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DNA Cloning and Organization of the *Staphylococcus aureus gyrA* and *gyrB* Genes: Close Homology among Gyrase Proteins and Implications for 4-Quinolone Action and Resistance

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***Staphylococcus aureus gyrA* and *gyrB* genes, which encode the DNA gyrase A and B proteins, have been isolated and found to map contiguously. DNA sequence analysis revealed close homology between the *S. aureus* gyrase subunits and their counterparts in *Bacillus subtilis* and *Escherichia coli*, including several conserved amino acid residues whose substitution in *E. coli* confers resistance to 4-quinolones. These results are discussed in regard to quinolone resistance mechanisms in *S. aureus*.**

Staphylococcal infections, particularly those involving methicillin-resistant *Staphylococcus aureus*, are a serious medical problem (17). Treatment with ciprofloxacin and other 4-quinolones, which are inhibitors of the essential bacterial enzyme DNA gyrase (7, 35), has been effective but has led to the widespread emergence of quinolone-resistant *Staphylococcus* strains (14, 23, 25–27).

Studies with *Escherichia coli* have shown that quinolones rapidly arrest DNA synthesis in vivo and inhibit ATP-dependent DNA supercoiling by purified DNA gyrase (3, 6, 8). Mutations conferring quinolone resistance in *E. coli* map largely in the unlinked *gyrA* or (less often) *gyrB* genes, which encode the A and B subunits of the A_2B_2 gyrase complex (2, 4, 6, 11, 36, 37). The gyrase A subunits are known to mediate transient DNA breakage and reunion during catalysis, a process interrupted by 4-quinolones, whereas the B subunits bind and hydrolyze ATP, providing the energetic requirement for DNA supercoiling (12, 19, 29, 34). Although *S. aureus* DNA gyrase has been partially purified by us and by others (31), abundant protease and nuclease activities present in crude extracts have hampered the study of the enzyme and its role in quinolone action and resistance (our unpublished results). We have therefore followed a genetic approach to the study of *S. aureus* gyrase, and we describe here the isolation and initial sequence characterization of the *S. aureus gyrA* and *gyrB* genes.

For these studies, we used *S. aureus* 81231, a methicillin-sensitive coagulase-positive clinical isolate taken from the neck wound of a patient at this hospital in 1987. The strain was susceptible to ciprofloxacin: there was no growth on brain heart infusion medium plates containing the drug at 0.5 μg/ml. Genomic DNA from the strain (16) was found to hybridize by Southern blotting on Hybond-N filters (28) to a 1.7-kilobase (kb) *Bam*HI-*Sac*I DNA fragment from within the *Bacillus subtilis gyrB* gene (21), which was isolated from plasmid pML2 (15) and radiolabeled by random priming with the Amersham Multiprime labeling kit (Fig. 1). Filters were probed under low-stringency conditions: hybridization was at 65°C in a solution containing 750 mM NaCl and 75 mM sodium citrate and filters were then washed twice at 65°C in a solution containing 300 mM NaCl and 30 mM sodium citrate, each time for 30 min. This Southern blot and others (data not shown) yielded a restriction map for the *gyrB* homolog in *S. aureus* 81231 (Fig. 2). Moreover, the absence of hybridization to *E. coli* DNA (Fig. 1) allowed the cloning of the 2.4-kb *Eco*RI-*Hin*dIII fragment from a plasmid library of 81231 DNA in *E. coli*.

S. aureus 81231 DNA was digested with *Eco*RI and *Hin*dIII, and fragments were separated by electrophoresis in a 0.8% agarose gel. DNA in the 2.4-kb size range was isolated by electrophoresis onto NA45 paper (Schleicher & Schuell, Inc.), ligated into plasmid pBR322, cut with *Eco*RI and *Hin*dIII, and used to transform *E. coli* DH5 *recA* by the FSII method of Hanahan (10). Ampicillin-resistant transformants were screened (following plasmid amplification on chloramphenicol plates) by low-stringency colony hybridization to the *B. subtilis gyrB* probe (9). Of the 35 strongly positive clones found in the library of 2,400 transformants, 7 were analyzed and found to contain pBR322 bearing the desired 2.4-kb insert.

