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# *Staphylococcus aureus* Has Clustered tRNA Genes

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**The polymerase chain reaction (PCR) was used to detect large tRNA gene clusters in *Bacillus subtilis*, *Bacillus badius*, *Bacillus megaterium*, *Lactobacillus brevis*, *Lactobacillus casei*, and *Staphylococcus aureus*. The primers were based on conserved sequences of known gram-positive bacterial tRNA$^{Arg}$ and tRNA$^{Phe}$ genes. This PCR procedure detected an unusually large tRNA gene cluster in *S. aureus*. PCR-generated probes were used to identify a 4.5-kb *Eco*RI fragment that contained 27 tRNA genes immediately 3′ to an rRNA operon. Some of these 27 tRNA genes are very similar, but only 1 is exactly repeated in the cluster. The 5′ end of this cluster has a gene order similar to that found in the 9- and 21-tRNA gene clusters of *B. subtilis*. The 3′ end of this *S. aureus* cluster exhibits more similarity to the 16-tRNA gene cluster of *B. subtilis*. The 24th, 25th, and 26th tRNA genes of this *S. aureus* tRNA gene cluster code for three similar, unusual Gly-tRNAs that may be used in the synthesis of the peptidoglycan in the cell wall but not in protein synthesis. Southern analysis of restriction digests of *S. aureus* DNA indicate that there are five to six rRNA operons in this bacterium's genome and that most or all may have large tRNA gene clusters at the 3′ end.**

The tRNA genes of *Bacillus subtilis* are clustered in groups of from 2 to 21 and are usually associated with rRNA operons (6, 7, 11, 16, 23, 32, 33, 36). Clusters of up to 11 tRNA genes with similar gene orders have been found in several types of mycoplasma, including the genera *Mycoplasma*, *Spiroplasma*, and *Acholeplasma* (14, 21, 25, 29). These cell wall-less bacteria have been grouped with the low-G+C gram-positive bacteria on the basis of their 5S and 16S rRNA sequences (20, 34). The similarities in tRNA gene organization indicate that large tRNA gene clusters may be widespread in the low-G+C gram-positive group of bacteria. The functions of this type of tRNA gene organization are not known. One possibility is that the very large tRNA gene clusters of *B. subtilis* are important in the rapid, coordinated production of tRNA during the germination of spores. One of the unifying traits of most of the low-G+C gram-positive bacteria is sporulation (35). The mycoplasmas may have a similar but smaller clustered tRNA gene organization that reflects their evolution from a sporulating ancestor.

Since rRNA-tRNA gene clusters contain the highly conserved rRNA and tRNA gene sequences, they are ideal targets for amplification by the polymerase chain reaction (PCR). The use of PCR primers that are designed to hybridize to tRNA genes can be used to quickly screen a large number of gram-positive bacteria for clusters of tRNA genes. The study of the tRNA gene clusters of the gram-positive group of bacteria may shed some light on the evolution and function of this type of gene organization. In addition, it may provide a method to determine the evolutionary relationships between closely related bacteria when rRNA comparisons alone cannot be used because of a lack of sequence differences (5).

In the work presented here, PCR is shown to be able to detect tRNA gene clusters in several gram-positive bacteria. A particularly large PCR product was detected in *Staphylococcus aureus*. This member of the low-G+C gram-positive group of bacteria does not form spores (35). Therefore, the comparison of its tRNA gene organization with that of *B. subtilis* would test the hypothesis that very large tRNA gene clusters are related to sporulation. The *S. aureus* PCR fragment was used to identify a clone of this tRNA gene cluster. Sequencing revealed that it contained 27 tRNA genes immediately 3′ to an rRNA operon. The *S. aureus* tRNA gene order had similarities to the 21-, 16-, and 9-tRNA gene clusters of *B. subtilis*. Three of the tRNA genes in this *S. aureus* cluster code for special tRNAs used in the synthesis of peptidoglycan.

## MATERIALS AND METHODS

**DNA synthesis.** All DNA oligomers were made on an Applied Biosystems DNA Synthesizer model 380B by using phosphoramidite chemistry. The PCR primers were designed to hybridize to all known tRNA$^{Arg}$ and tRNA$^{Phe}$ genes of the low-G+C gram-positive bacteria (28). Because of a small number of sequence differences in the known targets, some positions in the sequence contained equal amounts of more than one type of nucleotide. To facilitate cloning of the PCR fragments, nine bases were added to each primer at the 5′ end to accommodate either an *Eco*RI or a *Sal*I restriction site. The tRNA$^{Arg}$ primer sequence was 5′-CTCGAATTCTTGGATAGA(G,T)CG(C,T)TTGACTACG-3′ and was in the same sense as the tRNA. The tRNA$^{Phe}$ primer sequence was 5′-CTCGTCGACAC(A,G)GAAT(C,T)GAAC(C,T)GCCGACAC-3′ and was complementary to the tRNA. The residues in brackets indicate the presence of equal amounts of two different bases at that position.

