Mutations in Bartonella Bacilliformis gyrB Confer Resistance to Coumermycin A(1)

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Recent taxonomic reclassifications involving bacteria formerly constituting the \textit{Rochalimaea} and \textit{Grahamella} genera have rapidly expanded the number of species in the \textit{Bartonella} genus (5, 8, 10, 23, 47). Of these 12 species, 5 are presently considered to be etiologic agents of emerging infectious disease in humans: \textit{Bartonella bacilliformis}, \textit{B. clarridgeiae}, \textit{B. elizabethae}, \textit{B. henselae}, and \textit{B. quintana} (22, 23, 33). Hemotrophy and arthropod vector-mediated transmission are common parasitic strategies utilized by these small, gram-negative, facultatively intracellular pathogens.

Due to the lack of a system for site-specific genetic manipulation, few reports have been published concerning the molecular mechanisms involved in the pathogenesis, growth, and antibiotic resistance of \textit{Bartonella} species (3, 15, 16, 24, 27, 29, 31, 34, 42, 46, 49). Therefore, we initially address this problem by molecularly characterizing the pathogens’ \textit{gyrB} gene. DNA gyrase is the bacterial type II topoisomerase responsible for introducing negative supercoiling into DNA (reviewed in references 20 and 37), and it is the target of several types of antimicrobial agents. The holoenzyme is an A2B2 complex encoded by the \textit{gyrA} and \textit{gyrB} genes; the A subunit is responsible for DNA breakage and reunion, whereas the B subunit harbors the ATP binding site. The coumarin antibiotics coumermycin A1, novobiocin, and chlorobiocin impede DNA replication by inhibiting the ATP binding and hydrolysis catalyzed by GyrB (28). Several reports have demonstrated that single point mutations in the \textit{gyrB} gene confer resistance to coumarin antibiotics (11, 13, 19, 36, 39, 44) providing a locus and selectable phenotype for allelic exchange experiments.

In this study, we describe the isolation and characterization of the first spontaneous mutants of any \textit{Bartonella} species, as well as the first characterization of an antibiotic-resistant mutant. Analysis of coumermycin A1-resistant mutants revealed single nucleotide lesions corresponding to specific amino acid substitutions in the N-terminal domain of GyrB. These mutations confer an approximately three- to fivefold increase in the MIC of coumermycin A1 relative to the wild type. In addition, we show that the \textit{B. bacilliformis} \textit{gyrB} can functionally complement an \textit{E. coli} \textit{gyrB} mutant. Finally, we discuss the positions of the amino acid substitutions in \textit{B. bacilliformis} GyrB as they relate to recently solved high-resolution crystal structures and enzyme function (26, 48).

\textbf{MATERIALS AND METHODS}

\textbf{Bacterial strains and culture conditions.} \textit{E. coli} strains were grown overnight at 37°C in Luria-Bertani (LB) medium with standard antibiotic supplements when required (12). \textit{B. bacilliformis} was grown and harvested as previously described (34).

To isolate coumermycin A1-resistant mutants, suspensions of \textit{B. bacilliformis} KCS3 were plated on heart infusion agar supplemented with 5% erythrocytes and coumermycin A1 (0.1 \textmu g/ml Sigma Chemical Co., St. Louis, Mo.). Coumermycin A1-resistant mutants were usually observed after 5 days of growth and were harvested after 7 days. Resistant colonies were picked and resuspended in 150 \textmu l of heart infusion broth. Resistant mutants were maintained in the presence of 0.04 \textmu g of coumermycin A1 per ml. Strains of \textit{B. bacilliformis} and \textit{Escherichia coli} used or generated in this study are summarized in Table 1.

\textbf{Preparation and manipulation of DNA.} Chromosomal DNA from \textit{B. bacilliformis} for use in DNA hybridization or PCR analyses was prepared with CTAB (hexadecyldimethyl ammonium bromide) by the methods of Ausubel et al. (2). Plasmid DNA extraction and isolation from \textit{E. coli} for cloning were performed by the alkaline lysis procedure of Birnboim and Doly (4), and plasmid preparations for sequencing were made with either a MultiPrep kit (Qiagen, Chatsworth, Calif.) or a Perfect Prep kit (5 PRIME-3 PRIME, Boulder, Colo.) as per the manufacturer’s instructions. Cloning of individual DNA fragments was accomplished by two distinct methods. First, both lambda-ZAP Express (Stratagene Cloning Systems, La Jolla, Calif.) and lambda-GEM 11 (Promega, Madison, Wis.) genomic cloning systems were used as per the manufacturer’s recommendations to obtain a plasmid clone containing the entire wild-type \textit{gyrB} open reading frame (ORF) for gene expression and functional complementation analyses. When required, DNA was purified from ethidium bromide-stained agarose gels or PCR products with either a GeneClean kit (Bio 101, Inc., La Jolla, Calif.) or by a QIAquick kit (Qiagen). Plasmids and recombinants used or constructed in this study are summarized in Table 1.
TABLE 1. Bacterial strains and plasmids used in this study

