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Cloning, Sequencing, and Expression of the DNA Gyrase Genes from *Staphylococcus aureus*

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We have isolated and cloned the *gyrA* and *gyrB* genes from *Staphylococcus aureus*. These adjacent genes encode the subunits of DNA gyrase. The nucleotide sequence of a 5.9-kb region which includes part of an upstream *recF* gene, the whole of *gyrB* and *gyrA*, and about 1 kb of unknown downstream sequence has been determined. The *gyrB* and *gyrA* gene sequences predict proteins of 886 and 644 amino acid residues, respectively, which have significant homologies with the gyrase subunits of *Escherichia coli* and *Bacillus subtilis*. Residues thought to be important to the structure and function of the subunits are conserved. These genes have been expressed separately by using a T7 promoter vector. N-terminal sequencing of the cloned gene products suggests that the mature GyrB subunit exists mainly with its initial five residues removed. Protein sequencing also supports the interpretation of our DNA sequencing data, which are inconsistent in several places with the recently published sequence of the same genes (E. E. C. Margerrison, R. Hopewell, and L. M. Fisher, J. Bacteriol. 174:1596–1603, 1992).

DNA gyrase introduces negative supercoils into bacterial DNA (9). The DNA gyrase enzyme consists of an A_2B_2 tetramer (15, 16) in which the A subunit is responsible for the double-stranded breakage and reunion of DNA (35) while the B subunit mediates energy transduction via ATP hydrolysis (20, 34). DNA supercoiling affects the processes of DNA replication, recombination, and transcription (23, 31, 40). These physiological effects make gyrase essential to cell viability; two classes of antibacterial drugs, the 4-quinolones (6) and the coumarins (10), act by inhibiting it. The apparent absence of gyrase activity in eukaryotes and the lack of plasmid-borne resistance in bacteria (5) make DNA gyrase an attractive drug target.

Staphylococcus aureus is an important pathogen, and methicillin-resistant strains (which are often multiply resistant) are becoming more widespread. The latter remain susceptible, albeit with some reduction, to antigyrase drugs (27, 39). The 4-quinolone drugs are generally less active against gram-positive bacteria (including *S. aureus*) than gram-negative ones (28). It is not clear whether this is due to differences in drug accessibility in these bacterial groups or to differences in the structure of their gyrase proteins. One or both of the genes encoding the two subunits of gyrase (*gyrA* and *gyrB*) from a number of species have been sequenced, including *Escherichia coli* (1, 36), *Klebsiella pneumoniae* (8), *Pseudomonas putida* (26), *Neisseria gonorrhoeae* (32), *Mycoplasma pneumoniae* (3), *Haloferax* sp. (12), *Bacillus subtilis* (21), *Streptomyces sphaeroides* (38), and *S. aureus* (13, 19). Without cloning, the gyrase enzyme has also been purified from *Micrococcus luteus* (18) and *Citrobacter freundii* (2). From such studies it can be seen that the gyrase subunits fall into size ranges, with GyrA being about 90 to 100 kDa and GyrB being about 70 to 90 kDa. The proteins from gram-positive or -negative species correlate poorly into separate size (and structure?) classes (see also Reece and Maxwell [28]). This leaves unexplained the differences in antigyrase drug susceptibilities of these bacterial groups.

Although the purification of active DNA gyrase from *S. aureus* has been reported (22, 37), it has proved difficult to repeat (19; this laboratory, unpublished observations). This difficulty has hampered the study of the properties and drug susceptibilities of this enzyme. We embarked, therefore, on the cloning and expression of the gyrase genes from *S. aureus*, making use of the partial DNA sequence data already published (13). After completion of the work presented here, a very similar sequence for the *gyrB* and *gyrA* genes was published, although without any expression or protein sequence data (19). We provide evidence that some of the discrepancies between these two sequences are probably due to reading errors by Margerrison et al. (19), although others may represent minor differences between the gyrase genes of the two bacterial strains used.

MATERIALS AND METHODS

Bacterial strains, plasmids, and media. *E. coli* K-12 DH5α (*endA1 hsdR17 supE44 recA1 gyrA96 relA1* φ80*lacZ*ΔM15 *thi*) was used as a host for pUC vectors (30). BL21(DE3) (*hsdS gal* [λ *imm*^{21} *S*am7 *nin5 lac*UV5-T7 gene 1]) was used for expression of genes cloned under T7 promoter control (33). *S. aureus* 601055 is a methicillin- and quinolone-sensitive clinical isolate. Plasmid pUC19 was used as a cloning vector (42). Other plasmids are described in Table 1. The expression vector pTB361 is described in European patent application no. 92301465.8; it contains the T7 promoter (33). *E. coli* strains were grown in LB medium (30), and *S. aureus* was grown in Iso-Sensitest broth (Oxoid Ltd.). Antibiotics were used at the following final concentrations: ampicillin, 50 μg/ml; tetracycline, 10 μg/ml.

DNA manipulations. DNA was isolated, restricted, and analyzed, and plasmids were constructed by standard methods (30). We also used the Qiagen method for plasmid preparation (Qiagen Inc.), especially for sequencing reactions. DNA was isolated by standard procedures from cul-

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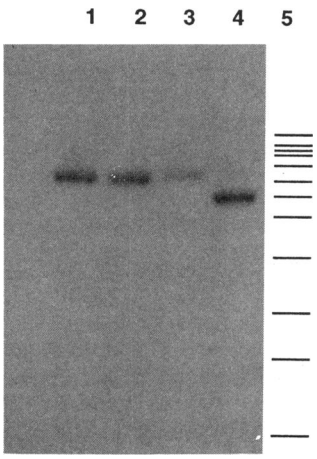

FIG. 1. Southern hybridizations of four clones. Tracks: 1 to 3, DNA from plasmids pTB362, -363, and -364, respectively, restricted with *Hin*dIII; 4, DNA from plasmid pTB365 restricted with *Eco*RI and electrophoresed in an agarose gel; 5, size markers (Bethesda Research Laboratories kilobase ladder). After transfer to a Hybond nylon membrane, the clones were hybridized with the A1 probe and autoradiographed.

tures of *S. aureus* after lysis with lysostaphin (Sigma) at 100 μg/ml.

DNA hybridization. DNA Southern blotting and colony hybridizations were done by using procedures similar to those previously described (30). Colonies were denatured on nitrocellulose filters and UV cross-linked (Stratalinker; 0.12 J). The colonies were then prehybridized at 50°C for 2 h in Blotto buffer and hybridized with ^{32}P-kinase-labelled oligonucleotide probe under the same conditions. They were washed three times with 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) at 50°C, dried, and visualized on an Autograph Beta-scanner or by autoradiography.