**Bacterial genomic DNA.** The *S. aureus* DNA was prepared from ATCC type strain 12600 by lysis of logarithmic cells in 100 U of mutanolysin (Sigma Chemical Co.) per ml–25 mM Tris-HCl (pH 7.0)–25 mM EDTA and subsequent phenol extraction. Genomic DNA from *B. subtilis* 168 was prepared by the same method. The other bacterial genomic DNAs that were used, from *Bacillus badius* 850529, *Bacillus brevis* ATCC 8185, *Bacillus megaterium* KM, *Bacillus stearothermophilus* 1430R, *Clostridium sporogenes* ATCC 3584, *Lactobacillus brevis* ATCC 14869, and *Lactobacillus casei*

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ATCC 7469, were the generous gift of Elisabeth Haas and Norman Pace, Indiana University.

**PCR.** Ten nanograms of each bacterial genomic DNA was used for each PCR in a volume of 100 μl of 10 mM Tris-HCl (pH 8.3)–50 mM KCl–1.5 mM $MgCl_2$–200 μM each deoxyribonucleotide triphosphate–1 μM each primer–1.5 U of *Thermus aquaticus* DNA polymerase (Perkin-Elmer Cetus). The reaction mixture was preincubated at 94°C for 5 min. A Perkin-Elmer Cetus DNA thermocycler was used for 30 cycles of 94°C, 30 s; 56°C, 30 s; and 72°C, 60 s.

**Nucleic acid probes.** The 1,000-bp *S. aureus* PCR product was isolated by agarose gel electrophoresis and labeled with $^{32}P$ by using the Multiprime DNA labeling system (Amersham). This labeled DNA was used to detect the 4.5-kb *Eco*RI fragment containing the rRNA-tRNA gene cluster. The 79-bp *Sau*3A fragment of the cloned *S. aureus* 23S rRNA gene found by sequencing M13 clones (see below) was labeled with [$\gamma$-$^{32}$P]ATP by using polynucleotide kinase. To isolate tRNA, *S. aureus* was grown in 100 ml of 2× YT media (24) at 37°C and was harvested in logarithmic phase, at an optical density at 600 nm of 1.0. The tRNA was extracted with phenol. The crude tRNA was bound to a 5-ml DEAE-cellulose (Whatman) column, washed with 0.2 M NaCl, and eluted with 1 M NaCl. The tRNA was then precipitated with ethanol. To remove small amounts of 5S rRNA, the tRNA was electrophoresed through a 5% acrylamide–7 M urea–Tris-borate-EDTA gel (24). The tRNA from 70 to 90 bases in length was excised from the gel, eluted, and precipitated with ethanol. The tRNA was nicked by heating at 100°C for 1 min and labeled with T4 polynucleotide kinase and [$\gamma$-$^{32}$P]ATP.

**Cloning of the *S. aureus* rRNA-tRNA gene cluster.** *Eco*RI-restricted DNA was electrophoresed on an agarose gel (SeaPlaque agarose, FMC Corp.). DNA fragments in the size range of 4.2 to 4.7 kb were excised from the gel and ligated into *Eco*RI-cut pUC18. This was transformed into *Escherichia coli* JM101. Approximately 500 colonies were screened by colony lifts on nitrocellulose filters (24) by using the PCR probe described above.

**DNA sequencing.** All DNA sequencing was performed with the Sequenase Sequencing Kit (U.S. Biochemicals). A *Sau*3A digest of the 4.5-kb *Eco*RI *S. aureus* rRNA-tRNA gene cluster was used to subclone the DNA into *Bam*HI-cut M13mp19. The *Sau*3A fragments were all sequenced in both directions by using the single stranded M13 subclones. Double-stranded sequencing of the pUC18 clone of the entire 4.5-kb *Eco*RI *S. aureus* rRNA-tRNA gene fragment was used to link the *Sau*3A fragments and to sequence the *Eco*RI ends.

**Nucleotide sequence accession number.** The 3,360-bp nucleotide sequence of the *S. aureus* tRNA gene cluster has been deposited in the GenBank data library under accession number L11530.