The insert from one plasmid, pRH1, was 3′ end labeled with ^{32}P at its *Eco*RI or *Hin*dIII ends or at internal *Cla*I or *Xba*I sites, and the labeled strands were partially sequenced by the method of Maxam and Gilbert (18). Open reading frames deduced from DNA sequences at the *Eco*RI, *Xba*I, and 5′ *Cla*I sites (Fig. 2) (PCGENE software package; IntelliGenetics, Inc.) exhibited strong homology to carboxy-terminal sequences of gyrase B protein from both *B. subtilis* and *E. coli* (1, 21, 36) (data not shown). On the other hand, amino acid sequences deduced from DNA sequence at the *Hin*dIII and 3′ *Cla*I sites were closely homologous to the N terminus of the *B. subtilis* and *E. coli* gyrase A subunits (21, 30, 37) (see below). These results indicated that the *gyrB* and *gyrA* genes are contiguous in *S. aureus*, as in *B. subtilis*, another gram-positive bacterium (15, 21). Because of the close similarity to *B. subtilis* genes, the likely extent of the *S. aureus gyrB* and *gyrA* genes could be estimated (Fig. 2). High-stringency screening of appropriate size-selected *S. aureus* DNA libraries in plasmid pUC19 with the 2.4-kb insert from pRH1 led to the isolation of plasmids pRH2 and pRH3, which carry the overlapping 3.5-kb *Xba*I-*Eco*RI and 6.5-kb *Hin*dIII fragments that span the *gyrB-gyrA* locus (Fig. 2).

To confirm the linkage of *S. aureus gyrB* and *gyrA* genes and to provide structural information on the functionally important N-terminal end of the gyrase A protein, we

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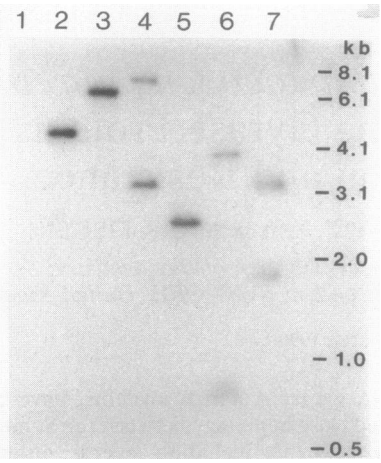


FIG. 1. Southern hybridization of *S. aureus* DNA to a *B. subtilis gyrB* probe. Genomic DNA from *S. aureus* clinical isolate 81231 (lanes 2 through 7) or *E. coli* K-12 strain RW1053 (lane 1) was digested with various restriction enzymes: *Eco*RI (lanes 1 and 2), *Hin*dIII (lane 3), *Xba*I (lane 4), *Eco*RI-*Hin*dIII (lane 5), *Eco*RI-*Xba*I (lane 6), or *Hin*dIII-*Xba*I (lane 7). DNA samples were electrophoresed in a 0.8% agarose gel and transferred to a nylon filter, and the blot was hybridized to a *B. subtilis gyrB* probe under low-stringency conditions prior to autoradiography. The positions of size markers are shown on the right.

sequenced a 630-base-pair (bp) intergenic region that overlaps the 3′ and 5′ ends of the *gyrB* and *gyrA* genes, respectively (Fig. 2 and 3). Two deduced amino acid sequences were found in the same reading frame separated by a short 39-bp region. These sequences were very closely homologous to the C-terminal end of gyrase B protein and to the N-terminal end of gyrase A protein in both *B. subtilis* and *E. coli* (1, 21, 30, 36, 37) (Fig. 4). The 52-amino-acid segment of the *S. aureus* gyrase B protein is identical to its *B. subtilis* and *E. coli* counterparts at 36 and 32 positions, i.e., 68 and 60% identity, respectively. Even closer homology is seen among the gyrase A protein sequences (Fig. 4B): 111 and 96 positions of the 146-amino-acid *S. aureus* sequence share