Protein analysis and N-terminal sequencing. Protein expression in bacterial cultures was analyzed by polyacrylamide gel electrophoresis and stained with Coomassie brilliant blue R250 (17). N-terminal sequencing was by Edman degradation (Applied Biosystems protein sequencer).

DNA sequencing. DNA was sequenced on both strands, with synthetic oligonucleotide primers on denatured double-stranded plasmid DNA, by the dideoxynucleotide termination method by using the Sequenase version 2.0 kit (U.S. Biochemicals Corp.). Oligonucleotides about 200 bp apart along the template were designed to prime the sequencing reaction. Sequence analysis was by the University of Wisconsin Genetics Computer Group programs (7).

Nucleotide sequence accession number. The nucleotide sequence data reported here have been submitted to the EMBL data base under accession number X71437.

TABLE 1. Plasmids[a]

Plasmid	Description
pTB361	pAT153 replicon; *cer*$^+$ *tetA*$^+$ *tetR*$^+$ (from RP4); T7 promoter (33)
pTB362	pUC19 (6.5-kb *Hin*dIII *S. aureus gyrB* insert B1)
pTB363	pUC19 (6.5-kb *Hin*dIII *S. aureus gyrB* insert B2)
pTB364	pUC19 (6.5-kb *Hin*dIII *S. aureus gyrB* insert B3)
pTB365	pUC19 (5.8-, 5.0-, and 0.9-kb *Eco*RI *S. aureus gyrA* insert A7)
pTB366	pUC19 (5.0-kb *Eco*RI insert recloned from pTB365)
pTB367	pUC19 (2.7-kb *Ssp*I *gyrB* fragment from pTB364)
pTB370	pTB361 (2.7-kb *Ssp*I *gyrB* fragment from pTB367)
pTB373	pTB361 (3.7-kb *Eco*47III-*Kpn*I *gyrA* fragment from pTB366)

[a] Plasmid pTB361 is described in European patent application no. 92301465.8. Construction of the other plasmids is described in the text.

RESULTS

Isolation and screening of clone banks. Chromosomal DNA isolated from *S. aureus* 601055 was digested with various restriction enzymes and ligated into suitably restricted DNA samples of plasmid pUC19. *E. coli* DH5α transformant colonies were selected by using ampicillin on rich medium with X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside). Clone banks were screened by hybridization with two synthetic 30-mer oligonucleotides which match the end of the *gyrB* gene (B1) or the start of the *gyrA* gene (A1) from a previous partial sequence (13). Potential positives were identified by autoradiography or by use of an Autograph Beta-scanner.

Four strongly hybridizing clones were isolated from this screen, three from a *Hin*dIII clone bank and one from an *Eco*RI clone bank. Plasmid DNA was isolated from these clones and subjected to restriction enzyme analysis. The three *Hin*dIII clones (pTB362, -363, and -364) contained an insert fragment of 6.3 kb, but the *Eco*RI clone (pTB365) contained three insert fragments of 5.8, 5.0, and 0.9 kb (Table 1). These gels were Southern blotted and hybridized with the A1 oligonucleotide probe. Figure 1 shows that the 6.3-kb *Hin*dIII fragment and the 5.0-kb *Eco*RI fragment from these clones are homologous to the probe. The 5.0-kb *Eco*RI fragment was recloned into pUC19. A confirmed clone was designated pTB366 (Table 1).

Restriction mapping of the cloned inserts. The 6.3-kb *Hin*dIII and 5.0-kb *Eco*RI fragments from the clones described above were mapped with a variety of 6-base recognition restriction enzymes. The results are presented as a combined map in Fig. 2. There was a clear region of identity of about 2.4 kb in the deduced maps of the two fragments; these maps have been overlapped (coordinates, 3.9 to 6.3 kb). The map is similar to that of Hopewell et al. (13). The

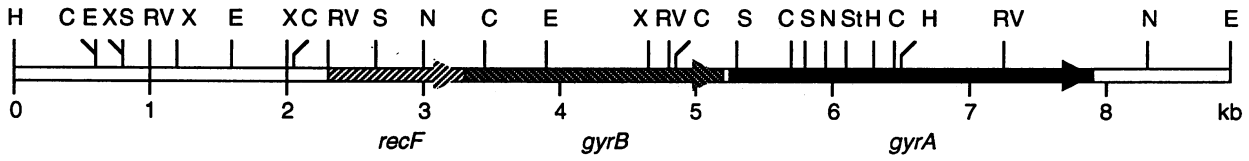

FIG. 2. Restriction map of the cloned *gyr* gene region. The 6.3-kb *Hin*dIII and 5.0-kb *Eco*RI cloned inserts were mapped with a variety of restriction enzymes. Abbreviations: C, *Cla*I; E, *Eco*RI; RV, *Eco*RV; H, *Hin*dIII; N, *Nde*I; S, *Ssp*I; St, *Sst*I; and X, *Xba*I. The two maps have been overlapped at the 2.4-kb region of identity between them. Positions of the genes are deduced from the sequencing data.

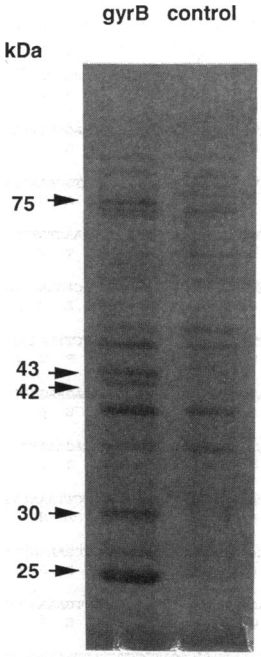

FIG. 3. Protein expression of the *gyrB* clone pTB370. The *gyrB* region cloned into the T7 promoter vector pTB361 was transformed into the expression host BL21(DE3) and induced with IPTG (33). Proteins were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Several new bands were visible compared with those of the control, which was the vector in the same host. These new bands were subjected to N-terminal sequence analysis (Table 2).

positions of the *gyr* genes are based on our DNA sequence data described below.

Expression of the *gyrB* gene. Our restriction data suggested that the *gyrB* gene could be subcloned on an *Ssp*I fragment of about 2.7 kb (Fig. 2, coordinates, 2.6 to 5.3 kb). This fragment was therefore gel isolated from *Ssp*I-cut pTB364 DNA and ligated into the *Sma*I site of pUC19. Transformant clones were screened by hybridization, and positive clones were mapped with restriction enzymes to determine the orientation of the insert. All of the clones tested had the fragment in the wrong orientation to have *gyrB* expressed by the *lac* promoter of the vector.