## RESULTS

The gel electrophoresis of the PCR products with the $tRNA^{Arg}$ and $tRNA^{Phe}$ gene primers is shown in Fig. 1. The amplification with *B. subtilis* DNA produced a single band of approximately 800 bp. This size of PCR product is consistent with the amplification of the 9-tRNA gene region between the $tRNA^{Arg}$ and $tRNA^{Phe}$ genes in the 21-tRNA gene cluster *trnB* (6, 33). The *B. badius* PCR produced two bands at approximately 500 and 900 bp. That of the *B. megaterium* produced a single band at 800 bp. The DNA of *L. brevis* and *L. casei* produced bands at 900 and 800 bp, respectively. The

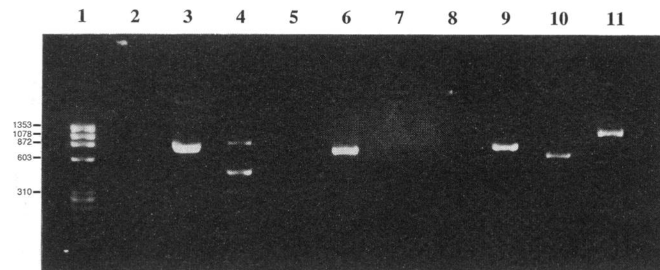


FIG. 1. Agarose gel electrophoresis of PCR-amplified gram-positive bacterial tRNA gene clusters between the $tRNA^{Arg}$ and $tRNA^{Phe}$ genes. PCR conditions are described in Materials and Methods. A total of 5 μl of each PCR mixture was loaded into lanes 2 to 11. Lane 1, molecular weight markers, 200 ng of *Hae*III-restricted φX174 phage DNA. The lengths, in base pairs, of the five largest bands are shown; lane 2, zero DNA PCR control; lane 3, *B. subtilis*; lane 4, *B. badius*; lane 5, *B. brevis*; lane 6, *B. megaterium*; lane 7, *B. stearothermophilus*; lane 8, *C. sporogenes*; lane 9, *L. brevis*; lane 10, *L. casei*; lane 11, *S. aureus*.

*S. aureus* PCR produced a 1,000-bp band. PCR with *B. brevis*, *B. stearothermopihlus*, and *C. sporogenes* did not produce any amplified product. The absence of PCR products may indicate that the primers were not a close enough match for their tRNA gene targets or that the $tRNA^{Arg}$ and $tRNA^{Phe}$ genes in these organisms are not closely linked.

Since the *S. aureus* amplicon was the largest found in these bacteria, it was used to probe a Southern blot of *Eco*RI-restricted *S. aureus* genomic DNA. This detected an intensely hybridizing band approximately 4.5 kb in length and a less strongly hybridizing band of 2.5 kb in length (data not shown). The 4.5-kb fragment was cloned as described in Materials and Methods. This cloned DNA was further digested with *Sau*3A and subcloned into M13mp19 for sequencing. The M13 clones were oriented by sequencing directly from the plasmid with newly synthesized primers to assemble the sequence shown in Fig. 2. All of the DNA piece shown was sequenced from both strands. There was another 1.1 kb of upstream 23S rRNA gene sequence that is not shown.

To determine if other tRNA gene clusters are present in the *S. aureus* genome, genomic DNA was restricted with *Eco*RI, *Hin*dIII, *Pst*I, and *Xba*I, electrophoresed, and probed separately with either *S. aureus* tRNA or a 23S rRNA gene fragment (Fig. 3). The 79-bp *Sau*3A 23S rRNA gene fragment from positions 1 to 79 of Fig. 2 was subcloned into M13 phage and used to probe the genomic DNA blot. This 23S rRNA probe detected five to six bands in the digested *S. aureus* genomic DNA, indicating the number of rRNA operons present in this bacterium. The tRNA probe of the same blot detected many more bands. The intensity of the tRNA-hybridizing bands varied considerably, with the 4.5-kb *Eco*RI band being surprisingly light. This variation in signal may have been caused by differences in the labeling efficiency of individual tRNA species. The same five to six bands that hybridized with the 23S probe also hybridized with the tRNA probe, indicating that all *S. aureus* rRNA operons may have tRNA genes associated with them. The *Eco*RI, *Hin*dIII, and *Xba*I digests had more, smaller fragments that hybridized to the tRNA probe but not the 23S rRNA probe, probably because several of the tRNA gene clusters contained sites for these restriction enzymes. However, the only *Pst*I fragments that were detected by the tRNA probe also were the only ones that hybridized to the