<table>
<thead>
<tr>
<th>Strain or plasmid</th>
<th>Relevant characteristic(s)</th>
<th>Source or reference</th>
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</thead>
<tbody>
<tr>
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<td></td>
<td></td>
</tr>
<tr>
<td>KC583</td>
<td>Wild-type strain</td>
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</tr>
<tr>
<td>CR1, 2, 6, 8, 9</td>
<td>KC583 GyrB1, Gly124&lt;sup&gt;-&lt;/sup&gt;, Cac</td>
<td>This study</td>
</tr>
<tr>
<td>CR4, 7, 11, 12</td>
<td>KC583 GyrB1&lt;sup&gt;-Arg164&lt;/sup&gt;, Gly124&lt;sup&gt;-&lt;/sup&gt;, Cac</td>
<td>This study</td>
</tr>
<tr>
<td>CR3</td>
<td>KC583 GyrB1&lt;sup&gt;-Thr214&lt;/sup&gt;, Gly124&lt;sup&gt;-&lt;/sup&gt;, Cac</td>
<td>This study</td>
</tr>
<tr>
<td>CR5, 10</td>
<td>KC583 GyrB1&lt;sup&gt;-Thr214&lt;/sup&gt;, Gly124&lt;sup&gt;-&lt;/sup&gt;, Cac</td>
<td>This study</td>
</tr>
<tr>
<td>E. coli</td>
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<td></td>
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<tr>
<td>HB101</td>
<td>Host strain used for cloning</td>
<td>6, Promega</td>
</tr>
<tr>
<td>TOP10&lt;sup&gt;f&lt;/sup&gt;</td>
<td>TOPO TA Cloning Kit host strain</td>
<td>Invitrogen</td>
</tr>
<tr>
<td>N99</td>
<td>Complementation analysis &lt;sup&gt;strA&lt;/sup&gt; &lt;sup&gt;galK&lt;/sup&gt;</td>
<td>30</td>
</tr>
<tr>
<td>N4177</td>
<td>Isogenic to N99 except gyrB221&lt;sup&gt;+&lt;/sup&gt; (Cac&lt;sup&gt;+&lt;/sup&gt;) and gyrB203 (TS)</td>
<td>30</td>
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<tr>
<td>Plasmids</td>
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<td>pBK-CMV</td>
<td>Phagemid cloning vector</td>
<td>Stratagene</td>
</tr>
<tr>
<td>pCR1-TOPO</td>
<td>Cloning vector</td>
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</tr>
<tr>
<td>pGYRB1</td>
<td>pBK-CMV recombinant containing 5&lt;sup&gt;'&lt;/sup&gt; portion of &lt;i&gt;B. bacilliformis&lt;/i&gt; gyrB in an ~2,000-bp Sau3AI fragment; derived from λ-ZAP library</td>
<td>This study</td>
</tr>
<tr>
<td>pGYRB2</td>
<td>pBK-CMV recombinant with an 13-kb SacI fragment containing the &lt;i&gt;B. bacilliformis&lt;/i&gt; gyrB; derived from λ-GEM 11 library</td>
<td>This study</td>
</tr>
<tr>
<td>pGYRB3</td>
<td>pCR1-TOPO recombinant containing entire gyrB gene in a 2,410-bp BamH1 fragment; derived from TA cloning strategy</td>
<td>This study</td>
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</table>

