
SHORT
COMMUNICATIONS

Phylogenetic Analysis of Type Strains of the *Salivarius* Group of the Genus *Streptococcus* Based on Their 16S rRNA Gene Sequences

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Phylogenetic relations of living organisms can be studied by comparing their genes and particular gene regions encoding ribosomal RNA. Data on the completely or partially sequenced rRNA genes of various microorganisms are deposited in international databases and are available via the Internet. The nucleotide sequences of ribosomal genes are widely used for taxonomic identification of bacteria [1, 2]. Relevant studies mostly use the nucleotide sequences of the small (16S) subunit of rRNA, whose gene is smaller and, hence, is less variable than that of the large (23S) rRNA subunit.

16S rRNA gene sequences are often used for the species identification of microflora in dairy products. Since *Streptococcus thermophilus* is the only streptococcal culture used as a starter in the production of fermented dairy products in Russia, the present work was aimed at studying the diversity of 16S rRNA gene sequences in various representatives of the genus *Streptococcus* in order to reveal intrageneric differences in these sequences and to elucidate the possibility of using this approach for the taxonomic identification of closely related species of this genus.

The objects for investigation were the nucleotide sequences of the 16S rRNA genes of ten typical representatives of streptococci deposited in the NCBI database.

The nucleotide sequences were analyzed using the Vector NTI: ContigExpress program. The sequences were aligned with the aid of the ClustalX program. The Mega 2.1 program was used to perform phylogenetic analysis, to construct dendrograms, and to calculate genetic distances.

First of all, we searched the available databases for the complete nucleotide sequences of the 16S rRNA genes of the ten typical representatives of the genus *Streptococcus*: *S. thermophilus* ATCC 19258 (AY188354), *S. salivarius* ATCC 7073 (AY188352),

S. vestibularis ATCC 49124 (AY188353), *S. macedonicus* ACA-DC-206 (SMZ94012), *S. bovis* ATCC 27960 (AB002481), *S. mitis* ATCC 903 (AY281078), *S. gordonii* ATCC 10558 (AY485606), *S. equinus* ATCC 9812 (AJ301607), *S. oralis* ATCC 35037 (AY485602), and *S. sanguinis* ATCC 29667 (AY281085). These species make up the *viridans* group of the genus *Streptococcus* [3]. The nucleotide sequences that we found were used for a comparative phylogenetic analysis. As is evident from Fig. 1, the 16S rRNA gene sequences of the streptococci under study fell into three clusters corresponding to three phylogenetic groups. The *salivarius* group included three type strains of the species *S. thermophilus*, *S. salivarius*, and *S. vestibularis*. The *bovis* group also included three species (*S. macedonicus*, *S. bovis*, and *S. equinus*) and the *mitis* group contained four species (*S. mitis*, *S. gordonii*, *S. oralis*, and *S. sanguinis*). The phylogenetic tree shown in Fig. 1 was constructed by the neighbor-joining method with the aid of the Mega 2.1 program. To assess the reliability of the branching points on the tree, the sequence data were subjected to bootstrap analysis. As is evident from the phylogenetic tree, the *salivarius* group includes three very close species (*S. thermophilus*, *S. salivarius*, and *S. vestibularis*). In the nucleotide sequences of the 16S rRNA genes and phylogenetic characteristics, this group is homologous to the *bovis*, *mitis*, and *angiosus* groups of streptococci [3, 4]. According to the data of Kawamura et al., the degree of homology of the 16S rRNA genes of the pairs *S. thermophilus*–*S. mitis* and *S. thermophilus*–*S. gordonii* is 94.5 and 95.4%, respectively [3]. On the other hand, Tsakalidou et al. reported that the degree of homology of the 16S rRNA genes of the pairs *S. thermophilus*–*S. salivarius*, *S. thermophilus*–*S. bovis*, and *S. thermophilus*–*S. mitis* is 99.0, 95.7, and 94.5%, respectively [4]. All of these species are phenotypically close. This fact does not allow their reliable taxonomic identification without the use of additional approaches. The homology of the complete nucleotide sequences of the 16S rRNA genes of the *viridans* group of the genus *Streptococcus* with respect to the 16S rRNA gene of

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[†] Descended.

