JOURNAL OF BACTERIOLOGY, Dec. 2000, p. 6913–6920 Vol. 182, No. 24
0021-9193/00/$04.00+0
Copyright © 2000, American Society for Microbiology. All Rights Reserved.

Evidence for Horizontal Gene Transfer in Evolution of Elongation Factor Tu in Enterococci

DANBING KE,[1,2] MAURICE BOISSINOT,[1,2] ANN HULETSKY,[1,2] FRANÇOIS J. PICARD,[1] JOHANNE FRENETTE,[1] MARC OUELLETTE,[1,2] PAUL H. ROY,[1,3] AND MICHEL G. BERGERON[1,2]*

Centre de Recherche en Infectiologie, Centre Hospitalier Universitaire de Québec (Pavillon CHUL), Sainte-Foy, Québec G1V 4G2,[1] and Division de Microbiologie, Faculté de Medicine,[2] and Département de Biochimie et de Microbiologie, Faculté des Sciences et de Genie,[3] Université Laval, Sainte-Foy, Québec G1K 7P4, Canada

Received 23 June 2000/Accepted 26 September 2000

The elongation factor Tu, encoded by *tuf* genes, is a GTP binding protein that plays a central role in protein synthesis. One to three *tuf* genes per genome are present, depending on the bacterial species. Most low-G+C-content gram-positive bacteria carry only one *tuf* gene. We have designed degenerate PCR primers derived from consensus sequences of the *tuf* gene to amplify partial *tuf* sequences from 17 enterococcal species and other phylogenetically related species. The amplified DNA fragments were sequenced either by direct sequencing or by sequencing cloned inserts containing putative amplicons. Two different *tuf* genes (*tufA* and *tufB*) were found in 11 enterococcal species, including *Enterococcus avium*, *Enterococcus casseliflavus*, *Enterococcus dispar*, *Enterococcus durans*, *Enterococcus faecium*, *Enterococcus gallinarum*, *Enterococcus hirae*, *Enterococcus malodoratus*, *Enterococcus mundtii*, *Enterococcus pseudoavium*, and *Enterococcus raffinosus*. For the other six enterococcal species (*Enterococcus cecorum*, *Enterococcus columbae*, *Enterococcus faecalis*, *Enterococcus sulfureus*, *Enterococcus saccharolyticus*, and *Enterococcus solitarius*), only the *tufA* gene was present. Based on 16S rRNA gene sequence analysis, the 11 species having two *tuf* genes all have a common ancestor, while the six species having only one copy diverged from the enterococcal lineage before that common ancestor. The presence of one or two copies of the *tuf* gene in enterococci was confirmed by Southern hybridization. Phylogenetic analysis of *tuf* sequences demonstrated that the enterococcal *tufA* gene branches with the *Bacillus*, *Listeria*, and *Staphylococcus* genera, while the enterococcal *tufB* gene clusters with the genera *Streptococcus* and *Lactococcus*. Primary structure analysis showed that four amino acid residues encoded within the sequenced regions are conserved and unique to the enterococcal *tufB* genes and the *tuf* genes of streptococci and *Lactococcus lactis*. The data suggest that an ancestral streptococcus or a streptococcus-related species may have horizontally transferred a *tuf* gene to the common ancestor of the 11 enterococcal species which now carry two *tuf* genes.

The elongation factor Tu (EF-Tu) is a GTP binding protein playing a central role in protein synthesis. It mediates the recognition and transport of aminoacyl-tRNAs and their positioning to the A site of the ribosome (20). The highly conserved function and ubiquitous distribution render the elongation factor a valuable phylogenetic marker among eubacteria and even throughout the archaebacterial and eukaryotic kingdoms (3, 31). The *tuf* genes encoding EF-Tu are present in various copy numbers per bacterial genome. Most gram-negative bacteria contain two *tuf* genes (5, 15, 19, 39, 41, 43). As found in *Escherichia coli*, the two genes, while being almost identical in sequence, are located in different parts of the bacterial chromosome (15, 20, 41). However, recently completed maps of microbial genomes revealed that only one *tuf* gene is found in *Helicobacter pylori* as well as in some obligate parasitic bacteria, such as *Borrelia burgdorferi*, *Rickettsia prowazekii*, and *Treponema pallidum*, and in some cyanobacteria (16, 18, 24, 32, 41, 44). In most gram-positive bacteria studied so far, only one *tuf* gene was found (8, 14, 17, 22, 28–30, 32, 35, 39). However, Southern hybridization showed that there are two *tuf* genes in some clostridia (39) as well as in *Streptomyces coelicolor* and *Streptomyces lividans* (46, 47). Up to three *tuf*-like genes have been identified in *Streptomyces ramocissimus* (48).

Although massive prokaryotic gene transfer is suggested to be one of the factors responsible for the evolution of bacterial genomes (12, 27, 42), the genes encoding components of the translation machinery are thought to be highly conserved and difficult to transfer horizontally due to the complexity of their interactions (23). However, a few recent studies demonstrated evidence that horizontal gene transfer has also occurred in the evolution of some genes coding for the translation apparatus, namely, 16S rRNA and some aminoacyl-tRNA synthetases (6, 27, 45, 48, 49). No further data suggest that such a mechanism is involved in the evolution of the elongation factors. Previous studies concluded that the two copies of *tuf* genes in the genomes of some bacteria resulted from an ancient event of gene duplication (10, 39). Moreover, a study of the *tuf* gene in *R. prowazekii* suggested that intrachromosomal recombination has taken place in the evolution of the genome of this organism (41).

To date, little is known about the *tuf* genes of enterococcal species. In this study, we analyzed partial sequences of *tuf* genes in 17 enterococcal species, namely, *Enterococcus avium*, *E. casseliflavus*, *E. cecorum*, *E. columbae*, *E. dispar*, *E. durans*, *E. faecalis*, *E. faecium*, *E. gallinarum*, *E. hirae*, *E. malodoratus*, *E. mundtii*, *E. pseudoavium*, *E. raffinosus*, *E. saccharolyticus*, *E. solitarius*, and *E. sulfureus*. We report here the presence of two divergent copies of *tuf* genes in 11 of these enterococcal spe-

* Corresponding author. Mailing address: Centre de Recherche en Infectiologie, Centre Hospitalier Universitaire de Québec, Pavillon CHUL, 2705 Boul. Laurier, Sainte-Foy, Québec G1V 4G2, Canada. Phone: (418) 654-2705. Fax: (418) 654-2715. E-mail: Michel.G.Bergeron@crchul.ulaval.ca.

TABLE 1. *tuf* gene sequences obtained in our laboratory

Species	Strain	Gene(s)	GenBank accession no.
Abiotrophia adiacens	ATCC 49175	*tuf*	AF124224
Enterococcus avium	ATCC 14025	*tufA*	AF124220
		tufB	AF274715
Enterococcus casseliflavus	ATCC 25788	*tufA*	AF274716
		tufB	AF274717
Enterococcus cecorum	ATCC 43198	*tuf*	AF274718
Enterococcus columbae	ATCC 51263	*tuf*	AF274719
Enterococcus dispar	ATCC 51266	*tufA*	AF274720
		tufB	AF274721
Enterococcus durans	ATCC 19432	*tufA*	AF274722
		tufB	AF274723
Enterococcus faecalis	ATCC 29212	*tuf*	AF124221
Enterococcus faecium	ATCC 19434	*tufA*	AF124222
		tufB	AF274724
Enterococcus gallinarum	ATCC 49573	*tufA*	AF124223
		tufB	AF274725
Enterococcus hirae	ATCC 8043	*tufA*	AF274726
		tufB	AF274727
Enterococcus malodoratus	ATCC 43197	*tufA*	AF274728
		tufB	AF274729
Enterococcus mundtii	ATCC 43186	*tufA*	AF274730
		tufB	AF274731
Enterococcus pseudoavium	ATCC 49372	*tufA*	AF274732
		tufB	AF274733
Enterococcus raffinosus	ATCC 49427	*tufA*	AF274734
		tufB	AF274735
Enterococcus saccharolyticus	ATCC 43076	*tuf*	AF274736
Enterococcus solitarius	ATCC 49428	*tuf*	AF274737
Enterococcus sulfureus	ATCC 49903	*tuf*	AF274738
Lactococcus lactis	ATCC 11154	*tuf*	AF274745
Listeria monocytogenes	ATCC 15313	*tuf*	AF274746
Listeria seeligeri	ATCC 35967	*tuf*	AF274747
Staphylococcus aureus	ATCC 25923	*tuf*	AF274739
Staphylococcus epidermidis	ATCC 14990	*tuf*	AF274740
Streptococcus mutans	ATCC 25175	*tuf*	AF274741
Streptococcus pneumoniae	ATCC 6303	*tuf*	AF274742
Streptococcus pyogenes	ATCC 19615	*tuf*	AF274743
Streptococcus suis	ATCC 43765	*tuf*	AF274744

cies. The six other species carried a single *tuf* gene. The evolutionary implications are discussed.

