli* cells.

DISCUSSION

We have demonstrated that substitution of cluster I of the *E. coli rpoB* gene by cluster I of the *S. citri rpoB* gene induces rifampin resistance in *E. coli* cells expressing the chimeric gene. The level of resistance was approximately four times that of susceptible cells. This level is rather low when compared with that in cells harboring plasmid pS531Y, but cells expressing the chimeric gene were affected in their growth. This phenomenon was due to the overexpression of sequences of *S. citri* origin, since in the absence of IPTG the cells exhibited normal growth (data not shown). In our construction, the DNA fragment from *S. citri rpoB* which was substituted in *E. coli rpoB* was not limited to cluster I but corresponded to 100 amino acids, 41 of which were different between the *S. citri* and the *E. coli* sequences. In cluster I itself, eight amino acids are different between *S. citri* and *E. coli*. It is possible that the changes in the protein altered the structure and/or the assembly of the enzyme, and the pleiotropic effects of mutations in the Rif region have been well documented (18, 20, 21).

Among the eight amino acid differences found between clusters I of *S. citri* and *E. coli*, two were good candidates for explaining the insusceptibility of *S. citri* RNA polymerase to rifampin, N and T replacing H526 and S531, respectively. A number of substitutions involving these positions have been associated with rifampin resistance not only in *E. coli* (19, 30, 37) but also in *M. tuberculosis* (3, 10, 17, 22, 41), *M. leprae* (16), and *N. meningitidis* (8). These two substitutions were engineered in the *E. coli rpoB* gene by site-directed mutagenesis. Replacement of S531 by a T did not induce any resistance to rifampin. This was not surprising since T and S are similar amino acids. In contrast, substitution of H526 by an N resulted in a significantly higher level of resistance (≤500 μg/ml) in cells carrying the mutated *rpoB* gene. This suggests that this amino acid of cluster I is responsible for the rifampin insusceptibility of *S. citri* and prevents the binding of rifampin to RNA polymerase. Indeed, it has been shown that in *E. coli*, residues 516 to 540 are part of the target of rifampin and participate with residues 1065 and 1237 in the formation of the initiation site when the β subunit is assembled in the RNA polymerase complex (27, 34, 35).

Recently, the complete nucleotide sequence of the *rpoB* genes of *Mycoplasma gallisepticum* and *Mycoplasma genitalium* became available in GenBank (13, 39). Fragments of *rpoB* from *Mycoplasma capricolum* have also been sequenced (5). Alignments of the deduced amino acid sequences indicated that in cluster I, H526 was replaced by an N in *S. citri*, *M. gallisepticum*, and *M. genitalium*, while S531 was conserved in *M. gallisepticum* and *M. genitalium* (Fig. 2). No data were available for *M. capricolum* in this region. This strengthens the role of N526 in the rifampin insusceptibility of *Mollicutes* species. The other differences observed between sequences were either not shared by the differente mollicutes tested, found in the sequences of susceptible RNA polymerases, or located in positions which have never been shown to be involved in rifampin resistance. In addition, two other positions outside of the Rif region, V146 and R687, are also conserved.

It seems that a single amino acid, N526, explains the insusceptibility of mollicutes to rifampin. This substitution corresponds to a single mutation, CAC→AAC. Rifampin insusceptibility has been demonstrated in vivo for all mollicutes species tested: *S. citri*, *S. melliferum*, *S. apis*, *Acholeplasma laidlawii*, *Mycoplasma mycoides*, *Mycoplasma orale*, *Mycoplasma pneumoniae*, and *Ureaplasma urealyticum* (14, 31, 38). These organisms are members of three of the four orders of mollicutes (42) and are representatives of the four phylogenetic groups of the class *Mollicutes* (44). In addition, three walled bacterial species, *C. ramosum*, *C. innocuum*, and *C. acidiurici*, which are phylogenetically related to mollicutes (44), were shown to be insusceptible to rifampin (31), suggesting that this property has been acquired from their common ancestor. Since a single point mutation seems to be responsible for that phenotype, it remains to be understood why it occurred. Maybe it is in relation to the low G+C contents of their DNAs or is a consequence of selection by natural rifamycins.