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1-GATCCCTCAAAGATGATGAGGTTAATAGGTTCGAGGTGGAAGCATGGTGACATGTGGAGCTGACGAATACTAATCGATCGAGGGCTTAACCAAAATAAATGTTTTGCGACAAGATCACTTTTACTTACTATCTAGTTTTGAATGTATAAT
        23S rRNA
151  TTACATTCATATGTCTGGTGACTATAGCAAGGAGGTCACACCTGTTCCCATGCCGAACACAGAAGTTAAGCTCCTTAGCGTCGATGGTAGTCGAACTTACGTTCCGCTAGAGTAGAACGTTGCCAGGCAGTTTTAAATCGGAGAATTAGC
        5S rRNA
301  TCAGCTGGGAGAGCATCTGCCTTACAAGCAGAGGGTCGGCGGTTCGAACCCGTCATTCTCCACCATTTATTCTTACATATTGCCGGCCTAGCTCAATTGGTAGAGCAACTGACTTGTAATCAGTAGGTTGGGGGTTCAAGTCCTCTGGCC
        Val                                        Thr1
451  GGCACCATGGAAGAGCCATTAGCTCAGTTGGTAGAGCATCTGACTTTTAATCAGAGGGTCAGAGGTTCGAATCCTCTATGGCTCACCATTTGCGGGTGTGGCGGAATTGGCAGACGCACTAGACTTAGGATCTAGCGCCTTTACGGCGTG
        Lys                                        Leu
601  GGGGTTCGACTCCCTTCACCCGCATATGCAGAAGTAGTTCAGCGGTAGAATACAACCTTGCCAAGGTTGGGGTCGCGGGTTCGAATCCCGTCTTCTGCTCCATTTTTATAGTGCCGGGGTGGCGGAACTGGCAGACGCACAGGACTTAAA
        Gly                                        Leu
751  ATCCTGCGGTGAGTGATCACCGTACCGGTTCGATTCCGGTCCTCGGCACCATTTTCAATAAAAACATATGCGCCCGTAGCTCAATTGGATAGAGCGTTTGACTACGGATCAAGAGGTTATGGGTTCGACTCCTATCGGGCGCGTTAATTA
        Arg
901  TACGGGAAGTAGCTCAGCTTGGTAGAGCACTTGGTTTGGGACCAAGGGGTCGCAGGTTCGAATCCTGTCTTCCCGATATACTGTAATTATTATGGGGGCTTAGCTCAGCTGGGAGAGCGCCTGCTTTGCACGCAGGAGGTCAGCGGTTCG
        Pro                                        Ala
1051 ATCCCGCTAGTCTCCACCATATTATTTACAAACTATATAAGGCGGTGTAGCTCAGCTGGCTAGAGCGTACGGTTCATACCCGTGAGGTCGGGGGTTCGATCCCCTCCACCGCCACTATTTATTAGTTGTAAAATTATATTTAGGACCTTT
        Met
1201 AGCTCAGTTGGTTAGAGCTAACGGCTCATAACCGTTCGGTCGCAGGTTCGAGTCCTGCAAGGTCCATATAATTTTGGAGGAATACCCAAGTCCGGCTGAAGGGATCGGTCTTGAAAACCGACAGGGGCTTAACGGCTCGCGGGGGTTCGA
        Ile                                        Ser1
1351 ATCCCTCTTCCTCCGTTTTACTAATGGTCTCGTAGTGTAGCGGTTAACACGCCTGCCTGTCACGCAGGAGATCGCGGGTTCGGTTCCCGTCGAGACCGCCATTTAATTTTATAATTAATAGCGATTTACCTATAATAATGGAGGAATACC
        Asp1
1501 CAAGTCCGGCTGAAGGGATCGGTCTTGAAAACCGACAGGGCCTTAACGGGCCGCGGGGGTTCGAATCCCTCTTCCTCCGCCATTATTTTTTATTATCGCGGGATGGAGCAGTTCGGTAGCTCGTCGGGCTCATAACCCGAAGGTCGGTGG
        Ser2                                       fMet
1650 TTCAAATCCGCCTCCCGCAATATTTTATAGGTCTCGTAGTGTAGCGGTTAACACGCCTGCCTGTCACGCAGGAGATCGCGGGTTCGATTCCCGTCGAGACCGCCATCATTACATTTTTATTATGGTTCAGTAGCTCAGTTGGTAGAGCAA
        Asp2