(This study was presented in part at the 100th General Meeting of the American Society for Microbiology, Los Angeles, Calif., 21 to 25 May 2000.)

MATERIALS AND METHODS

Bacterial strains. Seventeen enterococcal strains and other gram-positive bacterial strains obtained from the American Type Culture Collection (ATCC; Manassas, Va.) were used in this study (Table 1). All strains were grown on sheep blood agar or in brain heart infusion broth prior to DNA isolation.

DNA isolation. Bacterial DNAs were prepared using the G NOME DNA extraction kit (Bio101, Vista, Calif.) as previously described (25).

Sequencing of putative *tuf* genes. In order to obtain the *tuf* gene sequences of enterococci and other gram-positive bacteria, two sequencing approaches were used: (i) sequencing of cloned PCR products and (ii) direct sequencing of PCR products. A pair of degenerate primers (U1, 5′-AAYATGATIACIGGIG CIGCICARATGGA-3′, and U3, 5′-CCIACIGTICKICCRCCYTCRCG-3′) were used to amplify an 886-bp portion of the *tuf* genes from enterococcal species and other gram-positive bacteria as previously described (25). For *E. avium*, *E. casseliflavus*, *E. dispar*, *E. durans*, *E. faecium*, *E. gallinarum*, *E. hirae*, *E. mundtii*, *E. pseudoavium*, and *E. raffinosus*, the amplicons were cloned using the Original TA cloning kit (Invitrogen, Carlsbad, Calif.) as previously described (25). Five clones for each species were selected for sequencing. For *E. cecorum*, *E. faecalis*, *E. saccharolyticus*, and *E. solitarius* as well as the other gram-positive bacteria, the sequences of the 886-bp amplicons were obtained by direct sequencing. Based on the results obtained from the earlier rounds of sequencing, two pairs of primers were designed for obtaining the partial *tuf* sequences from the other enterococcal species by direct sequencing. One pair of primers (EntA1, 5′-ATCTTAGTAGTTTCTGCTGCTGA-3′, and EntA2, 5′-GTAGAATTCAG GACGGTAGTTAG-3′) was used to amplify the enterococcal *tuf* gene fragments from *E. columbae*, *E. malodoratus*, and *E. sulfureus*. Another pair of primers (U1 and EntB, 5′-GTAGAAYTGTGGWCGATARTTRT-3′) was used to amplify the second *tuf* gene fragments from *E. avium*, *E. malodoratus*, and *E. pseudoavium*.

Prior to direct sequencing, PCR products were electrophoresed on a 1% agarose gel at 120 V for 2 h. The gel was then stained with 0.02% methylene blue for 30 min and washed twice with autoclaved distilled water for 15 min. The gel slices containing PCR products of the expected sizes were cut out and purified with the QIAquick gel extraction kit (QIAgen Inc., Mississauga, Ontario, Canada) according to the manufacturer's instructions. PCR mixtures for sequencing were prepared as described previously (25). DNA sequencing was carried out with the Big Dye Terminator Ready Reaction cycle sequencing kit using a 377 DNA sequencer (PE Applied Biosystems, Foster City, Calif.). Both strands of the amplified DNA were sequenced. The sequence data were verified using the Sequencher 3.0 software (Gene Codes Corp., Ann Arbor, Mich.).

Sequence analysis and phylogenetic study. Nucleotide sequences of the *tuf* genes and their respective flanking regions in *E. faecalis*, *Staphylococcus aureus*, and *Streptococcus pneumoniae* were retrieved from The Institute for Genomic Research (http://www.tigr.org) microbial genome database, and sequences of *Streptococcus pyogenes* were obtained from the University of Oklahoma database (http://www.genome.ou.edu/strep.html). DNA sequences and deduced protein sequences obtained in this study were compared with those in all publicly available databases by using the BLAST (2) and FASTA programs. Unless specified, sequence analysis was conducted with the programs from the GCG package (version 10; Genetics Computer Group, Madison, Wis.). Sequence alignment of the *tuf* genes from 74 species representing all three kingdoms of life (Tables 1 and 2) was carried out by use of Pileup and was corrected upon visual analysis. The N- and C-terminus extremities of the sequences were trimmed to yield a common block of 201 amino acids, and equivocal residues were removed. Phylogenetic analysis was performed with the aid of PAUP 4.0b4, written by David L. Swofford (Sinauer Associates, Inc., Publishers, Sunderland, Mass.). The distance matrix and maximum parsimony were used to generate phylogenetic trees, and bootstrap resampling procedures were performed, using 500 and 100 replications in each analysis, respectively.

Protein structure analysis. The crystal structures of (i) *Thermus aquaticus* EF-Tu in complex with Phe-tRNA^{Phe} and a GTP analog (34) and (ii) *E. coli* EF-Tu in complex with GDP (40) served as templates for constructing the equivalent models for enterococcal EF-Tu. Homology modeling of protein structure was performed using the SWISS-MODEL server and inspected using the SWISS-PDB viewer version 3.1 (21).

Southern hybridization. In a previous study (25), we amplified and cloned an 803-bp PCR product of the *tuf* gene fragment from *E. faecium*. Two divergent sequences of the inserts, which we assumed to be *tufA* and *tufB* genes, were obtained. The recombinant plasmid carrying either *tufA* or *tufB* sequence was used to generate two probes labeled with digoxigenin (DIG)-11-dUTP by PCR incorporation following the instructions of the manufacturer (Boehringer Mannheim, Laval, Québec, Canada). Enterococcal genomic DNA samples (1 to 2 μg) were digested to completion with restriction endonucleases *Bgl*II and *Xba*I as recommended by the supplier (Amersham Pharmacia Biotech, Mississauga, Ontario, Canada). These restriction enzymes were chosen because no restriction sites were observed within the amplified *tuf* gene fragments of most enterococci. Southern blotting and filter hybridization were performed using positively charged nylon membranes (Boehringer Mannheim) and QuikHyb hybridization solution (Stratagene Cloning Systems, La Jolla, Calif.) according to the manufacturers' instructions, with modifications. Twenty microliters of each digest was electrophoresed for 2 h at 120 V on a 0.8% agarose gel. The DNA fragments were denatured with 0.5 M NaOH and transferred by Southern blotting onto a positively charged nylon membrane (Boehringer Mannheim). The filters were prehybridized for 15 min and then were hybridized for 2 h in the QuikHyb solution at 68°C with either DIG-labeled probe. Posthybridization washings were performed twice with 0.5× SSC–1% sodium dodecyl sulfate (SDS) at room temperature for 15 min and twice in the same solution at 60°C for 15 min (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate). Detection of bound probes was achieved using disodium 3-(4-methoxyspiro(1,2-dioxetane-3,2′-(5′-chloro) tricyclo(3,3.1.1^{3,7})decan)-4-yl) phenyl phosphate (CSPD) (Boehringer Mannheim) as specified by the manufacturer.

Nucleotide sequence accession numbers. The GenBank accession numbers for partial *tuf* gene sequences generated in this study are given in Table 1. Sequences were assigned accession no. AF124220 to AF124224 and AF274715 to AF274747.

RESULTS

Sequencing and nucleotide sequence analysis. In this study, all gram-positive bacteria other than enterococci yielded a single *tuf* sequence of 886 bp using primers U1 and U3 (Table 1). Each of the four enterococcal species *E. cecorum*, *E. faecalis*, *E. saccharolyticus*, and *E. solitarius* also yielded one 886-bp *tuf* sequence. On the other hand, for *E. avium*, *E. casseliflavus*,