**PCR and oligonucleotides.** PCR amplifications were achieved by using a GeneAmp 2400 Thermocycler (Perkin-Elmer, Norwalk, Conn.) following procedures developed by Mullis et al. (35). Reaction mixtures contained 10 mM Tris-HCl (pH 8.3), 50 mM KCl, a 200 μM concentration of each denucleotide triphosphate, 4 mM MgCl2, 2.5 U of AmpliTag DNA polymerase (Roche Molecular Systems, Branchburg, N.J.), 1 to 100 ng of template DNA, and 0.1 μg of each primer. The reactions were conducted for 30 cycles of 1 min at 94°C, 1 min at 50 to 60°C (depending on calculated primer melting temperature), and 1 min at 72°C, with an initial 5-min denaturation at 94°C and a final 7-min extension at 72°C. Single-stranded degenerate oligonucleotide primers (based on regions of conserved homology [21]) were synthesized with synthetic oligonucleotides prepared with a DNA synthesizer (model 394; Applied Biosystems, Foster City, Calif.). The nucleotide sequences for both DNA strands of the gyrB gene were determined by the dideoxy chain-termination method of Sanger et al. (41) using a Taq DyeDeoxy Terminator Cycle Sequencing Kit as per the manufacturer's instructions (Applied Biosystems). Sequencing was done on an Applied Biosystems Automated DNA Sequencer (model 373A). Sequence data were compiled and analyzed by using PC/GENE 6.8 software (IntelliGenetics, Mountain View, Calif.) for restriction site determination and ORF identification, BLAST (1) for database searches, CLUSTAL W 1.6 (45) for multiple sequence alignments, and BOXSHADE 3.2 (18) for sequence alignment formatting.

**Results.**

**Cloning the gyrB gene.** Two clones were required for sequence analysis of this gene. A positive plaque with the cloned <i>B. bacilliformis</i> gyrB gene was isolated from a λ-ZAP Express Library (Stratagene) by probing with a [α-<sup>32</sup>P]dCTP-labeled 300-bp PCR product generated from <i>B. bacilliformis</i> KC853 template DNA by using the degenerate oligonucleotide primers GYRB5 and GYRB3. A pBK-CMV phagemid clone was excised from the λ-ZAP Express clone and termed pGYRB1. Nucleotide sequence analysis revealed that only the 5<sup>'</sup> portion (1094 bp) of the gyrB gene was present in the ~2,000-bp Sau3AI insert of pGYRB1.

To obtain the remainder of the sequence for gyrB, the 1,101-bp HindIII fragment of pGYRB1 containing the 5<sup>'</sup> portion of the gyrB gene was labeled by random primer extension and used to probe a λ-GEM 11 genomic library (Promega) in hopes of obtaining a λ clone with a larger insert containing the entire gyrB gene. A second λ clone was identified and found to contain the entire gyrB gene in an ~13-kb SacI fragment by DNA hybridization. The SacI fragment was excised and cloned into pBK-CMV to generate pGYRB2. The insert in pGYRB2 was used to complete the nucleotide sequencing of the wild-type <i>B. bacilliformis</i> gyrB gene.

The complete gyrB gene (2,410 bp) was amplified from <i>B. bacilliformis</i> KC853 DNA by using the amplifier set GYRB-
F–GYRB-R and cloned into pCR2.1-TOPO. This gyrB recombinant was designated pGYRB3.

Nucleotide sequence of the gyrB gene. The nucleotide sequence of the wild-type (coumermycin A₁-sensitive) *B. bacilliformis* gyrB gene was determined from both DNA strands and is presented in Fig. 1. Computer-assisted analysis of the gyrB gene showed a 2,079-bp ORF. This ORF is characterized by a common initiation codon, ATG, that is preceded by putative −35 (TTCTAAA) and −10 (GATAAT) consensus regulatory elements and a potential ribosomal binding site (AGTA) (Fig. 1).

Further analysis of the ORF indicated that the encoded protein had a deduced length of 692 amino acid residues and a predicted molecular mass of approximately 77.5 kDa. BLAST (1) homology searches indicate that *B. bacilliformis GyrB* is most similar to *Bacillus subtilis GyrB*, with an amino acid sequence identity of 40.1%, whereas *B. bacilliformis GyrB* has only 18.4% identity with *B. subtilis ParC*, a GyrB homolog. The *B. bacilliformis* subunit has 34.1% identity with *E. coli GyrB*. Alignment of the deduced amino acid sequence from *B. bacilliformis gyrB* with the known amino acid sequences of GyrBs from *E. coli*, *B. subtilis*, and *Mycobacterium tuberculosis* (using CLUSTAL W 1.6) indicates multiple areas of strong homology (Fig. 2) and reveals that the *B. bacilliformis* GyrB has an unusually long N terminus. Sequence analysis of ~600 bp of flanking sequence indicate a possible gene upstream of gyrB with homology to lipote-protein ligase B, whereas 3’ flanking sequence produces no areas of strong homology to database sequences (data not shown).