The fragment was therefore excised from one of these constructs (pTB367) by using the flanking *Kpn*I and *Sal*I sites from the multicloning region of the pUC19 vector. This was inserted in the expression orientation by ligation into the *Kpn*I and *Xho*I sites of our vector pTB361 (Table 1), which carries the T7 promoter (33). Transformant colonies were screened by hybridization and checked for the *gyrB* fragment insert. A few positive clones were then transformed into the BL21(DE3) host (which contains the T7 RNA polymerase gene) and tested for protein expression after IPTG (isopropyl-β-D-thiogalactopyranoside) induction. Figure 3 shows the analysis of one of these (pTB370): several new bands appeared, compared with those of the control, including one running at about 75 kDa.

Sequence analysis of the proteins expressed from the *gyrB* clone. Each of the major bands expressed from the *gyrB* clone (Fig. 3) was subjected to N-terminal protein sequence analysis. The results are presented in Table 2. The sequence of the 75-kDa band is similar to the known *E. coli* and *B. subtilis* GyrB sequences (see below). The 43- and 42-kDa bands have identical N termini (presumably the smaller protein has a clipped C terminus). These match an internal sequence (from residue 314) in the GyrB protein, as predicted from our DNA sequencing (see below). These may be either protease degradation products or translational restarts. The latter seems more likely because no band corresponding to the expected other half of the protein is visible. Also, the sequence corresponds to a position with a putative ribosome binding site and an ATG start codon.

TABLE 2. N-terminal sequences of proteins expressed by the gyrase clones

Band	Sequence[a]
GyrB clone	
75 kDa[b]	VTALSDVNNTDNYGAGQIQVLEGLEA
43 and 42 kDa	MKEEKDRLSGEDTREGMTAIIS
30 kDa	MVPIVKGSPQIRRRFIDMELGQIXAVYLNDLAQYRIL
25 kDa	MLEVLNQQFAEYAMKVTDKRAH
GyrA clone, 120 kDa	AELPQSRINEXNITSE

[a] Amino acid residues are given in the standard one-letter code, with X as uncertain. These results are compiled from more than one sequencing analysis.

[b] The major part of the 75-kDa GyrB band was also found to contain a sequence four N-terminal residues shorter than that shown.

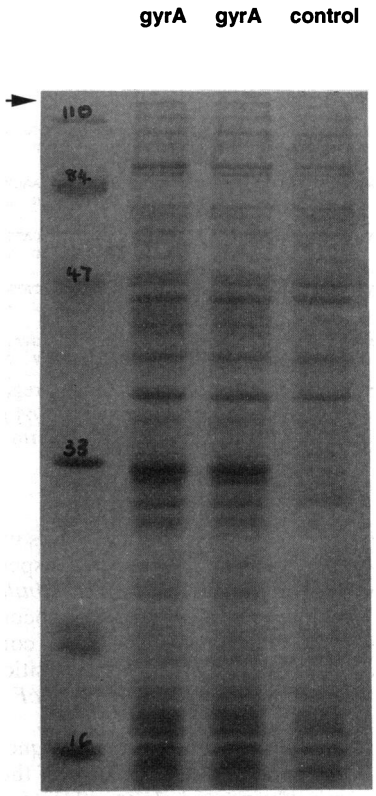

FIG. 4. Protein expression of the *gyrA* clone pTB373. Sodium dodecyl sulfate-polyacrylamide gel electrophoretic analysis of the clone containing the *gyrA* region inserted into the T7 promoter vector pTB361 in the BL21(DE3) host after induction by IPTG was done. A new band running at about 120 kDa is visible. This was N-terminally sequenced (Table 2).

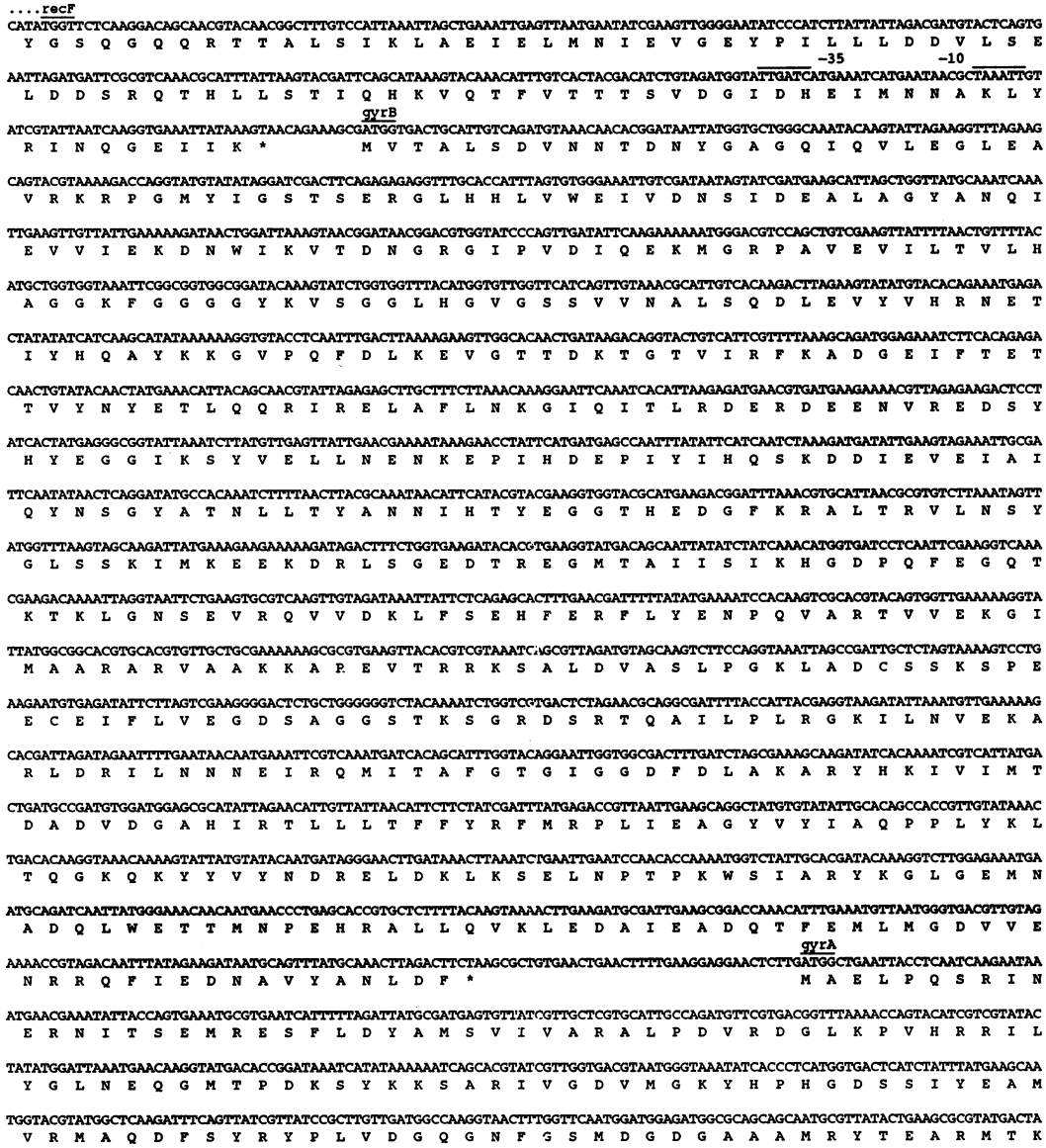

FIG. 5. Nucleotide sequence of the *gyrB-gyrA* region of *S. aureus*. The 5,909-bp region shows the predicted amino acid sequences for part of the *recF* gene and the whole of the *gyrB* and *gyrA* genes. A putative promoter for the expression of *gyrB* and *gyrA* is indicated within the *recF* gene. A putative transcription terminator (term.) is indicated just downstream of the *gyrA* gene.