TABLE 2. *tuf* gene sequences selected from databases for this study

Species	Gene(s)	Accession no.[a]
Agrobacterium tumefaciens	*tufA*	X99673
	tufB	X99674
Anacystis nidulans	*tuf*	X17442
Aquifex aeolicus	*tufA*	AE000657
	tufB	AE000657
Bacillus stearothermophilus	*tuf*	AJ000260
Bacillus subtilis	*tuf*	AL009126
Bacteroides fragilis	*tuf*	P33165
Borrelia burgdorferi	*tuf*	AE000783
Brevibacterium linens	*tuf*	X76863
Burkholderia cepacia	*tuf*	P33167
Campylobacter jejuni	*tufB*	Y17167
Chlamydia pneumoniae	*tuf*	AE001363
Chlamydia trachomatis	*tuf*	M74221
Corynebacterium glutamicum	*tuf*	X77034
Cytophaga lytica	*tuf*	X77035
Deinococcus radiodurans	*tuf*	AE000513
Escherichia coli	*tufA*	J01690
	tufB	J01717
Fervidobacterium islandicum	*tuf*	Y15788
Haemophilus influenzae	*tufA*	L42023
	tufB	L42023
Helicobacter pylori	*tuf*	AE000511
Homo sapiens (human)	*EF-1α*	X03558
Methanococcus jannaschii	*EF-1α*	U67486
Mycobacterium leprae	*tuf*	D13869
Mycobacterium tuberculosis	*tuf*	X63539
Mycoplasma genitalium	*tuf*	L43967
Mycoplasma pneumoniae	*tuf*	U00089
Neisseria gonorrhoeae	*tufA*	L36380
Nicotiana tabacum (tobacco)	*EF-1α*	U04632
Peptococcus niger	*tuf*	X76869
Planobispora rosea	*tuf1*	U67308
Saccharomyces cerevisiae (yeast)	*EF-1α*	X00779
Salmonella enterica serovar Typhimurium	*tufA*	X55116
	tufB	X55117
Shewanella putrefaciens	*tuf*	P33169
Spirochaeta aurantia	*tuf*	X76874
Spirulina platensis	*tufA*	X15646
Streptomyces aureofaciens	*tuf1*	AF007125
Streptomyces cinnamoneus	*tuf1*	X98831
Streptomyces coelicolor	*tuf1*	X77039
	tuf3	X77040
Streptomyces collinus	*tuf1*	S79408
Streptomyces ramocissimus	*tuf1*	X67057
	tuf2	X67058
	tuf3	X67059
Synechocystis sp.	*tuf*	AB001339
Taxeobacter ocellatus	*tuf*	X77036
Thermotoga maritima	*tuf*	AE000512
Thermus aquaticus	*tuf*	X66322
Thermus thermophilus	*tuf*	X06657
Thiobacillus cuprinus	*tuf*	U78300
Treponema pallidum	*tuf*	AE000520
Wolinella succinogenes	*tuf*	X76872

[a] Sequence data were obtained from GenBank, EMBL, and SWISSPROT databases. Genes were designated as they appear in the references.

E. dispar, *E. durans*, *E. faecium*, *E. gallinarum*, *E. hirae*, *E. mundtii*, *E. pseudoavium*, and *E. raffinosus*, direct sequencing of the 886-bp fragments revealed overlapping peaks according to their sequence chromatograms, suggesting the presence of additional copies of the *tuf* gene. Therefore, the *tuf* gene fragments of these 10 species were cloned first and then sequenced. Sequencing data revealed that two different types of *tuf* sequences (*tufA* and *tufB*) are found in eight of these species, namely, *E. casseliflavus*, *E. dispar*, *E. durans*, *E. faecium*, *E. gallinarum*, *E. hirae*, *E. mundtii*, and *E. raffinosus*. Five clones of both *E. avium* and *E. pseudoavium* yielded only a single *tuf* sequence. These new sequence data allowed the design of new primers specific for the enterococcal *tufA* or *tufB* sequences. Primers EntA1 and EntA2 were designed to amplify only enterococcal *tufA* sequences, and a 694-bp fragment was amplified from all 17 enterococcal species. The 694-bp sequences of *tufA* genes from *E. columbae*, *E. malodoratus*, and *E. sulfureus* were obtained by direct sequencing using these primers. Primers U1 and EntB were designed for the amplification of 730-bp portion of *tufB* genes and yielded the expected fragments from 11 enterococcal species, including *E. malodoratus* and the 10 enterococcal species in which heterogeneous *tuf* sequences were initially found. The sequences of the *tufB* fragments for *E. avium*, *E. malodoratus*, and *E. pseudoavium* were determined by direct sequencing using the primers U1 and EntB. Overall, *tufA* gene fragments were obtained from all 17 enterococcal species but *tufB* gene fragments were obtained from only 11 enterococcal species (Table 1).

The identities between *tufA* and *tufB* for each enterococcal species were 68 to 79% at the nucleotide level and 81 to 89% at the amino acid level. The *tufA* gene is highly conserved among all enterococcal species, with identities ranging from 87 to 99% for DNA and 93 to 99% for amino acid sequences, while the identities among *tufB* genes of enterococci ranged from 77 to 92% for DNA and from 91 to 99% for amino acid sequences, indicating their different origins and evolution (Table 3). Since *E. solitarius* has been transferred to the genus *Tetragenococcus* (13), which is also a low-G+C-content gram-positive bacterium, our sequence comparison did not include this species as an enterococcus. The G+C content of enterococcal *tufA* sequences ranged from 40.8 to 43.1%, while that of enterococcal *tufB* sequences ranged from 37.8 to 46.3%. Based on amino acid sequence comparison, the enterococcal *tufA* gene products shared higher identities with those of *Abiotrophia adiacens*, *Bacillus subtilis*, *Listeria monocytogenes*, *S. aureus*, and *Staphylococcus epidermidis*. On the other hand, the enterococcal *tufB* gene products shared higher percentages of amino acid identity with the *tuf* genes of *S. pneumoniae*, *S. pyogenes*, and *Lactococcus lactis* (Table 3).

In order to elucidate whether the two enterococcal *tuf* sequences encode genuine EF-Tu, the deduced amino acid sequences of both genes were aligned with other EF-Tu sequences available in SWISSPROT (release 38). Sequence alignment demonstrated that both gene products are highly conserved and carry all conserved residues present in this portion of prokaryotic EF-Tu (Fig. 1). Therefore, it appears that both gene products could fulfill the function of EF-Tu. The partial *tuf* gene sequences encode the portion of EF-Tu from residues 117 to 317, according to *E. coli* numbering (40). This portion makes up of the last four α-helices and two β-strands of domain I, the entire domain II, and the N-terminal part of domain III on the basis of the determined structures of *E. coli* EF-Tu (40).

Based on the deduced amino acid sequences, the enterococcal *tufB* genes have unique conserved residues, Lys129, Leu140, Ser230, and Asp234 (*E. coli* numbering), that are also conserved in streptococci and *L. lactis*, but not in the other bacteria (Fig. 1). All these residues are located in loops except for Ser230. In other bacteria the residue Ser230 is replaced by highly conserved Thr, which is the fifth residue of the third β-strand of domain II. This region is partially responsible for the interaction between the EF-Tu and aminoacyl-tRNA by the formation of a deep pocket for any of the 20 naturally occurring amino acids (34, 40). According to our three-dimen-

TABLE 3. Nucleotide and amino acid sequence identities of EF-Tu between different enterococci and other low-G+C-content gram-positive bacteria[a]