DNA hybridization analysis. In order to verify that the gyrB-containing fragment was of *Bartonella* origin, DNA hybridization analysis was done with BamHI-digested DNA from *B. bacilliformis* strains KC583 and KC584 and from *E. coli* HB101. As shown in Fig. 3B, Southern blots probed at high stringency (7% mismatch) with a 32P-labeled 2,410-bp PCR fragment derived from *B. bacilliformis* KC583 template (by using amplimers GYRB-F and GYRB-R) clearly demonstrated single hybridization bands from both strains of *B. bacilliformis* (Fig. 3B, lanes 3 and 4). No signal was observed in BamHI-digested *E. coli* HB101 DNA (Fig. 3, lane 2). In addition, the G+C content of the ORF (38.4 mol%) is in good agreement with the overall G+C content (39 mol%) of *B. bacilliformis* (7).

In vitro expression of gyrB. To determine if *E. coli* transcription-translation machinery would express the cloned *B. bacilliformis gyrB*, an *E. coli* S30 cell DNA expression kit (Promega) was used to produce polypeptides in vitro. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis of proteins expressed from pGYRB3 revealed a protein product consistent with the predicted molecular mass for GyrB of 77.5 kDa that was not expressed from the pCR2.1-TOPO control by this system (data not shown). The 77.5-kDa protein was the largest protein encoded, although additional insert-specific protein bands of approximately 68, 65, 52, and 38 kDa were observed and may have been produced by the *E. coli* S30 extract from abnormal ORFs on the noncoding strand of pGYRB3 or may be degradation products.

Functional complementation analysis. Since the S30 extract expressed the cloned gyrB, an isogenic pair of *E. coli* strains first described by Menzel and Gellert (30) was used to evaluate the in vivo function of the cloned *B. bacilliformis gyrB*. *E. coli* N99 carries a wild-type gyrB, and strain N4177 has two gyrB mutations, which together confer a coumermycin A₁-resistant (Coul) and temperature-sensitive (TS) phenotype. Growth of strain N4177 is permissive at 30°C but is restricted at 42°C unless a functional gyrB is supplied in trans to complement the Coul TS mutation. Therefore, we wanted to determine whether *B. bacilliformis gyrB* could functionally complement strain N4177. To address this question, the *B. bacilliformis gyrB* recombinant pGYRB3 was introduced into strains N99 and N4177, selected at 30°C, and subsequently replica plated and separately incubated at both permissive (30°C) and restrictive (42°C) temperatures. The *B. bacilliformis gyrB* recombinant, pGYRB3, was shown to increase the growth rate of strain N4177 at 42°C by approximately threefold relative to negative controls (Table 2). The presence of plasmids or varied incubation temperature did not affect the relative growth rates of host strain N99. The pattern of growth for this analysis was consistent and reproducible and shows that *B. bacilliformis gyrB* can functionally complement the Coul TS mutation of *E. coli* N4177.

Isolation of coumermycin A₁-resistant mutants. Spontaneous coumermycin A₁-resistant mutants were observed 7 days after inoculation and occurred at a frequency of ~6 × 10<sup>−9</sup> when selected in the presence of 0.1 μg of coumermycin A₁ per ml. After initial selection, mutant strains were cultured on heart infusion agar supplemented with 0.04 μg of coumermycin A₁ per ml. A total of 12 *B. bacilliformis* KC583 coumermycin-resistant mutants were selected in this manner and designated CR1 through CR12 (Table 1). In the absence of coumermycin A₁, the growth rate and gross morphology of the Coul colonies were indistinguishable from those of wild-type strains.