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                <-----------cluster I---------->
                                      526   531
                     ↓↓  ↓      ↓   ↓   ↓ ↓↓↓↓
Ec  SAAVKEFFGS SQLSQFMDQN NPLSEITHKR RISALGPGGL TRERAGFEVR  548
Sc  ..IIG...NL ........T ...A.L.N.. .LT....... S.....L...
Mge QIVI.D..NT H..T..L.HQ .....LSN.. ....M....I S..DPNLDI.
Mga QIVI.D..NT H..T..L..Q .....L.N.. ....M....I S..DPNLDI.


                    <-cluster II->
                     ↓↓        ↓ ↓
Ec  DVHPTHYGRV CPIETPEGPN IGLINSLSVY 588
Sc  ...YS....I .......... .....N.AT.
Mge ...YSQ...I ........M. ....M..ASF
Mga ...YS....I ........M. ....M..AF.
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FIG. 2. Amino acid sequence comparisons within the Rif regions (clusters I and II) of *E. coli* (Ec), *S. citri* (Sc), *M. genitalium* (Mge), and *M. gallisepticum* (Mga). Numbering is according to the *E. coli* sequence. Dots indicate identity to the *E. coli* sequence, and the positions involved in the Rif^r phenotype in *E. coli* are marked by vertical arrows.

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REFERENCES

1. **Aboshkiwa, M. A., G. C. Rowland, and G. Coleman.** 1995. Nucleotide and deduced amino acid sequence of the gene for a novel protein with a possible regulatory function encoded in the β operon of *Staphylococcus aureus*. FEMS Microbiol. Lett. **126:**305–310.
2. **Altshul, S. F., W. Gish, W. Miller, E. W. Myers, and D. J. Lipman.** 1990. Basic local alignment search tool. J. Mol. Biol. **215:**403–410.
3. **Bodmer, T., G. Zürcher, P. Imboden, and A. Telenti.** 1995. Mutation position and type of substitution in the β-subunit of the RNA polymerase influence in-vitro activity of rifamycins in rifampicin-resistant *Mycobacterium tuberculosis*. J. Antimicrob. Chemother. **35:**345–348.
4. **Boor, K. J., M. L. Duncan, and C. W. Price.** 1995. Genetic and transcriptional organization of the region encoding the β subunit of *Bacillus subtilis*