Bacterial *tuf* gene	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39
1. *E. avium tufA*		96	98	96	96	96	96	97	95	98	99	95	95	96	94	96	93	86	87	85	86	86	86	86	85	86	87	86	92	91	90	90	90	92	84	85	84	82	83
2. *E. casseliflavus tufA*	90		97	96	96	99	96	95	96	96	96	95	95	96	96	94	93	87	88	86	87	87	86	87	87	87	88	88	94	91	90	91	91	92	86	87	85	85	85
3. *E. dispar tufA*	93	90		95	95	96	95	96	95	97	97	91	90	95	95	95	93	86	87	85	87	87	86	87	86	87	87	87	93	90	89	90	90	92	85	86	84	85	84
4. *E. durans tufA*	90	89	90		98	96	99	93	99	95	96	90	91	94	95	94	92	87	87	86	86	86	85	86	87	87	88	87	94	90	90	90	90	91	85	86	84	84	84
5. *E. faecium tufA*	89	90	89	96		96	98	93	98	95	96	89	91	88	94	93	92	87	88	86	86	87	87	86	87	87	88	87	94	92	91	91	91	93	85	86	84	84	84
6. *E. gallinarum tufA*	90	97	89	89	89		96	93	95	96	96	88	89	89	96	93	92	87	87	86	87	87	87	86	87	87	88	87	93	92	90	90	90	93	85	86	84	83	84
7. *E. hirae tufA*	90	90	89	99	96	89		93	99	95	96	91	91	89	95	94	92	86	87	86	86	86	85	86	86	87	87	87	94	90	90	90	90	91	85	86	84	84	84
8. *E. malodoratus tufA*	96	91	94	90	89	90	89		92	97	97	89	89	90	93	96	92	86	85	82	85	85	85	85	83	85	86	86	92	90	88	88	89	91	83	84	83	83	82
9. *E. mundtii tufA*	89	89	88	96	93	89	96	88		94	95	88	90	88	94	94	92	87	87	86	86	86	85	86	87	87	88	87	94	90	89	90	89	91	85	86	84	84	84
10. *E. pseudoavium tufA*	97	92	93	90	89	91	89	97	89		98	90	90	91	95	96	94	87	87	86	87	87	86	87	86	87	88	88	93	90	89	90	90	91	85	86	85	85	84
11. *E. raffinosus tufA*	97	91	93	90	89	89	89	97	88	97		91	90	90	94	96	93	86	87	85	86	86	85	86	85	87	87	87	93	89	89	90	89	91	84	85	84	84	83
12. *E. cecorum tufA*	90	90	95	96	96	95	96	92	95	95	95		98	95	93	93	93	88	88	87	87	87	86	86	89	87	89	89	93	90	90	91	91	93	86	86	84	85	84
13. *E. columbae tufA*	90	90	95	96	97	96	96	93	95	95	95	97		95	94	92	92	89	88	86	87	88	88	87	87	87	89	89	94	92	91	91	92	93	86	86	85	86	85
14. *E. faecalis tufA*	91	91	90	89	96	97	94	94	94	95	96	90	89		94	94	93	87	87	86	87	87	86	86	87	87	88	87	93	91	89	90	91	93	86	86	86	85	85
15. *E. saccharolyticus tufA*	91	91	91	90	87	90	89	91	89	92	91	89	89	92		94	92	86	87	85	87	86	84	86	85	87	87	87	92	90	89	89	88	90	84	85	84	84	84
16. *E. sulfureus tufA*	91	89	90	91	88	88	90	91	89	92	91	88	89	91	94		91	85	84	81	84	85	84	84	81	84	85	85	91	90	87	88	89	91	82	83	83	82	82
17. *E. solitarius tuf*	83	84	83	83	84	83	82	84	83	84	84	84	83	84	83	83		88	87	86	87	87	86	87	88	88	88	89	92	91	89	90	90	91	86	85	85	85	84
18. *E. avium tufB*	77	77	78	78	76	77	78	78	77	78	77	78	78	78	77	76	77		93	93	94	94	94	92	98	93	99	97	87	86	87	86	85	86	89	88	87	85	86
19. *E. casseliflavus tufB*	71	72	72	72	70	72	72	70	71	72	72	72	70	72	72	68	72	79		93	95	95	96	95	93	95	94	94	87	86	88	88	84	85	90	90	89	88	88
20. *E. dispar tufB*	76	78	77	77	77	77	77	76	77	76	77	77	77	77	78	75	78	82	79		91	91	92	91	94	92	93	93	86	83	85	85	82	84	89	89	87	87	86
21. *E. durans tufB*	77	78	78	78	76	77	78	77	78	77	78	77	77	78	78	75	75	83	80	82		98	95	97	94	97	95	94	87	86	88	88	84	85	90	91	89	88	89
22. *E. faecium tufB*	76	75	76	76	75	77	76	76	76	75	76	77	77	77	76	74	74	80	78	79	86		96	97	95	97	95	94	87	87	88	88	84	86	90	90	89	87	87
23. *E. gallinarum tufB*	72	73	72	73	72	74	72	71	72	72	72	72	72	73	73	72	72	78	81	77	81	82		94	94	95	95	94	85	87	89	89	84	86	90	90	89	87	88
24. *E. hirae tufB*	75	74	75	75	75	75	75	75	76	75	75	74	74	74	75	72	74	80	79	79	84	83	79		93	97	93	94	87	85	86	88	83	85	89	90	88	88	87
25. *E. malodoratus tufB*	76	76	76	77	77	77	77	74	77	76	76	77	75	77	77	73	78	90	79	83	81	80	77	79		93	98	97	87	86	87	87	85	86	88	89	87	85	86
26. *E. mundtii tufB*	74	74	74	75	73	74	74	74	74	74	74	74	74	75	74	71	73	80	80	78	85	85	80	84	80		94	94	87	86	88	88	84	86	90	90	89	88	89
27. *E. pseudoavium tufB*	77	77	78	77	76	78	77	77	76	78	78	77	77	78	78	77	78	91	80	85	84	81	79	80	91	80		98	88	87	88	87	85	87	90	89	88	86	87
28. *E. raffinosus tufB*	78	79	79	78	77	77	78	78	77	79	79	78	78	78	79	77	79	90	79	84	84	81	77	80	90	81	92		87	85	87	88	84	86	90	89	88	88	87
29. *A. adiacens tuf*	88	87	87	86	88	86	86	89	86	88	88	87	88	88	88	90	82	77	70	76	77	76	71	73	77	73	78	78		90	88	89	90	91	85	86	84	85	83
30. *B. subtilis tuf*	81	80	79	79	80	80	79	79	79	80	81	80	81	81	80	78	78	73	69	73	73	71	70	71	72	71	74	74	78		91	92	90	90	82	82	83	82	84
31. *L. monocytogenes tuf*	82	81	82	82	82	82	82	81	81	81	82	81	81	81	81	79	79	76	71	75	75	75	73	74	75	73	78	76	79	82		99	88	90	84	84	84	84	84
32. *L. seeligeri tuf*	82	81	82	82	82	81	82	81	82	81	82	81	82	80	81	79	79	76	71	76	75	74	73	75	75	73	77	76	79	82	99		88	91	84	85	85	84	85
33. *S. aureus tuf*	84	84	83	83	83	84	84	82	84	83	84	86	86	84	82	81	79	75	69	75	75	73	69	72	74	72	74	74	83	79	81	81		96	81	82	82	80	82
34. *S. epidermidis tuf*	83	85	83	84	83	84	84	82	84	83	83	86	87	85	83	82	79	75	69	75	75	73	68	72	74	72	74	75	81	79	82	81	94		83	83	83	83	83
35. *S. mutans tuf*	76	77	76	76	76	77	76	75	76	76	76	77	76	76	76	74	78	79	72	77	78	77	74	75	78	75	78	81	77	75	76	77	74	73		97	96	94	88
36. *S. pneumoniae tuf*	76	77	76	77	77	77	77	75	78	76	76	77	76	77	75	74	75	76	72	76	78	76	73	74	77	75	75	78	75	76	77	76	74	74	87		96	96	89
37. *S. pyogenes tuf*	76	77	76	77	76	75	77	74	77	76	75	76	75	77	75	73	75	74	71	75	78	75	73	74	75	75	75	77	76	77	76	76	73	72	87	93		94	89
38. *S. suis tuf*	74	78	76	76	74	75	76	74	78	76	77	77	75	78	76	73	75	74	71	75	78	74	70	74	75	73	73	77	77	77	77	77	72	73	88	93	91		88
39. *L. lactis tuf*	75	76	75	76	75	75	76	75	76	76	76	77	76	76	75	72	74	75	72	75	77	76	71	75	74	75	75	75	75	75	77	76	74	74	80	73	82	81	

[a] The data are percent sequence identities. The data in the upper right triangle represent the deduced amino acid sequence identities of EF-Tu of gram-positive bacteria, while the data in the lower left triangle represent the DNA sequence identities of the corresponding *tuf* genes. The sequence identities between different enterococcal *tufA* genes are boxed, while those between enterococcal *tufB* genes are shaded.

sional model (data not illustrated), the substitution Thr230→Ser in domain II of EF-Tu may have little impact on the ability of the pocket to accommodate any amino acid. However, the high conservation of Thr230 compared to the unique Ser substitution found only in streptococci and 11 enterococci could suggest a subtle functional role for this residue.

The *tuf* gene sequences obtained for *E. faecalis*, *S. aureus*, *S. pneumoniae*, and *S. pyogenes* were compared with their respective incomplete genome sequences (http://www.tigr.org/tdb/mdb/mdbinprogress.html). Contigs with greater than 99% identity were identified. Analysis of the *E. faecalis* genome data revealed that the single *E. faecalis tuf* gene is located within an *str* operon in which *tuf* is preceded by *fus*, which encodes the elongation factor G. This *str* operon is present in *S. aureus* and *B. subtilis* but not in the two streptococcal genomes examined. The 700-bp or so sequence upstream of the *S. pneumoniae tuf* gene has no homology with any known gene sequences. In *S. pyogenes*, the gene upstream of *tuf* is similar to a cell division gene, *ftsW*, suggesting that the *tuf* genes in streptococci are not arranged in an *str* operon.

Phylogenetic analysis. Phylogenetic analysis of the *tuf* amino acid sequences with representatives of eubacteria, archaebacteria, and eukaryotes using neighbor-joining and maximum parsimony methods showed three major clusters representing the three kingdoms of life. Both methods yielded similar topologies consistent with the rRNA gene data (data not shown). Within the bacterial clade, the tree is polyphyletic, but *tufA* genes from all enterococcal species always clustered with those from other low-G+C-content gram-positive bacteria (except for streptococci and lactococci), while the *tufB* genes of the 11 enterococcal species form a distinct cluster with streptococci and *L. lactis* (Fig. 2). Duplicated genes from the same organism did not cluster together, thereby not suggesting evolution by recent gene duplication.