The sequences of the 30- and 25-kDa bands were found to have significant homologies (65 and 50%, respectively) with two regions of the RecF protein from *B. subtilis*. The *recF* gene is found just upstream of *gyrB* in that species (21). This is consistent with our cloned *gyrB* fragment containing part of the *S. aureus recF* gene in the same position. The high expression of these bands may be due to *recF* being closer than *gyrB* to the T7 promoter on the vector.

Expression of the *gyrA* gene. Our DNA sequence predicts an *Eco*47III site just upstream of the start of the *gyrA* gene. Restriction analysis of our *gyrA* clone, pTB365, showed that this site was unique. We therefore isolated a DNA fragment of about 3.7 kb from this *Eco*47III site to the *Sal*I site in the multicloning region of pTB365. The T7 promoter vector, pTB361, was cut with *Nde*I, and the 5′ ends were filled with Klenow polymerase III and then cut with *Xho*I. The *gyrA* fragment was ligated into this cut vector and then used to transform *E. coli* DH5α. Transformants were screened by hybridization and then checked by sequencing across the promoter-proximal junction. Several clones with the expected structure were transformed into BL21(DE3) and analyzed for protein expression after induction. (One such clone is designated pTB373 [Table 1]). Figure 4 shows that a band running at about 120 kDa as well as several at smaller molecular sizes is expressed by the *gyrA* clones. This upper band was analyzed by N-terminal Edman degradation. The sequence, shown in Table 2, is consistent with the protein predicted from our *gyrA* DNA sequencing (below).

DNA sequencing. We have determined the complete nucleotide sequence of the *S. aureus gyr* region on both strands by using the dideoxynucleotide termination method on plasmid DNA (mainly pTB364 and -365) templates. The total of

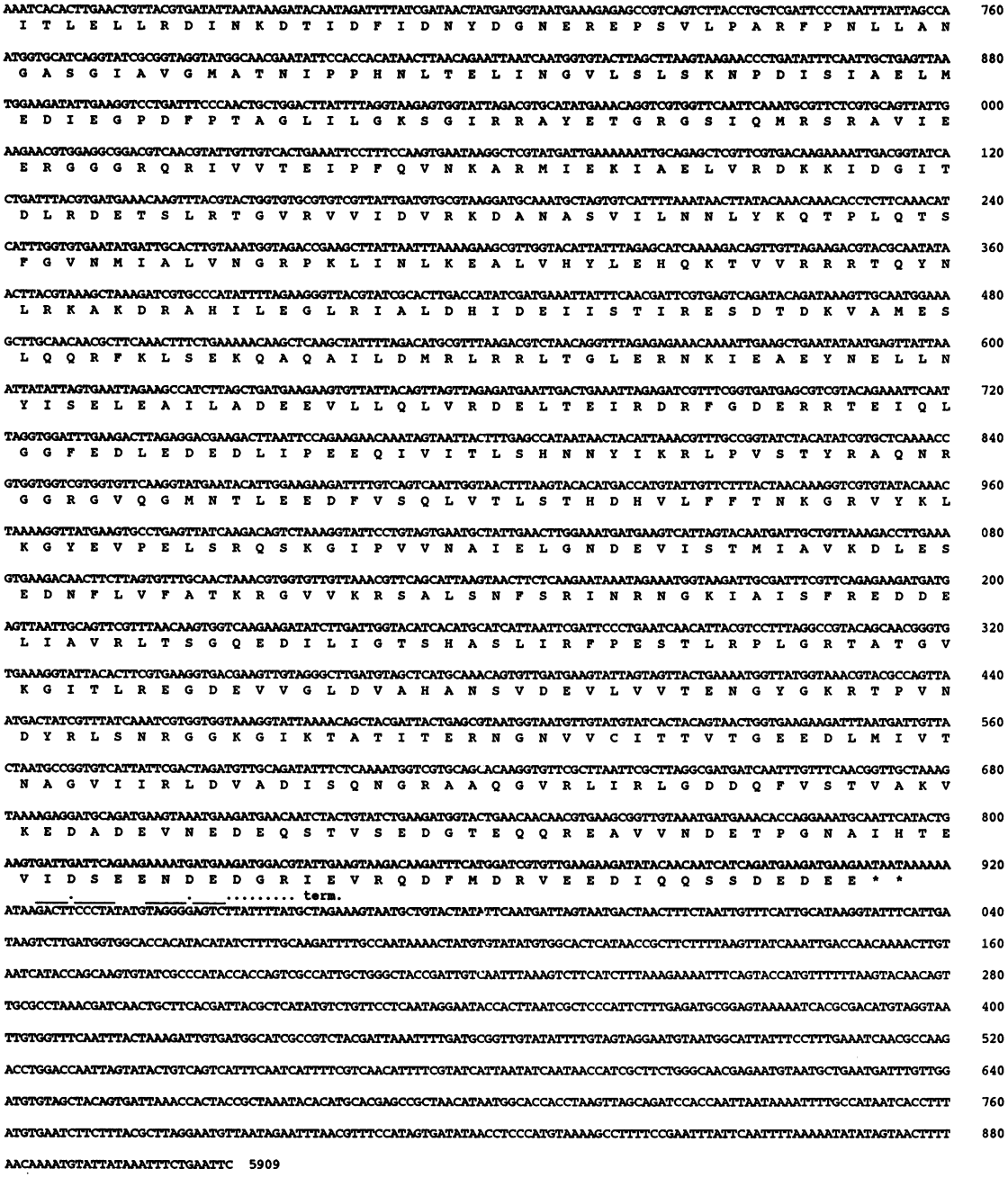

FIG. 5—*Continued.*

5.9 kb covers about 270 bp of *recF*, the whole of *gyrB* and gyrA, and about 1 kb of an unknown downstream sequence. This is the region from the *Nde*I site at coordinate 3.0 kb to the end of the map shown in Fig. 2. Sequencing was started by using the A1 and B1 probes as primers and also with primers reading from the pUC19 vector into the inserts. Further synthetic oligonucleotide primers were then designed from each new sequence determined.