RNA polymerase. J. Biol. Chem. **270:**20319–20336.
5. **Bork, P., C. Ouzounis, G. Casari, R. Schneider, C. Sander, M. Dolan, W. Gilbert, and P. M. Gillevet.** 1995. Exploring the *Mycoplasma capricolum* genome: a minimal cell reveals its physiology. Mol. Microbiol. **16:**955–967.
6. **Borukhov, S., K. Severinov, M. Kashlev, A. Lebedev, I. Bass, G. C. Rowland, P. P. Lim, R. E. Glass, V. Nikiforov, and A. Goldfarb.** 1991. Mapping the trypsin cleavage and antibody-binding sites and delineation of a dispensable domain in the β subunit of *Escherichia coli* RNA polymerase. J. Biol. Chem. **266:**23921–23926.
7. **Bustamante, V. H., J. L. Puente, F. Sanchez-Lopez, M. Bobadilla, and E. Calva.** 1995. Identification of *Campylobacter jejuni* and *C. coli* using the *rpoB* gene and a cryptic DNA fragment from *C. jejuni*. Gene **165:**1–8.
8. **Carter, P. E., F. J. R. Abadi, D. E. Yakubu, and T. H. Pennington.** 1994. Molecular characterization of rifamin-resistant *Neisseria meningitidis*. Antimicrob. Agents Chemother. **38:**1256–1261.
9. **Devereux, J., P. Haeberli, and O. Smithies.** 1984. A comprehensive set of sequence analysis programs for the VAX. Nucleic Acids Res. **12:**387–395.
10. **Donnabella, V., F. Martiniuk, D. Kinney, M. Bacerdo, S. Bonk, B. Hanna, and W. N. Rom.** 1994. Isolation of the gene for the β subunit of RNA polymerase from rifampicin-resistant *Mycobacterium tuberculosis* and identification of new mutations. Am. J. Respir. Cell. Mol. Biol. **11:**639–643.
11. **Dower, W. J., J. F. Miller, and C. W. Ragsdale.** 1988. High efficiency transformation of *E. coli* by high voltage electroporation. Nucleic Acids Res. **16:** 6127–6145.
12. **Engel, J. N., J. Pollack, F. Malik, and D. Ganem.** 1990. Cloning and characterization of RNA polymerase core subunits of *Chlamydia trachomatis* by using the polymerase chain reaction. J. Bacteriol. **172:**5732–5741.
13. **Fraser, C. M., J. D. Gocayne, O. White, M. D. Adams, R. A. Clayton, R. D. Fleischmann, C. J. Bult, A. R. Kerlavage, G. Sutton, J. M. Kelley, J. L. Fritchman, J. F. Weidman, K. V. Small, M. Sandusky, J. Fuhrmann, D. Nguyen, T. R. Utterback, D. M. Sauder, C. A. Phillips, J. M. Merrick, J. F. Tomb, B. A. Dougherty, K. F. Bott, P. C. Hu, T. S. Lucier, S. N. Peterson, H. O. Smith, C. A. Hutchison III, and J. G. Venter.** 1995. The minimal gene complement of *Mycoplasma genitalium*. Science **270:**397–403.
14. **Gadeau, A. P., C. Mouches, and J. M. Bové.** 1986. Probable insensitivity of mollicutes to rifampin and characterization of spiroplasmal DNA dependent-RNA polymerase. J. Bacteriol. **166:**824–828.
15. **Hartmann, G., K. O. Honikel, F. Knusel, and J. Nuesch.** 1967. The specific inhibition of the DNA-directed RNA synthesis by rifamycin. Biochem. Biophys. Acta **145:**843–844.
16. **Honoré, N., and S. T. Cole.** 1993. Molecular basis of rifampin resistance in *Mycobacterium leprae*. Antimicrob. Agents Chemother. **37:**414–418.
17. **Hunt, J. M., G. D. Roberts, L. Stockman, T. A. Felmlee, and D. H. Persing.** 1994. Detection of a genetic locus encoding resistance to rifampin in mycobacterial cultures and in clinical specimens. Diagn. Microbiol. Infect. Dis. **18:** 219–227.
18. **Jin, D. J., M. Cashel, D. I. Fridman, Y. Nakamura, W. A. Walter, and C. A. Gross.** 1988. Effects of rifampicin resistant *rpoB* mutations on antitermination and interaction with *nusA* in *Escherichia coli*. J. Mol. Biol. **204:**247–261.