Southern hybridization. Southern hybridization of *Bgl*II-*Xba*I-digested genomic DNA from 12 enterococcal species tested with the *tufA* probe (DIG-labeled *tufA* fragment from *E. faecium*) yielded two bands of different sizes in nine species, which also carried two divergent *tuf* sequences according to their sequencing data. For *E. faecalis* and *E. solitarius*, a single band was observed, indicating that one *tuf* gene is present (Fig. 3). A single band was also found when digested genomic DNAs from *S. aureus*, *S. pneumoniae*, and *S. pyogenes* were hybridized with the *tufA* probe (data not shown). For *E. faecium*, the presence of three bands can be explained by the existence of an *Xba*I restriction site in the middle of the *tufA* sequence, which was confirmed by sequencing data. Hybridization with the *tufB* probe (DIG-labeled *tufB* fragment of *E. faecium*) showed a banding profile similar to the one obtained with the *tufA* probe (data not shown).

DISCUSSION

In this study, we have shown that two divergent copies of genes encoding EF-Tu are present in some enterococcal species. Sequence data revealed that both genes are highly conserved at the amino acid level. One copy (*tufA*) is present in all enterococcal species, while the other (*tufB*) is present in only 11 of the 17 enterococcal species studied. Based on 16S rRNA sequence analysis, these 11 species are members of three different enterococcal subgroups (*E. avium*, *E. faecium*, and *E.*

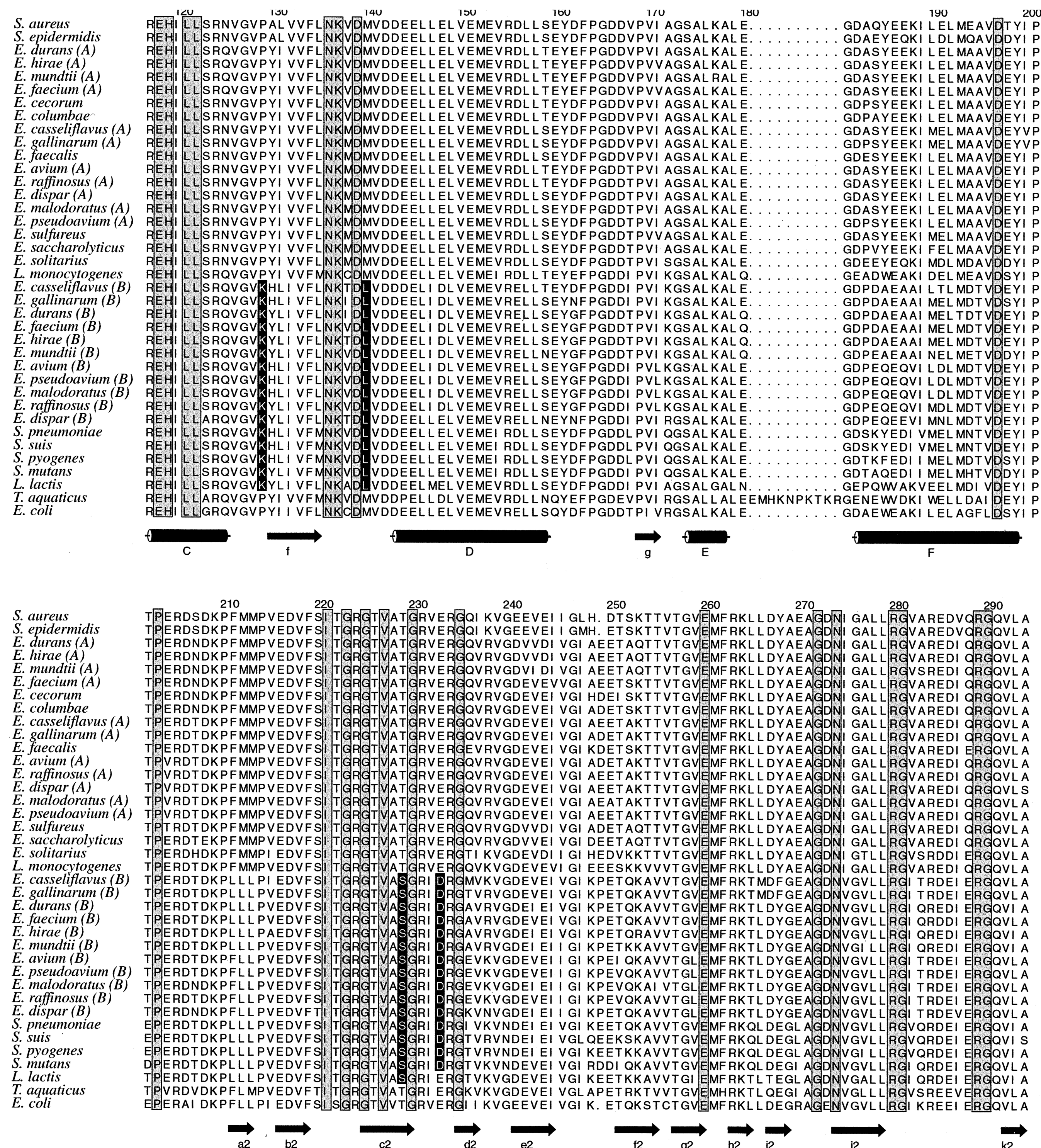

FIG. 1. Abridged multiple amino acid sequence alignment of the partial *tuf* gene products from selected species by the program Alscript (4). Residues highly conserved in bacteria (34) are boxed in grey and gaps are represented with dots. Residues in reverse print are unique to the enterococcal *tufB* gene as well as to streptococcal and lactococcal *tuf* gene products. Numbering is based on *E. coli* EF-Tu, and secondary structure elements of *E. coli* EF-Tu are represented by cylinders (α-helices) and arrows (β-strands) (40).

gallinarum species groups) and a distinct species (*E. dispar*). Moreover, 16S rDNA phylogeny suggests that the 11 species that possess two *tuf* genes all have a common ancestor from which they evolved further to become the current species (36). Since the six other species having only one copy diverged from the enterococcal lineage before that common ancestor, it appears that the presence of one *tuf* gene in these six species is not attributable to gene loss.

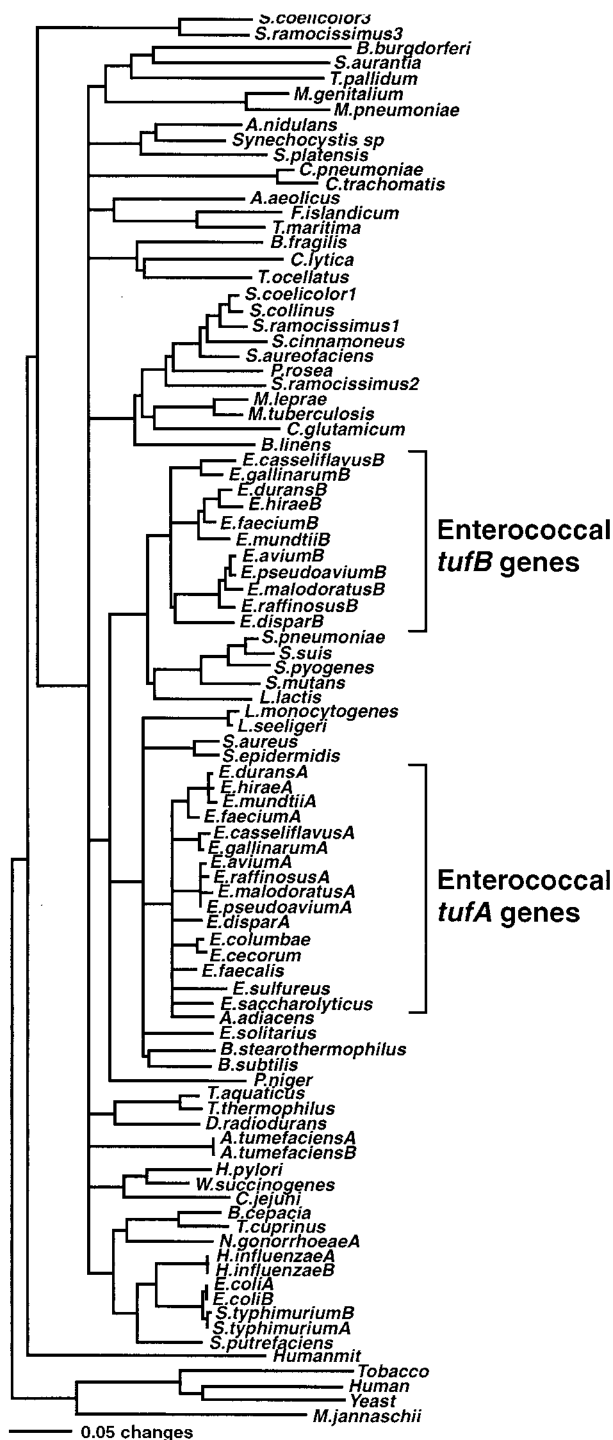

FIG. 2. Distance matrix tree of bacterial EF-Tu based on amino acid sequence homology. The tree was constructed by the neighbor-joining method. The tree was rooted using archaeal and eukaryotic EF-1α genes as the outgroup. The scale bar represents 5% changes in amino acid sequence, as determined by taking the sum of all of the horizontal lines connecting two species.