1801 TGGATTGAAGCTCCATGTGTCGGCAGTTCGACTCTGTCCTGAACCATTTCTTAGCCGGCCTAGCTCAATTGGTAGAGCAACTGACTTGTAATCAGTAGGTTGGGGGTTCAAGTCCTCTGGCCGGCACCATTTATGGAGGGGTAGCGAAGT
        Phe                                        Thr2
1951 GGCTAAACGCGGCGGACTGTAAATCCGCTCCTTCGGGGTTCGGCAGTTCGAATCTGCCCCCCTCCATTTATTATTTTTAATAGGGGCATAGTTCAACGGTAGAATAGAGGTCTCCAAAACCTTTGGTGTGGGTTCGATTCCTACTGCCCC
        Tyr                                        Trp
2101 TGCCATGGCGGCTGTGGTGAAGTGGTTAACACATCGGATTGTGGTTCCGACATTCGAGGGTTCGATCCCCTTCAGCCGCCCTTATTATTAATGGGCTATAGCCAAGCGGTAAGGCAACGGACTTTGACTCCGTCACTCGTTGGTTCGAAT
        His                                        Gln
2251 CCAGCTAGCCCAGTTATTGGCGGCATAGCCAAGTGGTAAGGCAGAGGTCTGCAAAACCTTTATCACCGGTTCAAATCCGGTTGCCGCCTCCAGGTTTATGCGGGAGTAGTTCAACTTTTAGAACACGTTCCTTCCCGGAACGAGGTATAG
        Cys                                        Gly1
2401 GTGCAAATCCTATCTTCCGCTCCATAATTTAATAATAATGCGGGAGTATTTCAACTCTTAGAATACATTCCTTCCTGGAATGAGGTATAGGTGTAAATCCTATCTTCCGCTCCATAATTTAATATTTGCGGGAGTAGTTCAACTTTTAGA
        Gly2
2551 ACACGTTCCTTCCCGGAACGAGGTATAGGTGTAAATCCTATCTTCCGCTCCATAATTGCTTCCAAAGGGAAGTTTTTTGTTTACCATTAAGCCGGTGTGGCGGAATTGGCAGACGCGCGGGACTCAAAATCCCGTTCCACTTGTGGAGTG
        Gly3                      att?                     Leu
2701 TCGGTTCGACCCCGACCACCGGTATAATTAACTGTTATTTACATAACATAACGTATTAGAAACCTTGTAAAACAAGGTTTCTTTTTATTTCTCTCTATACAATACAAATAAAAGTGGACTCAAATGGCACACGCTTTAATAGACTCTATG
        term?
2851 TCAAATTGTAATGATGAGTTCAATATTGGAAGTTAAGCAACTATGCATTGTTTAACGGAACTCACCAAATGTGGTGGGTATATAATTTAAAGAACTATTTTTAAAATACAACTTTTAGAGTTTTTATTATTAGGCGGCCAGTCCATTATT
        term?
3001 GGGCTTGGTTGTCTTCTTTTTTTCTCCTTTGTACAAGCTGAAAATCATCATTATACGTGCTTTAAAGTTGTTGAAATTTCTGTAACCAAAAGAAATTCACTTGATTAATTTTATCTTATTATTAATTCCTTCTATAGCACCATTATTAAA
3151 TGCTGGGTAATAAATTGTATTTCTTAACATCCTTTGATGTTTTCTATAATATTTAACCACTTTCCATACACCCTTACTCACAGACTTTTTACTAACTGAATTTAAACGATTAATAAATTTAGGCCAATTACATAACCTTAGGTCTTTTCG
3301 TAATCCTTGGACAAGTTCGTAGGAGTGTCGTAGTATATCGTCTTTTGAAAGCATGAATTC
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FIG. 2. Sequence of the *S. aureus* 27-tRNA gene cluster immediately 3′ to a rRNA operon. The gene coding sequences have a solid underlining. The anticodons of the tRNA genes are double underlined and labeled with their cognate amino acid. Putative attenuator and terminator base-paired hairpin sequences have a dotted underline.