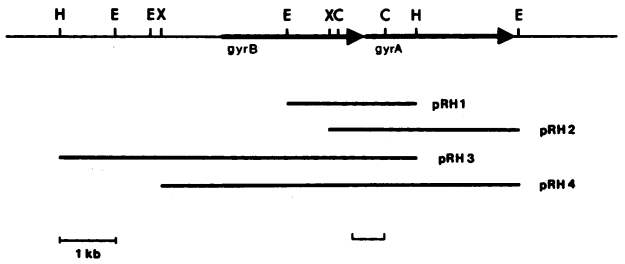
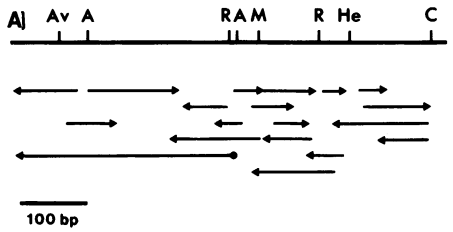


FIG. 2. Restriction map of the *gyrB-gyrA* locus in *S. aureus* clinical isolate 81231 and alignment of plasmid clones. The *gyrB* and *gyrA* genes are indicated by heavy lines. E, H, and X, restriction sites for *Eco*RI, *Hin*dIII, and *Xba*I. Two *Cla*I sites (C) within a 2.4-kb *Eco*RI-*Hin*dIII segment are also shown. The gyrase genes were localized and restriction maps were determined by Southern blotting of appropriately digested genomic DNA by using a *B. subtilis gyrB* probe. Plasmids RH1, RH2, and RH3 carrying the indicated genomic DNA inserts were isolated from size-selected plasmid libraries by colony hybridization to the *B. subtilis gyrB* probe (pRH1) or the pRH1 insert (pRH2 and pRH3). The insert in plasmid pRH4 was constructed by ligating the 3.1-kb *Xba*I insert fragment of pRH3 into pRH2 cut with *Xba*I. ⌊⌋, Region selected for sequence analysis (Fig. 3).



B) GYRB->

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...GluThrThrMetAsnProGluHisArgAlaLeuLeuGlnValLysLeuGluAspAla
...GAAACAACAATGAACCCTGAGCACCGCGCTCTTTTACAAGTAAAACTTGAAGATGCG

IleGluAlaAspGlnThrPheGluMetLeuMetGlyAspValValGluAsnArgArgGln
ATCGAAGCGGACCAAACATTTGAAATGTTAATGGGTGACGTTGTAGAAAACCGTAGACAA

PheIleGluAspAsnAlaValTyrAlaAsnLeuAspPhe***      ***
TTTATAGAAGATAATGCAGTTTATGCAAACTTAGACTTCTAAGCGCTGTGAACTGAACTT

                    GYRA->
                    METAlaGluLeuProGlnSerArgIleAsnGluArgAsnIle
TTGAAGGAGGAACTCTTGATGGCTGAATTACCTCAATCAAGAATAAATGAACGAAATATT
      SD                    1

ThrSerGluMetArgGluSerPheLeuAspTyrAlaMetSerValIleValAlaArgAla
ACCAGTGAAATGCGTGAATCATTTTTAGATTATGCGATGAGCGTTATCGTTGCTCGTGCA
                2

LeuProAspValArgAspGlyLeuLysProValHisArgArgIleLeuTyrGlyLeuAsn
TTGCCAGATGTTCGTGACGGTTTAAAACCAGTACATCGTCGTATACTATATGGATTAAAT

GluGlnGlyMetThrProAspLysSerTyrLysLysSerAlaArgIleValGlyAspVal
GAACAAGGTATGACACCGGATAAATCATATAAAAAATCAGCACGTATCGTTGGTGACGTA