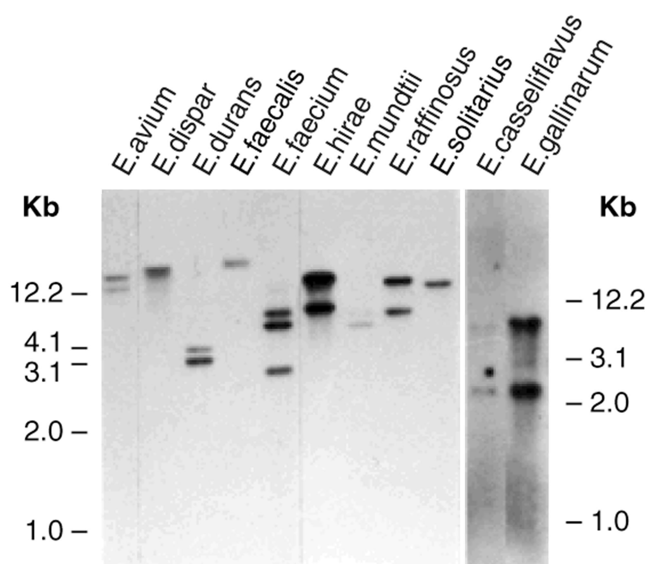

FIG. 3. Southern hybridization of *Bgl*II-*Xba*I-digested genomic DNAs of some enterococci (except for *E. casseliflavus* and *E. gallinarum*, whose genomic DNA was digested with *Bam*HI-*Pvu*II) using the *tufA* gene fragment of *E. faecium* as a probe. The sizes of hybridizing fragments are shown in kilobases. Strains tested are listed in Table 1.

Two clusters of low-G+C-content gram-positive bacteria were observed in the phylogenetic tree of the *tuf* genes: one contained a majority of low-G+C-content gram-positive bacteria and the other contained lactococci and streptococci. This is similar to a previous finding based on phylogenetic analysis of the 16S rRNA gene and the *hrcA* gene coding for a unique heat shock regulatory protein (1). The enterococcal *tufA* genes branched with most of the low-G+C-content gram-positive bacteria, suggesting that they originated from a common ancestor. On the other hand, the enterococcal *tufB* genes branched with the genera *Streptococcus* and *Lactococcus*, which form a distinct lineage separated from other low-G+C-content gram-positive bacteria (Fig. 2). The finding that these EF-Tu proteins share some conserved amino acid residues unique to this branch also supports the idea that they may have a common ancestor. Although these conserved residues might result from convergent evolution upon a specialized function, such convergence at the sequence level, even for a few residues, seems to be rare, making it an unlikely event. Moreover, no currently known selective pressure, if any, would account for keeping one versus two *tuf* genes in bacteria. The G+C contents of enterococcal *tufA* and *tufB* sequences are similar, indicating that they both originated from low-G+C-content gram-positive bacteria, in accordance with the phylogenetic analysis.

The *tuf* genes are present in various copy numbers in different bacteria. Furthermore, the two *tuf* genes are normally associated with characteristic flanking genes (10). The two *tuf* gene copies commonly encountered within gram-negative bacteria are part of either the bacterial *str* operon or the tRNA-*tufB* operon (5, 10, 41). The arrangement of *tufA* in the *str* operon was also found in a variety of bacteria, including *Thermotoga maritima*, the earliest divergent bacterium sequenced so far (33), *Aquifex aeolicus* (11), cyanobacteria (7, 24), *Bacillus* spp. (28, 29), *Micrococcus luteus* (35), *Mycobacterium tuberculosis* (9), and *Streptomyces* spp. (46, 47). Furthermore, the tRNA-*tufB* operon has also been identified in *A. aeolicus* (11), *Thermus thermophilus* (38), and *Chlamydia trachomatis* (10). The two widespread *tuf* gene arrangements argue in favor of their ancient origins (10). It is noteworthy that most obligate intracellular parasites, such as *Mycoplasma* spp. (17, 22), *R. prowazekii* (41), *B. burgdorferi* (16), and *T. pallidum* (18), contain only one *tuf* gene. Their flanking sequences are distinct

from the two conserved patterns as a result of selection for effective propagation by an extensive reduction in genome size by intragenomic recombination and rearrangement (10, 16, 18, 41).

Most gram-positive bacteria with low G+C content that have been sequenced to date contain only a single copy of the *tuf* gene as a part of the *str* operon. This is the case for *B. subtilis*, *S. aureus*, and *E. faecalis*. PCR amplification using a primer targeting a conserved region of the *fus* gene and the *tufA*-specific primer EntA2, but not the *tufB*-specific primer EntB, yielded the expected amplicons for all 17 enterococcal species tested, indicating the presence of the *fus-tuf* organization in all enterococci (data not shown). However, in the genomes of *S. pneumoniae* and *S. pyogenes*, the sequences flanking the *tuf* genes differ, although the *tuf* gene itself remains highly conserved. The enterococcal *tufB* genes are clustered with those of streptococci, but at present we do not have enough data to identify the genes flanking the enterococcal *tufB* genes. Furthermore, the functional role of the enterococcal *tufB* genes remains unknown. One can only postulate that the two divergent gene copies are expressed under different conditions.

The amino acid sequence identities between the enterococcal *tufA* and *tufB* genes are lower than either of (i) those between the enterococcal *tufA* and the *tuf* genes from other low-G+C-content gram-positive bacteria (streptococci and lactococci excluded) or (ii) those between the enterococcal *tufB* and streptococcal and lactococcal *tuf* genes. These findings suggest that the enterococcal *tufA* genes have a common ancestor with other low-G+C-content gram-positive bacteria via the simple scheme of vertical evolution, while the enterococcal *tufB* genes are more closely related to those of streptococci and lactococci. The facts that some enterococci possess an additional *tuf* gene and that the single streptococcal *tuf* gene is not clustered with those of other low-G+C-content gram-positive bacteria cannot be explained by the mechanism of gene duplication or intrachromosomal recombination. According to sequence and phylogenetic analysis, we propose that the presence of the additional copy of the *tuf* gene in 11 enterococcal species is due to horizontal gene transfer. The common ancestor of the 11 enterococcal species now carrying *tufB* genes acquired a *tuf* gene from an ancestral streptococcus or a streptococcus-related species through gene transfer during enterococcal evolution before the diversification of modern enterococci. Further study of the flanking regions of the gene may provide more clues to the origin and function of this gene in enterococci.

Recent studies of genes and genomes have demonstrated that considerable horizontal transfer occurred in the evolution of aminoacyl-tRNA synthetases in all three kingdoms of life (6, 26, 48). The heterogeneity of 16S rRNA is also attributable to horizontal gene transfer in some bacteria, such as *Streptomyces*, *Thermomonospora chromogena*, and *Mycobacterium celatum* (37, 45, 49). In this study, we provide the first example in support of a likely horizontal transfer of the *tuf* gene encoding EF-Tu. This may be an exception since stringent functional constraints do not allow for frequent horizontal transfer of the *tuf* gene as with other genes. However, enterococcal *tuf* genes should not be the only such exception as we have noticed that the phylogeny of *Streptomyces tuf* genes is at least as complex as that of enterococci. For example, the three *tuf*-like genes in one high-G+C-content gram-positive bacterium, *S. ramocissimus*, branched with the *tuf* genes of phylogenetically divergent groups of bacteria (Fig. 2). Another example may be the *tuf* genes in clostridia, which represent a phylogenetically very broad range of organisms and form a plethora of lines and groups of various complexities and depths. Four species belonging to three different clusters within the genus *Clostridium* have been shown by Southern hybridization to carry two copies of the *tuf* gene (39). Further sequence data and phylogenetic analysis may help in interpreting the evolution of EF-Tu in these gram-positive bacteria. Since the *tuf* genes and 16S rRNA genes are often used for phylogenetic study, the existence of duplicate genes originating from horizontal gene transfer may alter the phylogeny of microorganisms when the laterally acquired copy of the gene is used for such analyses. Hence, caution should be taken in interpreting phylogenetic data. In addition, the two *tuf* genes in enterococci have evolved separately and are distantly related to each other phylogenetically. The enterococcal *tufB* genes are less conserved and unique to the 11 enterococcal species. We previously demonstrated that the enterococcal *tufA* genes could serve as a target to develop a DNA-based assay for identification of enterococci (25). The enterococcal *tufB* genes would also be useful in the identification of these 11 enterococcal species.