The DNA sequence (which has a GC content of 36.3%) is marked in Fig. 5 with protein translations for one partial and two complete open reading frames. The polypeptide predicted from the partial open reading frame has homologies of 65 and 30% to the RecF proteins of *B. subtilis* (21) and *E. coli* (1), respectively. The two open reading frames which follow predict proteins with high homology to the GyrB and GyrA subunits of *B. subtilis* and *E. coli* as shown in the Lineup analysis (Fig. 6 and 7). Our DNA sequence and protein predictions to the end of *gyrA* are very similar to the recent results of Margerrison et al. (19), although there are significant differences (discussed below). However, 14 nucleotides beyond the stop codon of *gyrA*, the sequences completely

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Saugyrb  MVTALSDVNN TDNYGAGQIQ VLEGLEAVRK RPGMYIGST. SERGLHHLVW  50
Bsugyrb  M......EQQ QNSYDENQIQ VLEGLEAVRK RPGMYIGST. NSKGLHHLVW
Ecogyrb  M......... SNSYDSSSIK VLKGLDAVRK RPGMYIGDTD DGTGLHHMVF
   Gyrb  M......... .nsYd..qIq VLeGLeAVRK RPGMYIGsT. ...GLHHlVw

Saugyrb  EIVDNSIDEA LAGYANQIEV VIEKDNWIKV TDNGRGIPVD IQEKMGRPAV 100
Bsugyrb  EIVDNSIDEA LAGYCTDINI QIEKDNSITV VDNGRGIPVG IHEKMGRPAV
Ecogyrb  EVVDNAIDEA LAGHCKEIIV TIHADNSVSV QDDGRGIPTG IHPEEGVSAA
   Gyrb  EiVDNsIDEA LAGyc..I.v .IekDNsi.V .DnGRGIPvg IhekmGrpAv

Saugyrb  EVILTVLHAG GKFGGGGYKV SGGLHGVGSS VVNALSQDLE VYVHRNETIY 150
Bsugyrb  EVIMTVLHAG GKFDGSGYKV SGGLHGVGAS VVNALSTELD VTVHRDGKIH
Ecogyrb  EVIMTVLHAG GKFDDNSYKV SGGLHGVGVS VVNALSQKLE LVIQREGKIH
   Gyrb  EVImTVLHAG GKFdg.gYKV SGGLHGVG.S VVNALSq.Le v.vhR.gkIh

Saugyrb  HQAYKKGVPQ FDLKEVGTTD KTGTVIRFKA DGEIFTETTV YNYETLQQRI 200
Bsugyrb  RQTYKRGVPV TDLEIIGETD HTGTTTHFVP DPEIFSETTE YDYDLLANRV
Ecogyrb  RQIYEHGVPQ APLAVTGETE KTGTMVRFWP SLETFTNVTE FEYEILAKRL
   Gyrb  rQ.Yk.GVPq .dL...GeTd kTGT..rF.p d.EiFtetTe y.Ye.La.R.

Saugyrb  RELAFLNKGI QITLRDERDE ENVREDSYHY EGGIKSYVEL LNENKEPIHD 250
Bsugyrb  RELAFLTKGV NITIEDKREG QE.RKNEYHY EGGIKSYVEY LNRSKEVVHE
Ecogyrb  RELSFLNSGV SIRLRDKRDG KE...DHFHY EGGIKAFVEY LNKNKTPIHP
   Gyrb  RELaFLnkGv .ItlrDkRdg .e.r.d.yHY EGGIKsyVEy LN.nKepiH.

Saugyrb  EPIYIHQSKD DIEVEIAIQY NSGYATNLLT YANNIHTYEG GTHEDGFKRA 300
Bsugyrb  EPIYIEGEKD GITVEVALQY NDSYTSNIYS FTNNINTYEG GTHEAGFKTG
Ecogyrb  NIFYFSTEKD GIGVEVALQW NDGFQENIYC FTNNIPQRDG GTHLAGFRAA
   Gyrb  epiYi..eKD gI.VEvAlQy Ndgy..Niy. ftNNI.tyeG GTHeaGFk.a

Saugyrb  LTRVLNSYGL SSKIMKEEKD RLSGEDTREG MTAIISIKHG DPQFEGQTKT 350
Bsugyrb  LTRVINDYAR KKGLIKENDP NLSGDDVREG LTAIISIKHP DPQFEGQTKT
Ecogyrb  MTRTLNAYMD KEGYSKKAKV SATGDDAREG LIAVVSVKVP DPKFSSQTKD
   Gyrb  lTRvlN.Y.. k.g..Ke.k. .lsGdD.REG ltAiiSiKhp DPqFegQTKt

Saugyrb  KLGNSEVRQV VDKLFSEHFE RFLYENPQVA RTVVEKGIMA ARARVAAKKA 400
Bsugyrb  KLGNSEARTI TDTLFSTAME TFMLENPDAA KKIVDKGLMA ARARMAAKKA
Ecogyrb  KLVSSEVKSA VEQQMNELLA EYLLENPTDA KIVVGKIIDA ARAREAARRA
   Gyrb  KLgnSEvr.. vd.lfse..e .fllENP..A k.vV.KgimA ARAR.AAkkA

Saugyrb  REVTRRKSAL DVASLPGKLA DCSSKSPEEC EIFLVEGDSA GGSTKSGRDS 450
Bsugyrb  RELTRRKSAL EISNLPGKLA DCSSKDPSIS ELYIVEGDSA GGSAKQGRDR
Ecogyrb  REMTRRKGAL DLAGLPGKLA DCQERDPALS ELYLVEGDSA GGSAKQGRNR
   Gyrb  RE.TRRKsAL d.a.LPGKLA DCsskdP..s ElylVEGDSA GGSaKqGRdr

Saugyrb  RTQAILPLRG KILNVEKARL DRILNNNEIR QMITAFGTGI G.GDFDLAKA 500
Bsugyrb  HFQAILPLRG KILNVEKARL DKILSNNEVR SMITALGTGI G.EDFNLEKA
Ecogyrb  KNQAILPLKG KILNVEKARF DKMLSSQEVA TLITALGCGI GRDEYNPDKL
   Gyrb  ..QAILPLrG KILNVEKARl DkiLsnnEvr .mITAlGtGI G..dfnl.Ka

Saugyrb  RYHKIVIMTD ADVDGAHIRT LLLTFFYRFM RPLIEAGYVY IAQPPLYKLT 550
Bsugyrb  RYHKVVIMTD ADVDGAHIRT LLLTFFYRYM RQIIENGYVY IAQPPLYKVQ
Ecogyrb  RYHSIIIMTD ADVDGSHIRT LLLTFFYRQM PEIVERGHVY IAQPPLYKVK
   Gyrb  RYHkivIMTD ADVDGaHIRT LLLTFFYR.M r.iiE.GyVY IAQPPLYKv.