23S rRNA probe, indicating that virtually all of the *S. aureus* tRNA genes are associated with rRNA.

## DISCUSSION

The PCR data indicate that the clustering of tRNA genes is widespread in the low-G+C gram-positive bacteria. The absence of a PCR-amplified product for some of the bacteria may be due to sequence differences in the tRNA genes or to different tRNA gene orders. The sequence of the 27-tRNA gene cluster from *S. aureus* demonstrates again that the gene orders of the low-G+C gram-positive group of bacteria are remarkably similar (Fig. 4).

The first nine tRNA genes of the *S. aureus* cluster have an order identical to that of *trnJ*, the nine-tRNA gene cluster of *B. subtilis* (7). With the exception of the substitution of a $tRNA^{Leu}_{UAG}$ gene in the *S. aureus* sequence for that of a $tRNA^{Leu}_{CAG}$, the first 12 genes of the *S. aureus* cluster have the same order as the first 12 of the *B. subtilis trnB* 21-tRNA gene cluster (6, 33). The 15th through 22nd tRNA gene of the *S. aureus* cluster has the same gene order as the 5th through 12th gene of the *trnD* 16-tRNA gene *B. subtilis* cluster (33).

The $tRNA^{Leu}_{CAA}$ gene at the end of the *S. aureus* cluster not only is placed identically to the same gene in the *B. subtilis trnD* cluster but also shares some 5′ and 3′ secondary structural features. Immediately upstream of the *B. subtilis* $tRNA^{Leu}_{CAA}$ gene is a base-paired attenuator structure followed by a promoter sequence separating it from the rest of

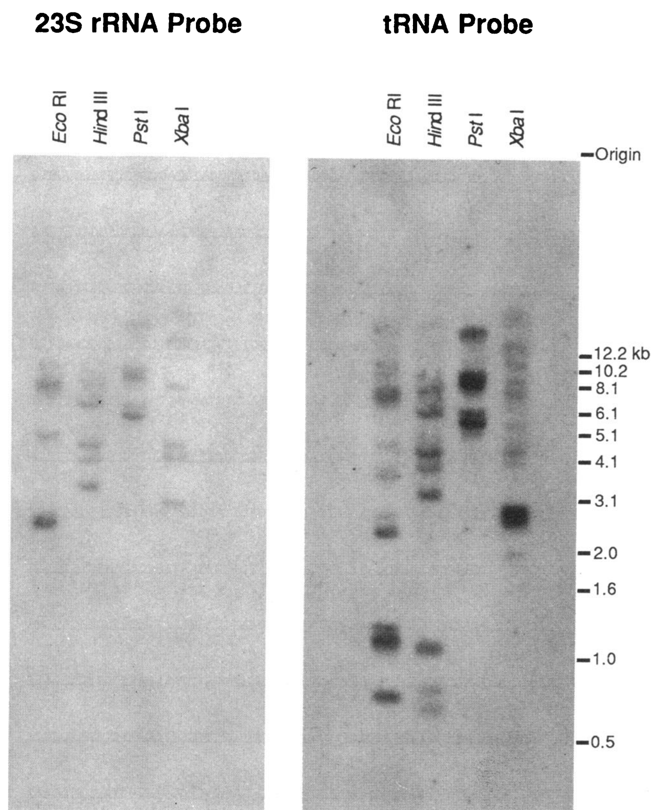


FIG. 3. Autoradiograph of the Southern analysis of *S. aureus* genomic DNA. A single nitrocellulose blot was probed with the $^{32}$P-labeled 79-bp fragment of the *S. aureus* 23S rRNA gene (left). The blot was stripped and reprobed with $^{32}$P-labeled *S. aureus* tRNA (right). From left to right on both autoradiographs, the restriction enzymes used are *Eco*RI, *Hin*dIII, *Pst*I, and *Xba*I. The positions of double-stranded DNA molecular weight markers are shown on the right.

the cluster (Fig. 4) (30). Immediately following the *B. subtilis* $tRNA^{Leu}_{CAA}$ gene sequence, there is a strong double terminator that appears to end the combined rRNA-tRNA operon's transcription (30). Although there is no obvious promoter sequence in the *S. aureus* cluster, very similar attenuator and terminator-like hairpin structures are found in similar positions relative to the *B. subtilis* $tRNA^{Leu}_{CAA}$ gene (Fig. 2 and 4). By analogy with the *B. subtilis trnD* transcription (30), this may indicate that the putative attenuator reduces the transcription of the $tRNA^{Leu}_{CAA}$ gene in the *S. aureus* cluster compared to the 26 upstream tRNA genes.

The *B. subtilis trnB* 21-tRNA gene cluster has been shown to have a functional promoter between the second and third tRNA genes (31). A putative promoter with an identical sequence is found in *trnJ*, the nine-tRNA gene cluster of *B. subtilis* (7). Although the gene order in these two *B. subtilis* operons is very similar to the *S. aureus* tRNA gene cluster, the latter does not appear to have such a promoter. The promoter sequences used by *S. aureus* are not as well studied as those of *B. subtilis*, and therefore they may not be obvious from the sequence. However, the space between the second and third tRNA genes in the *S. aureus* sequence is only 5 bp compared with the 37 bp in the *B. subtilis* sequence, making it unlikely that there is a *S. aureus* promoter present in this region unless it is entirely buried within the structural sequence of the second tRNA gene.