ACKNOWLEDGMENTS

We thank members of the Rapid Diagnostic group at the Centre de Recherche en Infectiologie of Laval University for their help in obtaining the *tuf* sequences. We thank Sonia Paradis and Pascal Lapierre for their help with phylogenetic analysis and Dominique Boudreau for his contribution to the three-dimensional structure analysis of EF-Tu and preparation of figures. Sequencing of *E. faecalis*, *S. aureus*, and *S. pneumoniae* genomes by the Institute for Genomic Research was accomplished with support from The National Institute of Allergy and Infectious Diseases, National Institutes of Health. We also thank the Streptococcal Genome Sequencing Project funded by USPHS/NIH grant no. AI38406 and B. A. Roe, S. P. Linn, L. Song, X. Yuan, S. Clifton, R. E. McLaughlin, M. McShan, and J. Ferretti from Department of Chemistry and Biochemistry, the University of Oklahoma, Norman, and the University of Oklahoma Health Science Center, Department of Microbiology and Immunology, Oklahoma City, for making available the *S. pyogenes* genomic sequence before publication.

This study was supported by grant PA-15586 from the Medical Research Council (MRC) of Canada and by Infectio Diagnostic (I.D.I.) Inc., Sainte-Foy, Québec, Canada. M. Ouellette is an MRC Scientist.

REFERENCES

1. **Ahmad, S., A. Selvapandiyan, and R. K. Bhatnagar.** 1999. A protein-based phylogenetic tree for gram-positive bacteria derived from *hrcA*, a unique heat-shock regulatory gene. Int. J. Syst. Bacteriol. **49:**1387–1394.
2. **Altschul, S. F., T. L. Madden, A. A. Schaffer, J. Zhang, Z. Zhang, W. Miller, and D. J. Lipman.** 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. **25:**3389–3402.
3. **Baldauf, S. L., J. D. Palmer, and W. F. Doolittle.** 1996. The root of the universal tree and the origin of eukaryotes based on elongation factor phylogeny. Proc. Natl. Acad. Sci. USA **93:**7749–7754.
4. **Barton, G. J.** 1993. ALSCRIPT: a tool to format multiple sequence alignments. Protein Eng. **6:**37–40.
5. **Bremaud, L., C. Fremaux, S. Laalami, and Y. Cenatiempo.** 1995. Genetic and molecular analysis of the tRNA-*tufB* operon of the myxobacterium *Stigmatella aurantiaca*. Nucleic Acids Res. **23:**1737–1743.
6. **Brown, J. R., and W. F. Doolittle.** 1999. Gene descent, duplication, and horizontal transfer in the evolution of glutamyl- and glutaminyl-tRNA synthetases. J. Mol. Biol. **49:**485–495.
7. **Buttarelli, F. R., R. A. Calogero, O. Tiboni, C. O. Gualerzi, and C. L. Pon.** 1989. Characterisation of the *str* operon genes from *Spirulina platensis* and their evolutionary relationship to those of other prokaryotes. Mol. Gen. Genet. **217:**97–104.
8. **Carlin, N. I. A., S. Lofdahl, and M. Magnusson.** 1992. Monoclonal antibodies specific for elongation factor Tu and complete nucleotide sequence of the *tuf* gene in *Mycobacterium tuberculosis*. Infect. Immun. **60:**3136–3142.
9. **Cole, S. T., R. Brosch, J. Parkhill, T. Garnier, C. Churcher, D. Harris, S. V. Gordon, K. Eiglmeier, S. Gas, C. E. Barry III, F. Tekaia, K. Badcock, D. Basham, D. Brown, T. Chillingworth, R. Connor, R. Davies, K. Devlin, T. Feltwell, S. Gentles, N. Hamlin, S. Holroyd, T. Hornsby, K. Jagels, B. G. Barrell, et al.** 1998. Deciphering the biology of *Mycobacterium tuberculosis* from the complete genome sequence. Nature **393:**537–544.