Coumermycin A₁ resistance is correlated with mutations in the gyrB gene. Genomic DNA was isolated from wild-type *B. bacilliformis* KC583 and the 12 coumermycin A₁-resistant mutants. The region of the gyrB gene encoding the N-terminal domain was amplified by PCR with LESION-F and LESION-R primers and subsequently sequenced with the LESION-F primer. Further analysis of these sequences revealed single nucleotide transitions at three separate loci that resulted in four distinct amino acid substitutions. First, in 5 of the 12 coumermycin A₁-resistant strains (CR1, CR2, CR6, CR8, and CR9), identical G-to-A transitions at base 370 of the 2,079-bp ORF resulted in a deduced Gly124-to-Ser (Gly124→Ser) substitution. Second, 4 of the 12 resistant strains (CR4, CR7, CR11, and CR12) carried a G-to-A transition at base 550 that resulted in a deduced Arg184-to-Gln substitution. The third loci at which lesions were detected occurred in the Thr214 codon, in which two different transitions were observed with two distinct deduced substitutions; the ACA-to-ATA transition resulted in a Thr214→Ala substitution (CR3), whereas the ACA-to-ATA transition resulted in a Thr214→Ile substitution (CR5, CR10). These data demonstrate that spontaneous coumermycin A₁-resistant mutants are correlated with specific and localized lesions in the gyrB gene. Table 3 summarizes several genotypic and phenotypic attributes of the coumermycin A₁-resistant strains.

In vitro coumermycin A₁ susceptibilities. We assessed the antibiotic susceptibility of wild-type *B. bacilliformis* KC583 to coumermycin A₁ by using agar dilution techniques. At coumermycin A₁ concentrations above 0.03 g/ml, growth rates were noticeably decreased, and at those above 0.06 μg/ml, growth appeared to be completely inhibited. Thus, the MIC for KC583 was determined to be 0.06 μg/ml. One representative of each of the four different gyrB mutant types was assayed for coumermycin A₁ susceptibility. MICs for mutant strains CR3, CR4, and CR9 were 0.2 μg/ml, whereas CR5 demonstrated a slightly higher level of resistance, with a MIC of 0.3 μg/ml (Table 3).

**DISCUSSION**

We have described the first isolation and molecular characterization of spontaneous mutant strains conferring natural
FIG. 1. Nucleotide and predicted amino acid sequence of \textit{B. bacilliformis} gyrB. The nucleotide sequence of a 2,250-bp fragment containing the wild-type coumermycin A1-sensitive \textit{B. bacilliformis} gyrB is shown. Nucleotides within the 2,079-bp ORF are given in uppercase letters, and the deduced 692-residue amino acid sequence is shown below each corresponding codon. Putative consensus regulatory elements are indicated (235, 210, ribosomal binding site [RBS]). The stop codon is marked with an asterisk. The three codons (and their corresponding amino acids) in which single nucleotide substitutions resulting in coumermycin A1 resistance were found are boxed. The unusually long 52-residue N terminus is shown in boldface type. The predicted molecular mass of the mature protein is 77.5 kDa. The GenBank accession number for the gyrB gene is U82225.
resistance to an antibiotic for any Bartonella species. Generation of the mutant strains was accomplished by exposure to inhibitory (0.1—mg/ml) levels of the DNA gyrase inhibitor coumermycin A1 and occurred at a frequency of $6 \times 10^{-9}$.

Based upon amino acid sequence alignments, B. bacilliformis GyrB belongs to the shorter, 650-amino-acid size class represented by homologs of enzymes from B. subtilis, Mycoplasma pneumoniae, Staphylococcus aureus, Borrelia burgdorferi, and...
Halofex sp. (20). In the larger, 800-amino-acid size class, represented by *E. coli*, an extra 150-amino-acid block is found in the C-terminal domain of the protein (20) (Fig. 2). The commonly recognized ATP binding motif GXXGXG is found at positions 162 to 167 of *B. bacilliformis* GyrB, corresponding to positions 114 to 119 of *E. coli* GyrB.