Saugyrb  QGKQKYY... ..........  ..........  ..........  .......... 600
Bsugyrb  QGKRVEY... ..........  ..........  ..........  ..........
Ecogyrb  KGKQEQYIKD DEAMDQYQIS IALDGATLHT NASAPALAGE ALEKLVSEYN
   Gyrb  qGKq..Y... ..........  ..........  ..........  ..........


Saugyrb  ..........  ..........  ..........  ..........  .......VYN 650
Bsugyrb  ..........  ..........  ..........  ..........  .......AYN
Ecogyrb  ATQKMINRME RRYPKAMLKE LIYQPTLTEA DLSDEQTVTR WVNALVSELN
   Gyrb  ..........  ..........  ..........  ..........  ........yN

Saugyrb  DRE.......  ..........  ..........  ..........  .......... 700
Bsugyrb  DKE.......  ..........  ..........  ..........  ..........
Ecogyrb  DKEQHGSQWK FDVHTNAEQN LFEPIVRVRT HGVDTDYPLD HEFITGGEYR
   Gyrb  DkE.......  ..........  ..........  ..........  ..........

Saugyrb  ..........  ..........  ..........  LDKLKSELNP TPKW..SIAR 750
Bsugyrb  ..........  ..........  ..........  LEELLKTLPQ TPKPGL..QR
Ecogyrb  RICTLGEKLR GLLEEDAFIE RGERRQPVAS FEQALDWLVK ESRRGLSIQR
   Gyrb  ..........  ..........  ..........  le.ll..L.. tpk.glsiqR

Saugyrb  YKGLGEMNAD QLWETTMNPE HRALLQVKLE DAIEADQTFE MLMGDVVENR 800
Bsugyrb  YKGLGEMNAT QLWETTMDPS SRTLLQVTLE DAMDADETFE MLMGDKVEPR
Ecogyrb  YKGLGEMNPE QLWETTMDPE SRRMLRVTVK DAIAADQLFT TLMGDAVEPR
   Gyrb  YKGLGEMNa. QLWETTMdPe sR.lLqVtle DAi.ADqtFe mLMGD.VEpR

Saugyrb  RQFIEDNAVY A.NLDF 816
Bsugyrb  RNFIEANARY VKNLDI
Ecogyrb  RAFIEENALK AANIDI
   Gyrb  R.FIE.NA.y a.NlDi
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FIG. 6. Comparison between the GyrB proteins from *S. aureus* (Sau), *B. subtilis* (Bsu), and *E. coli* (Eco). The University of Wisconsin Genetics Computer Group Bestfit and Lineup programs were used to compare our predicted *S. aureus* sequence with those from the other two species. The consensus is shown in the bottom line. Capital letters indicate complete conservation and lowercase letters indicate partial agreements at each position. Important conserved residues are dealt with in Discussion.

diverge. This presumably reflects the different strains of *S. aureus* used in the two laboratories.

DISCUSSION

Our DNA sequence data predict open reading frames for *gyrB* and *gyrA* consisting of 644 and 886 amino acid residues, respectively. N-terminal sequence analysis of the expressed proteins (Table 2) confirm our predicted start sequences, but the initiating methionine has been removed from the mature proteins. This gives calculated molecular weights for the GyrB and GyrA proteins of 72,408 and 99,085, respectively. GyrB is also found in higher amounts (about threefold) with its initial five residues removed (Table 2). The N-terminal arm is implicated in the cross-binding of the two GyrB subunits (41); thus, the fact that the *S. aureus* GyrB is translated with a longer N-terminal arm than its counterparts in *E. coli* or *B. subtilis* (Fig. 6) suggests that this processed form may be the active one.

A putative promoter for *gyrB* transcription is visible within the upstream *recF* sequence, and, immediately downstream of *gyrA*, there is a potential transcription termination sequence (Fig. 5). This suggests that both gene products are produced from a single transcript. This putative terminator is not found in the sequence of Margerrison et al. (19) since it lies beyond the sequence divergence point.

The *S. aureus* GyrB protein has 57 and 69% identity with its homologs from *E. coli* and *B. subtilis*, respectively (Fig. 6). The closer relationship to the *B. subtilis* protein is emphasized by the absence in both of a 170-residue region located near the C terminus of the *E. coli* GyrB. X-ray crystallography of the N-terminal fragment of GyrB from *E. coli* (41) has indicated that certain amino acid residues are involved in ATP binding (such as Tyr-5, Asn-46, Asp-73, Lys-103, Tyr-109, Gln-335, and Lys-337). Two consecutive glycines (220 and 221) are thought to form a flexible link between two domains. Also, Arg-136 and Gly-164 have been shown to be important in susceptibility to coumarins (4). All of these residues (given above in *E. coli* amino acid numbers) are conserved in the proteins from the three species (Fig. 6). Other extensive regions of conserved homology are also visible.

The GyrA protein from *S. aureus* has 52 and 65% identity with GyrA from *E. coli* and *B. subtilis*, respectively (Fig. 7). It also more closely resembles the *B. subtilis* GyrA because of the absence of a 34-residue region found approximately in the middle of the *E. coli* protein. There is great divergence among all three proteins at their C termini. Less is known about the structure of the GyrA subunit; however, significant regions of conserved homology can be seen in the alignment. This includes the Tyr-122 residue which is involved in the DNA breakage and rejoining reaction of gyrase (14).