19. **Jin, D. J., and C. A. Gross.** 1988. Mapping and sequencing of mutations in the *Escherichia coli rpoB* gene that lead to rifampicin resistance. J. Mol. Biol. **202:**45–58.
20. **Jin, D. J., and C. A. Gross.** 1989. Characterization of the pleitropic phenotypes of rifampin-resistant *rpoB* mutants of *Escherichia coli*. J. Bacteriol. **171:** 5229–5231.
21. **Jin, D. J., W. A. Walter, and C. A. Gross.** 1988. Characterization of the termination phenotypes of rifampin-resistant mutants. J. Mol. Biol. **202:** 245–253.
22. **Kapur, V., L. L. Li, S. Iordanescu, M. R. Hamrick, A. Wanger, B. N. Kreiswirth, and J. M. Musser.** 1994. Characterization by automated DNA sequencing of mutations in the gene (*rpoB*) encoding the RNA polymerase β subunit in rifampin-resistant *Mycobacterium tuberculosis* strains from New York City and Texas. J. Clin. Microbiol. **32:**1095–1098.
23. **Laigret, F., P. Gaurivaud, and J. M. Bové.** Unique organization of the *rpoB* region of *Spiroplasma citri*: a restriction and modification system gene is adjacent to *rpoB*. Gene, in press.
24. **Levin, M. E., and G. F. Hatfull.** 1993. *Mycobacterium smegmatis* RNA polymerase: DNA supercoiling, action of rifampicin and mechanism of rifampicin resistance. Mol. Microbiol. **8:**277–285.
25. **Lisitsyn, N. A., E. D. Sverdlov, E. P. Moiseyeva, O. N. Danilevskaya, and V. G. Nikiforov.** 1984. Mutation to rifampicin resistance in the beginning of the RNA polymerase β subunit gene in *Escherichia coli*. Mol. Gen. Genet. **196:**173–174.
26. **Miller, L. P., J. T. Crawford, and T. M. Shinnick.** 1994. The *rpoB* gene of *Mycobacterium tuberculosis*. Antimicrob. Agents Chemother. **38:**805–811.
27. **Mustaev, A., M. Kashlev, J. Lee, A. Polyakov, A. Lebedev, A. Grachev, A. Goldfarb, and V. Nikiforov.** 1991. Mapping of the priming substrate contacts in the active center of *Escherichia coli* RNA polymerase. J. Biol. Chem. **266:** 23927–23931.
28. **National Committee for Clinical Laboratory Standards.** 1990. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Standard M7-A2. National Committee for Clinical Laboratory Standards, Villanova, Pa.
29. **Ovchinnikov, Y. A., G. S. Monastyrskaya, V. V. Gubanov, S. O. Guryev, O. U. Chertov, N. N. Modyanov, V. A. Grinkevich, I. A. Makarova, T. V. Marchenko, I. N. Polovnikova, V. M. Lipkin, and E. D. Sverdlov.** 1981. The primary structure of *Escherichia coli* RNA polymerase: nucleotide sequence of the *rpoB* gene and amino acid sequence of the beta-subunit. Eur. J. Biochem. **116:**621–629.
30. **Ovchinnikov, Y. A., G. S. Monastyrskaya, S. O. Guriev, N. F. Kalinina, E. D. Sverdlov, A. I. Gragerov, I. A. Bass, I. F. Kiver, E. P. Moiseyeva, V. N. Igumnov, S. Z. Mindlin, V. G. Nikiforov, and R. B. Khesin.** 1983. RNA polymerase rifampicin resistance mutations in *Escherichia coli*: sequence changes and dominance. Mol. Gen. Genet. **190:**344–348.
31. **Pellegrin, J. L., J. Maugein, M. T. Clerc, B. Leng, J. M. Bové, and C. Bébéar.** 1990. Activity of rifampin against Mollicutes, clostridia and L forms. Recent advances in mycoplasmology. Zentralbl. Bakteriol. **1990**(Suppl. 20)**:**810–812.
32. **Renaudin, J., M. C. Pascarel, C. Saillard, C. Chevalier, and J. M. Bové.** 1986. Chez les spiroplasmes le codon UGA n'est pas non sens et semble coder pour le tryptophane. C. R. Acad. Sci. Paris **303:**539–540.