The *B. subtilis trnD* operon contains a functional promoter immediately 5′ to the 5S rRNA gene (30). At a similar position in the *S. aureus* rRNA-tRNA gene cluster (positions 145 to 150), there is a TATAAT sequence that may be the −10 part of a promoter, although there is no good match for the typical −35 consensus sequence. The same TATAAT sequence can be found at several of the spacer regions between tRNA genes. This is probably because of the high A/T concentrations in these regions rather than the presence of promoters. However, without performing transcriptional analyses of this region, the presence of promoters that can increase the transcription of some of the downstream *S. aureus* tRNA genes cannot be ruled out.

Twelve of the 27 tRNA genes in the *S. aureus* cluster do not encode the 3′-terminal CCA sequence. Although all *E. coli* tRNA genes do encode this feature (9), 14 of the 62 known tRNA genes in *B. subtilis* do not (8). There is no obvious pattern in the sequence or gene order for *S. aureus* and *B. subtilis* tRNA genes that lack the CCA sequence.

The 11th gene in the *S. aureus* tRNA gene cluster has a CAU anticodon. However, on the basis of homology with similar tRNA genes in *B. subtilis* and *Mycoplasma capricolum*, it is almost certain that it codes for an isoleucine tRNA with a modified C residue in the "wobble" position that allows it to base pair with AUA isoleucine codons (1, 6, 8, 14). Therefore, we have designated this a gene for $tRNA^{Ile}_{NAU}$, with the N representing an uncharacterized base modification.

The 24th, 25th, and 26th tRNA genes in the *S. aureus* cluster all code for unusual tRNA genes with a Gly UCC anticodon. Their most unusual aspect is the replacement of the typical GTTC sequence in the fourth loop of these tRNA genes with a GTGC in the $tRNA^{Gly-1}_{UCC}$ gene and a GTGT sequence in the $tRNA^{Gly-2}_{UCC}$ and $tRNA^{Gly-3}_{UCC}$ genes. These tRNA genes code for tRNAs that are almost identical to two $tRNA^{Gly}_{UCC}$ found in *S. epidermidis* (18). Neither of these two *S. epidermidis* tRNAs participate in protein synthesis but are instead used to make the peptide cross-links in the peptidoglycan of the *Staphylococcus* cell wall (17, 19). This same type of $tRNA^{Gly}$-mediated peptidoglycan synthesis has been found in *S. aureus* (2, 12, 13). Three chromatographically distinct $tRNA^{Gly}$ fractions have been found in *S. aureus*. All of these fractions can participate in making the peptidoglycan cross-links, but one of the three fractions cannot support polypeptide synthesis (2). Although there is no direct evidence that the three *S. aureus* $tRNA^{Gly}_{UCC}$ genes are actually expressed, their near identity with the *S. epidermidis* tRNAs make it likely that they code for the tRNAs used exclusively for peptidoglycan synthesis. The very similar sequences of these three putative tRNAs may have made it difficult to resolve them by chromatography.

Since these unusual $tRNA^{Gly}_{UCC}$ genes appear to be on a single transcriptional unit with many other normal tRNA genes, it would seem to make it impossible to regulate their transcription independently of the normal upstream genes. Any regulation of the relative concentrations of these unusual tRNAs would therefore likely be due to processing or turnover. Perhaps the presence of three almost identical copies of these unusual $tRNA^{Gly}_{UCC}$ genes increases the production of their tRNAs relative to that of the normal tRNAs by a simple gene dosage effect. In addition, the rate of synthesis of the cell wall peptidoglycan is likely to be closely linked to the rate of synthesis of protein so that the two types of tRNA may not need separate regulatory mechanisms.

Since the 27-tRNA gene cluster of the nonsporulating bacterium *S. aureus* is larger than any of those found in the