MetGlyLysTyrHisProHisGlyAspSerSerIleTyrGluAlaMetValArgMetAla
ATGGGTAAATATCACCCTCATGGTGACTCATCTATTTATGAAGCAATGGTACGTATGGCT

GlnAspPheSerTyrArgTyrProLeuValAspGlyGlnGlyAsnPheGlySerMetAsp
CAAGATTTCAGTTATCGTTATCCGCTTGTTGATGGCCAAGGTAACTTTGGTTCAATGGAT

GlyAspGlyAlaAlaAlaMetArgTyrThrGluAlaArgMetThrLysIleThrLeuGlu
GGAGATGGCGCAGCAGCAATGCGTTATACTGAAGCGCGTATGACTAAAATCACACTTGAA

LeuLeuArgAspIleAsnLysAspThrIleAspPhe.....
CTGTTACGTGATATTAATAAAGATACAATAGATTTT.....
```

FIG. 3. DNA sequence analysis of an intergenic 630-bp DNA region from *S. aureus* spanning the 3′ and 5′ ends of the *gyrB* and *gyrA* genes. (A) DNA sequencing strategy. A, Av, C, He, M and R, sites for *Acc*I, *Ava*II, *Cla*I, *Hae*III, *Msp*I, and *Rsa*I, respectively. DNA restriction fragments were 5′ end labeled with ^{32}P and sequenced by the method of Maxam and Gilbert (18). DNA sequence from one *Rsa*I site was obtained by the dideoxy chain termination technique (24). (B) Nucleotide sequence of the DNA region and the predicted protein sequence of the longest open reading frame. *, Stop codons; SD, potential Shine-Dalgarno sequence. Two short inverted repeat sequences (numbered 1 and 2) are underlined; hairpin structures involving these sequences in RNA have low calculated free energies of formation (32).

identity with the respective *B. subtilis* and *E. coli* proteins, i.e., 76 and 66% identity. These data establish that we have isolated genes encoding the *S. aureus* gyrase A and B proteins. This study supports the proposal that linkage of *gyrB* and *gyrA* genes may be the normal arrangement in bacteria, with unlinked genes in *E. coli* being the exception (22). We have yet to determine whether the *S. aureus gyrA* and *gyrB* genes are in the replication origin region, as in *B. subtilis* (22).

The *S. aureus* gyrase A and B sequences contain a number of extended peptide homologies that deserve comment (Fig. 4B). First, a highly conserved sequence, Ala-Ala-Ala-Met-

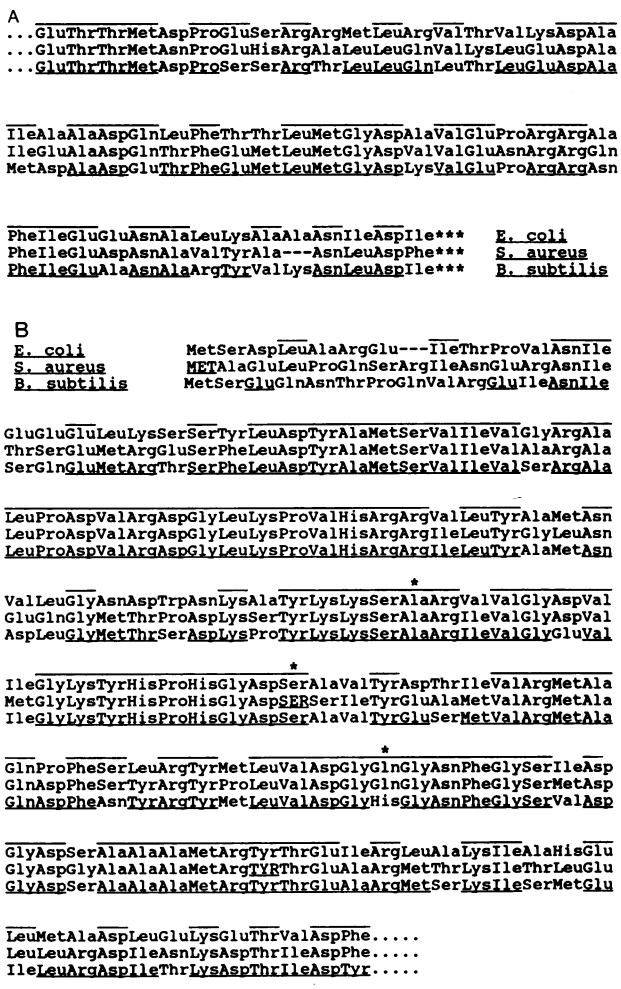

FIG. 4. Sequence comparison of *E. coli*, *S. aureus*, and *B. subtilis* gyrase B (C-terminal sequences) (A) and gyrase A (N-terminal sequences) (B) proteins. Positions of amino acid identity between the *E. coli* and *B. subtilis* gyrase proteins and those of *S. aureus* are indicated by overlining and underlining, respectively (1, 30, 36, 37). In panel B, the conserved catalytic tyrosine, Tyr-122, is indicated in capitals (13). *, Residues in the gyrase A protein, e.g., Ser-83, whose substitution confers resistance to quinolones in *E. coli* (2, 37).

Arg-Tyr-Thr-Glu, has been shown in the *E. coli* A protein to carry the catalytic tyrosine, Tyr-122, responsible for transient DNA scission via a DNA-phosphotyrosine intermediate (13).