33. **Severinov, K., M. Kashlev, E. Severinova, I. Bass, K. McWilliams, E. Kutter, V. Nikiforov, L. Snyder, and A. Goldfarb.** 1994. A non-essential domain of *Escherichia coli* RNA polymerase required for the action of the termination factor Alc. J. Biol. Chem. **269:**14254–14259.
34. **Severinov, K., A. Mustaev, E. Severinova, I. Bass, M. Kashlev, R. Landick, V. Nikiforov, A. Goldfarb, and S. A. Darst.** 1995. Assembly of functional *Escherichia coli* RNA polymerase containing β subunit fragments. Proc. Natl. Acad. Sci. USA **92:**4591–4595.
35. **Severinov, K., A. Mustaev, E. Severinova, M. Kozlov, S. A. Darst, and A. Goldfarb.** 1995. The β subunit Rif-cluster I is only angstroms away from the active center of *Escherichia coli* RNA polymerase. J. Biol. Chem. **270:**29428–29432.
36. **Severinov, K., M. Soushko, A. Goldfarb, and V. Nikiforov.** 1993. Rifampicin region revisited. New rifampicin-resistant and streptolydigin-resistant mutants in the β subunit of *Escherichia coli* RNA polymerase. J. Biol. Chem. **268:**14820–14825.
37. **Severinov, K., M. Soushko, A. Goldfarb, and V. Nikiforov.** 1994. Rif^r mutations in the beginning of the *Escherichia coli rpoB* gene. Mol. Gen. Genet. **244:**120–126.
38. **Shepard, M. C., C. D. Lunceford, D. K. Ford, R. H. Purcell, D. Taylor-Robinson, S. Razin, and F. T. Black.** 1974. *Ureaplasma urealyticum* gen. nov., sp. nov.: proposed nomenclature for the human T (T-strain) mycoplasmas. Int. J. Syst. Bacteriol. **24:**160–171.
39. **Skamrov, A., T. Rozovskaya, M. Goldman, E. Feoktistova, and R. S. Beabealashvilli.** 1995. Unpublished data. GenBank accession number L38402.
40. **Sweetser, D., M. Nonet, and R. A. Young.** 1987. Prokaryotic and eukaryotic RNA polymerases have homologous core subunits. Proc. Natl. Acad. Sci. USA **84:**1192–1196.
41. **Telenti, A., P. Imboden, F. Marchesi, D. Lowrie, S. Cole, M. J. Colston, L. Matter, K. Schopfer, and T. Bodmer.** 1993. Detection of rifampicin-resistance mutations in *Mycobacterium tuberculosis*. Lancet **341:**647–650.
42. **Tully, J. G., J. M. Bové, F. Laigret, and R. F. Whitcomb.** 1993. Revised taxonomy of the class *Mollicutes*: proposed elevation of a monophyletic cluster of arthropod-associated mollicutes to ordinal rank (*Entomoplasmatales*, ord. nov.), with provision for familial rank to separate species with non-helical morphology (*Entomoplasmataceae*, fam. nov.) from helical species (*Spiroplasmataceae*), and emended descriptions of the order *Mycoplasmatales*, family *Mycoplasmataceae*. Int. J. Syst. Bacteriol. **43:**378–385.
43. **Wehrli, W., F. Knusel, K. Schmid, and M. Staehelin.** 1968. Interaction of rifamycin with bacterial RNA polymerase. Proc. Natl. Acad. Sci. USA **61:** 667–673.
44. **Weisburg, W. G., J. G. Tully, D. L. Rose, J. P. Petzel, H. Oyaizu, D. Yang, L. Mandelco, J. Sechrest, T. G. Lawrence, J. Van Etten, J. Maniloff, and C. R. Woese.** 1989. A phylogenetic analysis of the mycoplasmas: basis for their classification. J. Bacteriol. **171:**6455–6467.
45. **Woese, C. R.** 1987. Bacterial evolution. Microbiol. Rev. **51:**221–271.
46. **Woese, C. R., E. Stackebrandt, and W. Ludwig.** 1985. What are mycoplasmas: the relationship of tempo and mode in bacterial evolution. J. Mol. Evol. **21:**305–316.
47. **Zillig, W., K. Zechel, D. Rabussay, M. Schachner, V. S. Sethi, P. Palm, A. Heil, and W. Seifert.** 1970. On the role of different subunits of DNA-dependent RNA polymerase from *E. coli* in the transcription process. Cold Spring Harbor Symp. Quant. Biol. **33:**47–58.