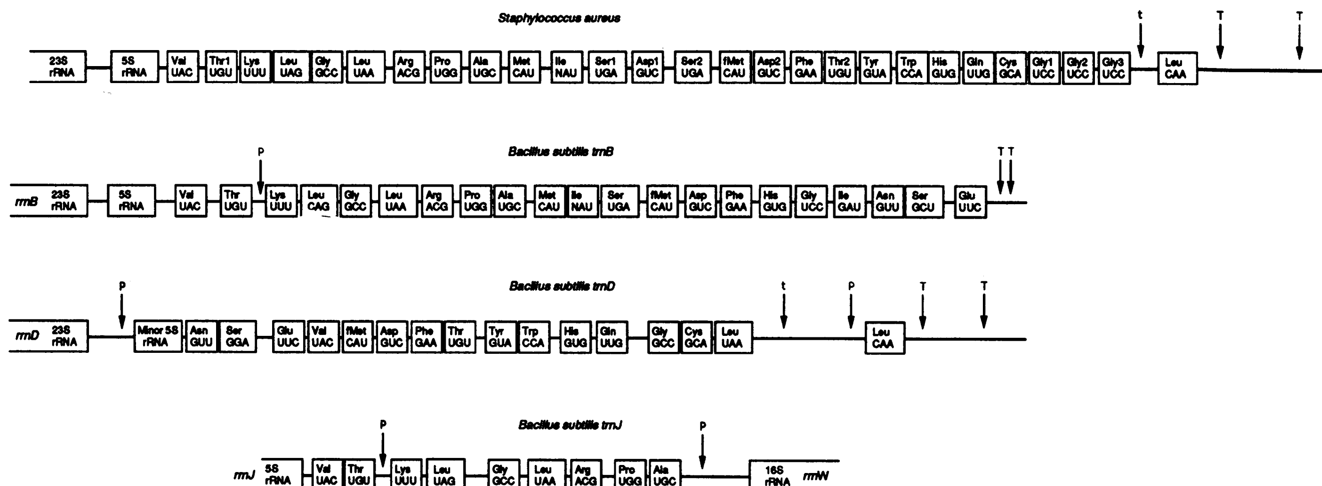


FIG. 4. Comparison of the *S. aureus* rRNA-tRNA gene cluster with *B. subtilis* clusters *trnB*, *trnD*, and *trnJ*, drawn to scale. Putative promoters, terminators, and attenuators are labeled P, T, and t, respectively.

sporulating bacterium *B. subtilis*, it is not likely that a clustered tRNA gene organization is needed exclusively for sporulation. Since sporulation is a common trait in most of the low-G+C gram-positive bacteria (35), it is likely that *S. aureus* evolved from a sporulating ancestor while preserving a clustered tRNA gene organization. Perhaps we should think of extensive tRNA gene clustering as being normal and ask why *E. coli* does not use the same type of organization. One advantage of tRNA gene clustering is that it reduces the number of tRNA transcriptional units. *B. subtilis* has 62 known tRNA genes on only eight transcriptional units (8). In *E. coli*, there are 79 tRNA genes in 41 transcriptional units (9). tRNA ratios in gram-positive bacteria may be maintained by a simple gene dosage effect, with the most common tRNA species being encoded by genes appearing in more than one cluster or repeated within the same cluster.

A disadvantage to having a highly clustered tRNA gene organization is that it is more difficult to finely tune the transcription of individual tRNA genes during different growth conditions. *E. coli* may have a greater need and ability to separately regulate the transcription of some specialized tRNA genes under differing growth conditions (3, 22). The possibility that *E. coli* has more specialized tRNAs for specific uses than are found in the gram-positive bacteria is also suggested by the comparison of the total complement of different tRNAs. The 79 tRNA genes of *E. coli* produce tRNAs with 42 different types of anticodons, including both the initiator and elongator $tRNA^{Met}$ (9). The 62 known genes of *B. subtilis* can produce tRNAs with only 29 different types of anticodons (8). Although there may be a few more *B. subtilis* tRNA genes that have not yet been found, almost all of the tRNA-hybridizing bands on Southern analysis can be accounted for by known tRNA gene clusters (7). This disparity in anticodon complement may have several explanations. Gram-positive bacteria may allow more wobble in the translation of codons and therefore do not need as large a complement of tRNAs (1, 26). *E. coli* may require more special tRNAs to carry out functions such as the one that inserts selenocysteine at certain UGA codons (10). Codon usage and context sensitivity (4, 27) may put more restrictions on which tRNAs can be used to translate a given mRNA in *E. coli*. There is a pronounced codon usage bias in *E. coli*, while *B. subtilis* has a much more uniform codon usage (15). *E. coli* may rely on a more precisely adjusted tRNA population to control translation (3, 22) while the gram-positive bacteria use other means.

The presence of large tRNA gene clusters in the gram-positive bacteria is one of the major distinguishing features of the genomic organization of this group of organisms. The presence of large tRNA gene clusters may be useful in identifying gram-positive bacteria. This type of gene organization is consistent with a less diverse tRNA population and less stringent requirements for codon-anticodon pairing.

## ACKNOWLEDGMENT

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