Comparison of our sequence data with those previously published. Our DNA sequence data for *parF*, *gyrB*, and *gyrA* are very similar to those of Margerrison et al. (19). A comparison of the differences is shown in Fig. 8. For example, within *gyrB*, our sequence predicts residues 40 to 49 to be SERGLHHLVW, which contains the conserved GLHHLV motif (Fig. 6); this is in comparison with a sequence two residues shorter (QRELHISV) predicted previously (19). Also, in the comparison of residues 312 to 321 of the two sequences, although there is little homology between the proteins aligned in Fig. 6, our prediction is supported by the N-terminal sequence of a protein fragment (Table 2). These discrepancies can be interpreted as a few

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Saugyra  MAELPQSRIN ERNITSEMRE SFLDYAMSVI VARALPDVRD GLKPVHRRIL  50
Bsugyra  MSEQNTPQVR EINISQEMRT SFLDYAMSVI VSRALPDVRD GLKPVHRRIL
Ecogyra  MSDL.AREIT PVNIEEELKS SYLDYAMSVI VGRALPDVRD GLKPVHRRVL
   Gyra  Msel....i. e.NI..Emr. SfLDYAMSVI V.RALPDVRD GLKPVHRRiL

Saugyra  YGLNEQGMTP DKSYKKSARI VGDVMGKYHP HGDSSIYEAM VRMAQDFSYR 100
Bsugyra  YAMNDLGMTS DKPYKKSARI VGEVIGKYHP HGDSAVYESM VRMAQDFNYR
Ecogyra  YAMNVLGNDW NKAYKKSARV VGDVIGKYHP HGDSAVYDTI VRMAQPFSLR
   Gyra  YamN.lGmt. dK.YKKSARi VGdViGKYHP HGDSavYe.m VRMAQdFsyR

Saugyra  YPLVDGQGNF GSMDGDGAAA MRYTEARMTK ITLELLRDIN KDTIDFIDNY 150
Bsugyra  YMLVDGHGNF GSVDGDSAAA MRYTEARMSK ISMEILRDIT KDTIDYQDNY
Ecogyra  YMLVDGQGNF GSIDGDSAAA MRYTEIRLAK IAHELMADLE KETVDFVDNY
   Gyra  YmLVDGqGNF GS.DGDsAAA MRYTEaRm.K I..EllrDi. KdTiDf.DNY

Saugyra  DGNEREPSVL PARFPNLLAN GASGIAVGMA TNIPPHNLTE LINGVLSLSK 200
Bsugyra  DGSEREPVVM PSRFPNLLVN GAAGIAVGMA TNIPPHQLGE IIDGVLAVSE
Ecogyra  DGTEKIPDVM PTKIPNLLVN GSSGIAVGMA TNIPPHNLTE VINGCLAYID
   Gyra  DG.EreP.Vm P.rfPNLLvN GasGIAVGMA TNIPPHnLtE .InGvLa.s.

Saugyra  NPDISIAELM EDIEGPDFPT AGLILGKSGI RRAYETGRGS IQMRSRAVIE 250
Bsugyra  NPDITIPELM EVIPGPDFPT AGQILGRSGI RKAYESGRGS ITIRAKAEIE
Ecogyra  DEDISIEGLM EHIPGPDFPT AAIINGRRGI EEAYRTGRGK VYIRARAEVE
   Gyra  npDIsI.eLM E.IpGPDFPT Ag.IlGrsGI r.AYetGRGs i.iRarAeiE

Saugyra  .ERGGGRQRI VVTEIPFQVN KARMIEKIAE LVRDKKIDGI TDLRDETSLR 300
Bsugyra  .QTSSGKERI IVTELPYQVN KAKLIEKIAD LVRDKKIEGI TDLRDE.SDR
Ecogyra  VDAKTGRETI IVHEIPYQVN KARLIEKIAE LVKEKRVEGI SALRDE.SDK
   Gyra  .....GrerI iVtEiPyQVN KArlIEKIAe LVrdKkieGI tdLRDE.Sdr

Saugyra  TGVRVVIDVR KDANASVILN NLYKQTPLQT SFGVNMIALV NGRPKLINLK 350
Bsugyra  TGMRIVIEIR RDANANVILN NLYKQTALQT SFGINLLALV DGQPKVLTLK
Ecogyra  DGMRIVIEVK RDAVGEVVLN NLYSQTQLQV SFGINMVALH HGQPKIMNLK
   Gyra  tGmRiVIevr rDAna.ViLN NLYkQT.LQt SFGiNm.ALv .GqPK..nLK

Saugyra  EALVHYLEHQ KTVVRRRTQY NLRKAKDRAH ILEGLRIALD HIDEIISTIR 400
Bsugyra  QCLEHYLDHQ KVVIRRRTAY ELRKAEARAH ILEGLRVALD HLDAVISLIR
Ecogyra  DIIAAFVRHR REVVTRRTIF ELRKARDRAH ILEALAVALA NIDPIIELIR
   Gyra  ..l.hyl.Hq k.VvrRRT.y eLRKA.dRAH ILEgLrvALd hiD.iIslIR

Saugyra  ESDTDKVAME SLQQR..... ..........  .........  .........F 450
Bsugyra  NSQTAEIART GLIEQ..... ..........  .........  .........F
Ecogyra  HAPTPAEAKT ALVANPWQLG NVAAMLERAG DDAARPEWLE PEFGVRDGLY
   Gyra  .s.T...A.t .L.....  .........  .........  .........f

Saugyra  KLSEKQAQAI LDMRLRRLTG LERNKIEAEY NELLNYISEL EAILADEEVL 500
Bsugyra  SLTEKQAQAI LDMRLQRLTG LEREKIEEEY QSLVKLIAEL KDILANEYKV
Ecogyra  YLTEQQAQAI LDLRLQKLTG LEHEKLLDEY KELLDQIAEL LRILGSADRL
   Gyra  .LtEkQAQAI LDmRLqrLTG LEreKie.EY .eLl..IaEL ..ILa.e..l

Saugyra  LQLVRDELTE IRDRFGDERR TEIQLGGFED LEDEDLIPEE QIVITLSHNN 550
Bsugyra  LEIIREELTE IKERFNDERR TEIVTSGLET IEDEDLIERE NIVVTLTHNG
Ecogyra  MEVIREELEL VREQFGDKRR TEI.TANSAD INLEDLITQE DVVVTLSHQG
   Gyra  le.iReELte irerFgDeRR TEI.t.g.ed iedEDLI..E .iVvTLsHng.

Saugyra  YIKRLPVSTY RAQNRGGRGV QGMNTLEEDF VSQLVTLSTH DHVLFFTNKG 600
Bsugyra  YVKRLPASTY RSQKRGGKGV QGMGTNEDDF VEHLISTSTH DTILFFSNKG
Ecogyra  YVKYQPLSEY EAQRRGGKGK SAARIKEEDF IDRLLVANTH DHILCFSSRG
   Gyra  YvKrlP.StY raQ.RGGkGv qgm.t.EeDF v..L...sTH DhiLfFsnkG

Saugyra  RVYKLKGYEV PELSRQSKGI PVVNAIELGN DEVISTMIAV KDLESEDNFL 650
Bsugyra  KVYRAKGYEI PEYGRTAKGI PIINLLEVEK GEWINAIIPV TEFNAE.LYL
Ecogyra  RVYSMKVYQL PEATRGARGR PIVNLLPLEQ DERITAILPV TEFEEG.VKV
   Gyra  rVY..KgYe. PE..R.akGi PivNllele. dE.I.aiipV tefe.e...l