10. **Cousineau, B., C. Cerpa, J. Lefebvre, and R. Cedergren.** 1992. The sequence of the gene encoding elongation factor Tu from *Chlamydia trachomatis* compared with those of other organisms. Gene **120:**33–41.
11. **Deckert, G., P. V. Warren, T. Gaasterland, W. G. Young, A. L. Lenox, D. E. Graham, R. Overbeek, M. A. Snead, M. Keller, M. Aujay, R. Huber, R. A. Feldman, J. M. Short, G. J. Olsen, and R. V. Swanson.** 1998. The complete genome of the hyperthermophilic bacterium *Aquifex aeolicus*. Nature **392:** 353–358.
12. **Doolittle, R. F.** 1998. Microbial genomes opened up. Nature **392:**339–342.
13. **Facklam, R. R., D. F. Sahm, and L. M. Teixeira.** 1999. *Enterococcus*, p. 297–305. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.), Manual of clinical microbiology, 7th ed. ASM Press, Washington, D.C.
14. **Filer, D., and A. V. Furano.** 1981. Duplication of the *tuf* gene, which encodes peptide chain elongation factor Tu, is widespread in gram-negative bacteria. J. Bacteriol. **148:**1006–1011.
15. **Fleischmann, R. D., M. D. Adams, O. White, R. A. Clayton, E. F. Kirkness, A. R. Kerlavage, C. J. Bult, J. F. Tomb, B. A. Dougherty, J. M. Merrick, et al.** 1995. Whole-genome random sequencing and assembly of *Haemophilus influenzae* Rd. Science **269:**496–512.
16. **Fraser, C. M., S. Casjens, W. M. Huang, G. G. Sutton, R. Clayton, R. Lathigra, O. White, K. A. Ketchum, R. Dodson, E. K. Hickey, M. Gwinn, B. Dougherty, J. F. Tomb, R. D. Fleischmann, D. Richardson, J. Peterson, A. R. Kerlavage, J. Quackenbush, S. Salzberg, M. Hanson, R. van Vugt, N. Palmer, M. D. Adams, J. Gocayne, J. C. Venter, et al.** 1997. Genomic sequence of a Lyme disease spirochaete, *Borrelia burgdorferi*. Nature **390:**580–586.
17. **Fraser, C. M., J. D. Gocayne, O. White, M. D. Adams, R. A. Clayton, R. D. Fleischmann, C. J. Bult, A. R. Kerlavage, G. Sutton, J. M. Kelley, et al.** 1995. The minimal gene complement of *Mycoplasma genitalium*. Science **270:**397–403.
18. **Fraser, C. M., S. J. Norris, G. M. Weinstock, O. White, G. G. Sutton, R. Dodson, M. Gwinn, E. K. Hickey, R. Clayton, K. A. Ketchum, E. Sodergren, J. M. Hardham, M. P. McLeod, S. Salzberg, J. Peterson, H. Khalak, D. Richardson, J. K. Howell, M. Chidambaram, T. Utterback, L. McDonald, P. Artiach, C. Bowman, M. D. Cotton, J. C. Venter, et al.** 1998. Complete genome sequence of *Treponema pallidum*, the syphilis spirochete. Science **281:**375–388.
19. **Goldstein, B. P., G. Zaffaroni, O. Tiboni, B. Amiri, and M. Denaro.** 1989. Determination of the number of *tuf* genes in *Chlamydia trachomatis* and *Neisseria gonorrhoeae*. FEMS Microbiol. Lett. **60:**305–310.
20. **Grunberg-Manago, M.** 1996. Regulation of the expression of aminoacyl-tRNA synthetases and translation factors, p. 1432–1457. *In* F. C. Neidhardt, R. Curtiss III, J. L. Ingraham, E. C. C. Lin, K. B. Low, B. Magasanik, W. S. Reznikoff, M. Riley, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella*: cellular and molecular biology, 2nd ed., vol. 2. ASM Press, Washington, D.C.
21. **Guex, N., and M. C. Peitsch.** 1997. SWISS-MODEL and the Swiss-Pdb-Viewer: an environment for comparative protein modeling. Electrophoresis **18:**2714–2723.
22. **Himmelreich, R., H. Hilbert, H. Plagens, E. Pirkl, B. C. Li, and R. Herrmann.** 1996. Complete sequence analysis of the genome of the bacterium *Mycoplasma pneumoniae*. Nucleic Acids Res. **24:**4420–4449.
23. **Jain, R., M. C. Rivera, and J. A. Lake.** 1999. Horizontal gene transfer among genomes: the complexity hypothesis. Proc. Natl. Acad. Sci. USA **96:**3801–3806.
24. **Kaneko, T., S. Sato, H. Kotani, A. Tanaka, E. Asamizu, Y. Nakamura, N. Miyajima, M. Hirosawa, M. Sugiura, S. Sasamoto, T. Kimura, T. Hosouchi, A. Matsuno, A. Muraki, N. Nakazaki, K. Naruo, S. Okumura, S. Shimpo, C. Takeuchi, T. Wada, A. Watanabe, M. Yamada, M. Yasuda, and S. Tabata.** 1996. Sequence analysis of the genome of the unicellular cyanobacterium Synechocystis sp. strain PCC6803. II. Sequence determination of the entire genome and assignment of potential protein-coding regions. DNA Res. **3:**109–136.
25. **Ke, D., F. J. Picard, F. Martineau, C. Ménard, P. H. Roy, M. Ouellette, and M. G. Bergeron.** 1999. Development of a PCR assay for detection of enterococci at the genus level. J. Clin. Microbiol. **37:**3497–3503.
26. **Koonin, E. V., and L. Aravind.** 1998. Genomics: re-evaluation of translation machinery evolution. Curr. Biol. **8:**R266–R269.
27. **Koonin, E. V., and M. Y. Galperin.** 1997. Prokaryotic genomes: the emerging paradigm of genome-based microbiology. Curr. Opin. Genet. Dev. **7:**757–763.
28. **Krasny, L., J. R. Mesters, L. N. Tieleman, B. Kraal, V. Fucik, R. Hilgenfeld, and J. Jonak.** 1998. Structure and expression of elongation factor Tu from *Bacillus stearothermophilus*. J. Mol. Biol. **283:**371–381.
29. **Kunst, F., N. Ogasawara, I. Moszer, A. M. Albertini, G. Alloni, V. Azevedo, M. G. Bertero, P. Bessieres, A. Bolotin, S. Borchert, R. Borriss, L. Boursier, A. Brans, M. Braun, S. C. Brignell, S. Bron, S. Brouillet, C. V. Bruschi, B. Caldwell, V. Capuano, N. M. Carter, S. K. Choi, J. J. Codani, I. F. Connerton, A. Danchin, et al.** 1997. The complete genome sequence of the gram-positive bacterium *Bacillus subtilis*. Nature **390:**249–256.
30. **Ladefoged, S. A., and G. Christiansen.** 1991. Analysis of the nucleotide sequence of the *Mycoplasma hominis tuf* gene and its flanking region. FEMS Microbiol. Lett. **63:**133–139.
31. **Ludwig, W., J. Neumaier, N. Klugbauer, E. Brockmann, C. Roller, S. Jilg, K. Reetz, I. Schachtner, A. Ludvigsen, M. Bachleitner, U. Fischer, and K. H. Schleifer.** 1993. Phylogenetic relationships of Bacteria based on comparative sequence analysis of elongation factor Tu and ATP-synthase β-subunit genes. Antonie Leeuwenhoek **64:**285–305.
32. **Ludwig, W., M. Weizenegger, D. Betzl, E. Leidel, T. Lenz, A. Ludvigsen, D. Mollenhoff, P. Wenzig, and K. H. Schleifer.** 1990. Complete nucleotide sequences of seven eubacterial genes coding for the elongation factor Tu: functional, structural and phylogenetic evaluations. Arch. Microbiol. **153:** 241–247.
33. **Nelson, K. E., R. A. Clayton, S. R. Gill, M. L. Gwinn, R. J. Dodson, D. H. Haft, E. K. Hickey, J. D. Peterson, W. C. Nelson, K. A. Ketchum, L. McDonald, T. R. Utterback, J. A. Malek, K. D. Linher, M. M. Garrett, A. M. Stewart, M. D. Cotton, M. S. Pratt, C. A. Phillips, D. Richardson, J. Heidelberg, G. G. Sutton, R. D. Fleischmann, J. A. Eisen, C. M. Fraser, et al.** 1999. Evidence for lateral gene transfer between Archaea and bacteria from genome sequence of *Thermotoga maritima*. Nature **399:**323–329.
34. **Nissen, P., M. Kjeldgaard, S. Thirup, G. Polekhina, L. Reshetnikova, B. F. Clark, and J. Nyborg.** 1995. Crystal structure of the ternary complex of Phe-tRNA^{Phe}, EF-Tu, and a GTP analog. Science **270:**1464–1472.
35. **Ohama, T., F. Yamao, A. Muto, and S. Osawa.** 1987. Organization and codon usage of the streptomycin operon in *Micrococcus luteus*, a bacterium with a high genomic G+C content. J. Bacteriol. **169:**4770–4777.
36. **Patel, R., K. E. Piper, M. S. Rouse, J. M. Steckelberg, J. R. Uhl, P. Kohner, M. K. Hopkins, F. R. Cockerill III, and B. C. Kline.** 1998. Determination of 16S rRNA sequences of enterococci and application to species identification of nonmotile *Enterococcus gallinarum* isolates. J. Clin. Microbiol. **36:**3399–3407.
37. **Reischl, U., K. Feldmann, L. Naumann, B. J. M. Gaugler, B. Ninet, B. Hirschel, and S. Emler.** 1998. 16S rRNA sequence diversity in *Mycobacterium celatum* strains caused by presence of two different copies of 16S rRNA gene. J. Clin. Microbiol. **36:**1761–1764.
38. **Satoh, M., T. Tanaka, A. Kushiro, T. Hakoshima, and K. Tomita.** 1991. Molecular cloning, nucleotide sequence and expression of the *tufB* gene encoding elongation factor Tu from *Thermus thermophilus* HB8. FEBS Lett. **288:**98–100.
39. **Sela, S., D. Yogev, S. Razin, and H. Bercovier.** 1989. Duplication of the *tuf* gene: a new insight into the phylogeny of eubacteria. J. Bacteriol. **171:**581–584.
40. **Song, H., M. R. Parsons, S. Rowsell, G. Leonard, and S. E. Phillips.** 1999. Crystal structure of intact elongation factor EF-Tu from *Escherichia coli* in GDP conformation at 2.05 Å resolution. J. Mol. Biol. **285:**1245–1256.
41. **Syvanen, A. C., H. Amiri, A. Jamal, S. G. E. Andersson, and C. G. Kurland.** 1996. A chimeric disposition of the elongation factor genes in *Rickettsia prowazekii*. J. Bacteriol. **178:**6192–6199.
42. **Syvanen, M.** 1994. Horizontal gene transfer: evidence and possible consequences. Annu. Rev. Genet. **28:**237–261.
43. **Tiboni, O., G. D. Pasquale, and O. Ciferri.** 1984. Two *tuf* genes in the cyanobacterium *Spirulina platensis*. J. Bacteriol. **159:**407–409.
44. **Tomb, J. F., O. White, A. R. Kerlavage, R. A. Clayton, G. G. Sutton, R. D. Fleischmann, K. A. Ketchum, H. P. Klenk, S. Gill, B. A. Dougherty, K. Nelson, J. Quackenbush, L. Zhou, E. F. Kirkness, S. Peterson, B. Loftus, D. Richardson, R. Dodson, H. G. Khalak, A. Glodek, K. McKenney, L. M. Fitzegerald, N. Lee, M. D. Adams, J. C. Venter, et al.** 1997. The complete genome sequence of the gastric pathogen *Helicobacter pylori*. Nature **388:** 539–547.
45. **Ueda, K., T. Seki, T. Kudo, T. Yoshida, and M. Kataoka.** 1999. Two distinct mechanisms cause heterogeneity of 16S rRNA. J. Bacteriol. **181:**78–82.
46. **van Wezel, G. P., L. P. Woudt, R. Vervenne, M. L. A. Verdurmen, E. Vijgenboom, and L. Bosch.** 1994. Cloning and sequencing of the *tuf* genes of *Streptomyces coelicolor* A3(2). Biochim. Biophys. Acta **1219:**543–547.
47. **Vijgenboom, E., L. P. Woudt, P. W. H. Heinstra, K. Rietveld, J. van Haarlem, G. P. van Wezel, S. Shochat, and L. Bosch.** 1994. Three *tuf*-like genes in the kirromycin producer *Streptomyces ramocissimus*. Microbiology **140:**983–998.
48. **Wolf, Y. I., L. Aravind, N. V. Grishin, and E. V. Koonin.** 1999. Evolution of aminoacyl-tRNA synthetases—analysis of unique domain architectures and phylogenetic trees reveals a complex history of horizontal gene transfer events. Genome Res. **9:**689–710.
49. **Yap, W. H., Z. Zhang, and Y. Wang.** 1999. Distinct types of rRNA operons exist in the genome of the actinomycete *Thermomonospora chromogena* and evidence for horizontal transfer of an entire rRNA operon. J. Bacteriol. **181:**5201–5209.