The structure of the *B. bacilliformis* GyrB is unusual in two ways. First, in GyrBs sequenced to date, the first N-terminal amino acid that demonstrates universal conservation throughout bacteria is a Tyr residue represented by *E. coli* Tyr5, corresponding to Tyr53 of *B. bacilliformis* (Fig. 2). The side chain of Tyr5 hydrogen bonds to the bound ATP analog (48). The number of amino acids preceding this conserved Tyr is less than 13 for nearly all bacteria examined to date. *B. bacilliformis* GyrB is unusual in this respect in that 52 amino acid residues precede the *E. coli* Tyr5 homolog, making it the longest N-terminal extension reported to date. Only *M. tuberculosis* has an N-terminal extension of this magnitude, with 50 amino acids (26). The crystal structure of the *E. coli* GyrB N-terminal domain complexed with a nonhydrolyzable ATP analog shows that the N-terminal 13 residues form a protrusion that interacts with the other GyrB protomer (48). This interaction stabilizes the dimer interface and forms part of the ATP binding site (48). However, the N terminus is apparently not ordered in the cocystal structure with the coumarin inhibitor novobiocin (26). The function of the unusually long N-terminal extensions of *M. tuberculosis* and *B. bacilliformis* GyrBs is intriguing and remains to be determined. A second primary structural feature of *B. bacilliformis* GyrB that we have noted is Glu128 (*E. coli* equivalent, Gly81). In all wild-type GyrBs reported thus far, this residue is either glycine or aspartate, with the exception of those found in the *Mycobacteria*, which have alanine or glutamate at this position. In this respect, *B. bacilliformis* GyrB is also more similar to the mycobacterial GyrB. This position is one of three loci that is mutated in a novobiocin-resistant *Halofex* (Asp82→Gly) (19), although it is distant from the coumarin binding site (26). Although both *B. bacilliformis* and *M. tuberculosis* are slow-growing bacteria and have several similar GyrB structural features, the effect of these properties on interactions with ATP or coumarins is unknown.

The mechanism of coumermycin A₁ resistance in *B. bacilliformis* mutants was identified by sequencing PCR fragments generated with primers amplifying the portion of the gyrB gene that encodes the N-terminal domain. We have isolated 12 coumermycin A₁-resistant mutants and have identified single nucleotide transitions at three separate loci resulting in single amino acid substitutions in the N-terminal domain of the GyrB protein. Lesions detected in the resistant *B. bacilliformis* gyrB genes are analogous in location and residue substitution to previously characterized resistant gyrB genes (11, 13, 19, 39, 40, 44). The crystal structure has revealed important interactions for each of the lesion sites. First, the side group of the *E. coli* Arg136 residue (*B. bacilliformis* Arg184) makes critical hydrogen bonds with the coumarins and with *E. coli* Tyr5 (B. bacilliformis Tyr53) on the other protomer (which is involved in ATP binding) (26). The second and third residues associated with coumarin resistance, *E. coli* Gly77 (*B. bacilliformis* Gly124) and *E. coli* Thr165 (*B. bacilliformis* Thr214), specifically interact with each other as well as stabilize interactions with ATP and coumarins (26).

These data demonstrate that the *B. bacilliformis* DNA gyrase B protein is a target for coumarin antibiotics. Wild-type *B. bacilliformis* (MIC, 0.06 μg/ml) was shown to be more susceptible to growth inhibition by coumermycin A₁ than almost all other bacteria tested (50) and is 250 times more susceptible than *E. coli* (17). These data are consistent with the finding that *Bartonella* is extremely susceptible to a variety of antibac-

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**TABLE 2. Complementation with *B. bacilliformis* gyrB**

<table>
<thead>
<tr>
<th><em>E. coli</em> strain</th>
<th>Growth at 42°C with the following plasmid*</th>
<th>None</th>
<th>pCR2.1-TOPO</th>
<th>pGYRB3</th>
</tr>
</thead>
<tbody>
<tr>
<td>N99</td>
<td>+++</td>
<td>+++</td>
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<td>+/−</td>
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<td>+++</td>
</tr>
</tbody>
</table>

* Growth is based upon colony size and logarithmic growth kinetics as measured by optical density at 600 nm. The temperature chosen (42°C) is the restrictive temperature for N4177 (30). Symbols: ++++, robust growth; +/−, slight growth.
material agents in vitro (27). The mutant strains demonstrated an approximately fivefold increase in resistance levels. The MICs for GyrB mutants represented by strains CR3, CR4, and CR9 were determined to be 0.2 μg/ml, whereas the MIC for CR5 was 0.3 μg/ml. This suggests that a Thr214—Ile substitution confers a higher level of resistance than Thr214—Ala, Gly124—Ser, or Arg184—Glu, consistent with findings in B. burgdorferi (40).

The transition between the diverse thermal environments of the arthropod vector and the human host, as well as the presentation of the verruga peruana on the extremities (<37°C), suggests that there is a close relationship between temperature and gene expression in B. bacilliformis. Yersinia enterocolitica DNA gyrase mutants simulate thermoinduced alterations of DNA supercoiling with coincident phenotypic changes (38). Likewise, DNA topology regulated by DNA gyrase may play an important role in the survival or virulence of B. bacilliformis in both the vector and host, and DNA gyrase mutants may provide a method for analysis of thermoregulation.

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