Saugyra  VFATKRGVVK RSALSNFSRI NRNGKIAISF REDDELIAVR LTSGQEDILI 700
Bsugyra  FFTTKHGVSK RTSLSQFANI RNNGLIALSL REDDELMGVR LTDGTKQIII
Ecogyra  FMATANGTVK KTVLTEFNRL RTAGKVAIKL VDGDELIGVD LTSGEDEVML
   Gyra  ffaTk.GvvK rt.Ls.F.ri r.nGkiAisl redDELigVr LTsG...i.i

Saugyra  GTSHASLIRF PESTLRPLGR TATGVKGITL REGDEVVGLD VAHANSVDEV 750
Bsugyra  GTKNGLLIRF PETDVREMGR TAAGVKGITL TDDDVVVGME ILEEES..HV
Ecogyra  FSAEGKVVRF KESSVRAMGC NTTGVRGIRL GEGDKVVSLI VPRGDG..AI
   Gyra  gt..g.liRF pEs.vR.mGr tatGVkGItL .egD.VVgl. v....s...v

Saugyra  LVVTENGYGK RTPVNDYRLS NRGGKGIKTA TITERNGNVV CITTVTGEED 800
Bsugyra  LIVTEKGYGK RTPAEEYRTQ SRGGKGLKTA KITENNGQLV AVKATKGEED
Ecogyra  LTATQNGYGK RTAVAEYPTK SRATKGVISI KVTERNGLVV GAVQVDDCDQ
   Gyra  L.vTenGYGK RTpv.eYrt. sRggKG.kta kiTErNG.vV ....v.geed

Saugyra  LMIVTNAGVI IRLDVADISQ NGRAAQGVRL IRLGDDQFVS TVAKVKEDAD 850
Bsugyra  LMIITASGVL IRMDINDISI TGRVTQGVRL IRMAEEEHVA TVALVEKNEE
Ecogyra  IMMITDAGTL VRTRVSEISI VGRNTQGVIL IRTAEDENVV GLQRV...AE
   Gyra  lMiiT.aGvl iR.dv.dISi .GR.tQGVrL IR.aede.V. tva.V...ae

Saugyra  EVNEDEQSTV SEDGTEQQRE AVVNDETPGN AIHTEVIDSE ENDEDGRIEV 900
Bsugyra  DENEEEQEEV
Ecogyra  PVDEEDLDTI DGSAAEGDDE .......... .IAPEVDVDD EPEEE
   Gyra  .vnEeeq.tv .....E...E .......... .I..EV.... E..E.GRIEV

Saugyra  RQDFMDRVEE DIQQSSDEDE E 921
   Gyra  RQDFMDRVEE DIQQSSDEDE E
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FIG. 7. Comparison of the GyrA proteins from *S. aureus* (Sau), *B. subtilis* (Bsu), and *E. coli* (Eco). Sequences were compared as described in the legend to Fig. 6.

```
recF

A  GGGGAATATCCCATCTTATTATTAGACGAT  110
   G  E  Y  P  I  L  L  L  D  D

B  GGG AATATCCCATCTTATTATT G CGAT
   G   N  I  P  S  Y  Y  C   D

gyrB

A  ACTTCAGAGAGAGGTTTGCACCATTTAGTGTGGGAA  431   AATCAAATT  482
   T  S  E  R  G  L  H  H  L  V  W  E         N  Q  I

B  ACT CAGAGAGAG  TTGCAC ATT AGTGT GGAA        AATAAAATT
   T   Q  R  E    L  H   I   S  V   E         N  K  I

A  AGCAAGATTATGAAAGAAGAAAAAGATAGACTTTCT  1247
   S  K  I  M  K  E  E  K  D  R  L  S

B  AGC AGA TATGAA GAAGAAAA GATAG C TTCT
   S   R   Y  E   E  E  K   I  A   S

A  AGTAAAAGT  1556      TTAACATTC  1850      GCACGATAC  2021
   S  K  S              L  T  F             A  R  Y

B  AGTCAAAGT            TTAATATTC            GCGCTATAC
   S  Q  S              L  I  F             A  L  Y

gyrA

A  AGAAACAAA  3572      GATGAGCGT  3704      CTTGGAAAT  4037
   R  N  K              D  E  R             L  G  N

B  AGAGACAAA            GATGATCGT            CTTGAAAAT
   R  D  K              D  D  R             L  E  N

A  GCTCATGCAAACAGT  4385   GCAGATGAAGTAAAT  4706  TCTACTGTA  4727
   A  H  A  N  S           A  D  E  V  N          S  T  V

B  GCTCACGAAAATAGT         GCAGAAGATGAAACGAAT     TCTACTTCAACTGTA
   A  H  E  N  S           A  E  D  E  T  N       S  T  S  T  V

A  ATTGATTCA  4814      GAAAATGATGAAGAT  4832     TCATCAGAT  4898
   I  D  S              E  N  D  E  D            S  S  D

B  ATTGAATCA            GAAACTGATGACGAT           TCATTAGAT
   I  E  S              E  T  D  D  D             S  L  D
```

FIG. 8. Differences between amino acid predictions of our data and those of Margerrison et al. (19). Within the *recF*, *gyrB*, and *gyrA* genes, our DNA sequence data (rows A) are aligned with those of Margerrison et al. (rows B) for each difference in the predicted amino acid residue of the encoded protein. One codon on each side of the difference is given. The numbers indicate the nucleotide coordinates of the adjacent base from our sequence shown in Fig. 5.

nucleotides having been missed in the latter data (19), leading to a few short frame-shift errors (Fig. 8).

The other differences within *gyrB* and *gyrA* occur as single or double amino acid residues due to small nucleotide differences. Mostly, our predicted residues match the *B. subtilis* or consensus sequence better than the data in reference 19 do (compare Fig. 6, 7, and 8). In addition, there are 53 silent differences that are presumably due to strain differences. Overall, there are about 40 amino acid residue differences between our sequencing data and those of Margerrison et al. We predict GyrB to have four more and GyrA to have three fewer residues than the sequences in Margerrison et al. (19). On the basis of the arguments described above, however, there may be very few real differences between the gyrase proteins in the two strains of *S. aureus* studied.

Replication origin region. The finding of the *recF* gene upstream of *gyrB* in *S. aureus* is consistent with the observed conservation of genes (*dnaA*, *dnaN*, *recF*, *gyrB*, *gyrA*) in the replication origin region of the eubacterial chromosome (24, 25). These genes have a direct role in the process of DNA replication except, apparently, for *recF*. However, *recF* is closely linked to adjacent genes: in our sequence it contains the putative promoter for *gyrB*, and

there are only 9 bp between its stop codon and the start codon of *gyrB*. Its position may be explained by its proposed role in postreplication DNA repair (29), although the finding that the RecF protein has strong single-stranded DNA-binding properties (11) suggests that it could also have a more direct role in DNA replication.

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