Second, there is conservation of five residues in the gyrase A and B subunits whose substitution in *E. coli* is responsible for bacterial resistance to nalidixic acid and the 4-quinolones; these residues are Ala-67, Ser-83, and Gln-106 in the gyrase A protein (Fig. 4B, asterisks) and the gyrase B residues Asp-426 and Lys-447 (data not shown) (2, 35, 37). Mutation at codon 83 of the gyrase A protein has been shown frequently to confer high-level quinolone resistance in *E. coli* (2, 5, 35), whereas changes at the other positions result in low-level resistance. Interestingly, the equivalent residues were identical in the *S. aureus* and *E. coli* proteins, with the exception of Lys-447, which in the gram-positive proteins is replaced by arginine, a residue which is almost isosteric with lysine and which preserves its positive charge. Although the mechanisms underlying quinolone resistance in *S. aureus* are currently unknown, these sequence homologies (particularly at and around Ser-83) suggest that *E. coli* and *S. aureus* may share similar mutational pathways involving gyrase that result in quinolone resistance. Recently, Ubukata et al. have cloned a DNA fragment which is responsible for fluoroquinolone resistance in an *S. aureus* clinical isolate and which hybridizes to *E. coli gyrA* and *gyrB* probes on Southern blots (33). This observation and the demonstration here that the *S. aureus gyrA* and *gyrB* genes are indeed linked suggest that quinolone resistance in *S. aureus* can involve gyrase genes.

Finally, the linkage of *S. aureus gyrB* and *gyrA* genes could permit coordinate control of expression through a polycistronic message. The linked *gyrB* and *gyrA* genes in *B. subtilis* are transcribed separately, as shown by S1 mapping analysis: the 213-bp intergenic region carries a perfect 14-bp inverted repeat just 3′ of the *gyrB* gene which presumably functions as a transcription termination signal (21). By comparison, computer analysis of the 39-bp intergenic region in *S. aureus* did not reveal any strong transcription termination signal (20, 32). Second, although there is a potential Shine-Dalgarno sequence, appropriately located 5′ of the *gyrA* gene (Fig. 3), from which translation of *gyrA* message could take place, no promoter sequences are apparent. Thus, the lack of obvious transcription initiation and termination signals in the intergenic region admits the possibility of coordinate control. The availability of the cloned *gyrB* and *gyrA* genes will enable their expression and other aspects of gyrase function in *S. aureus* to be elucidated.

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