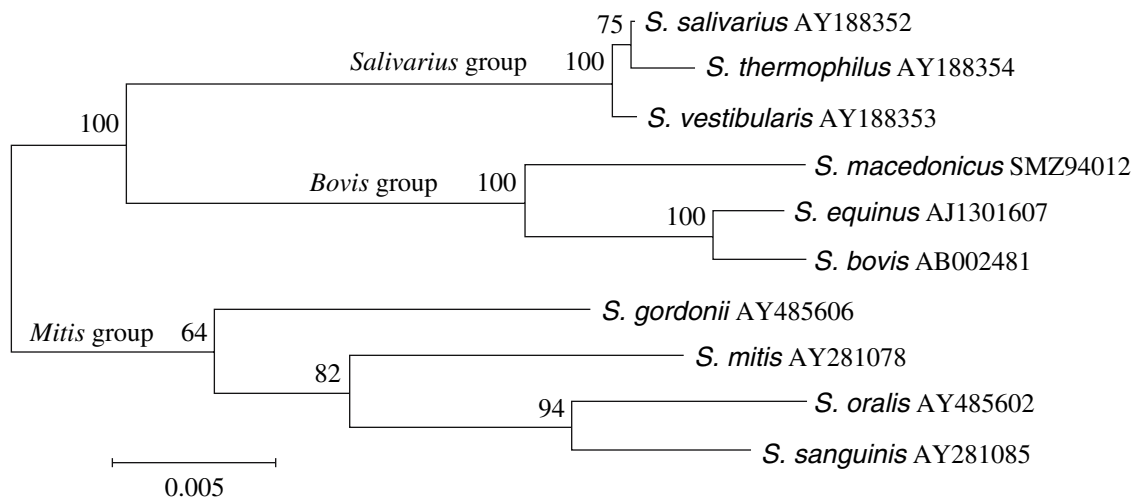


Fig. 1. Phylogenetic tree constructed on the basis of a comparative analysis of the nucleotide sequences of the 16S rRNA genes of ten species belonging to the *viridans* group of the genus *Streptococcus*. The sequence data were subjected to bootstrap analysis.

<i>S. salivarius</i> AY188352	141	CCTTGAGCG	GGGGATAACT	ATTGGAAACG	ATAGCTAATA	CCGCATAACA	ATGGATGACA	CATGTCATT	210
<i>S. vestibularis</i> AY188353							..A.G....		
<i>S. thermophilus</i> AY188354							..G.A....		
<i>S. salivarius</i> AY188352	211	ATTTGAAAGG	GGCAATTGCT	CCACTACAAG	ATGGACCTGC	GTTGTATTAG	CTAGTAGGTG	AGGTAACGGC	280
<i>S. vestibularis</i> AY188353								C...	
<i>S. thermophilus</i> AY188354								T...	
<i>S. salivarius</i> AY188352	421	GTGAAGAAGG	TTTTCGGATC	GTAAGCTCT	GTTGTAAGTC	AAGAACGAGT	GTGAGAGTGG	AAAGTTCACA	490
<i>S. vestibularis</i> AY188353								A...	
<i>S. thermophilus</i> AY188354								G...	
<i>S. salivarius</i> AY188352	491	CTGTGACGGT	AGCTTACCAG	AAAGGGACGG	CTAACTACGT	GCCAGCAGCC	GCGGTAATAC	GTAGGTCGCC	560
<i>S. vestibularis</i> AY188353									
<i>S. thermophilus</i> AY188354				A.....					
<i>S. salivarius</i> AY188352	1269	GGTACAACGA	GTTGCGAGTC	GGTGACGGCA	AGCTAATCTC	TTAAAGCCAA	TCTCAGTTTCG	GATTGTAGGC	1330
<i>S. vestibularis</i> AY188353				A.....					
<i>S. thermophilus</i> AY188354				G.....					
<i>S. salivarius</i> AY188352	1471	GGAGCCAGCC	GCCTAAGGTG	GGATAGATGA	TTGGGGTGAA	GTCGTAACAA	GGTAGCCGTA	TCGGAAGGTG	1540
<i>S. vestibularis</i> AY188353				T.....					
<i>S. thermophilus</i> AY188354				C.....					

Fig. 2. Regions of the nucleotide sequences of the 16S rRNA genes of *S. thermophilus*, *S. salivarius*, and *S. vestibularis* containing nucleotide substitutions.

S. thermophilus ranges from 96.1 to 96.4% for the *bovis* group and from 95.3 to 96.2% for the *mitis* group.

The taxonomic status of *S. thermophilus* has long remained the subject of debate. Some researchers proposed to reclassify the species *S. thermophilus* into the subspecies *S. salivarius*. The study of the degree of relatedness between *S. thermophilus* and *S. salivarius* by the DNA–DNA hybridization technique showed a high similarity of their genomes (more than 80%). This fact allowed Farrow and Collins [5] to unite these two species into one, with the names *S. salivarius* and *S. salivarius* ssp. *thermophilus*, respectively. Later, however, *S. thermophilus* and *S. salivarius* were again classified as two separate species, since closer examination of these species showed the DNA hybridization level between them of to be no higher than 80% [6].

According to our data, the degree of homology between the 16S rRNA gene sequences of *S. thermophilus* and *S. salivarius* is 99.8%, and that between the 16S rRNA gene sequences of *S. thermophilus* and *S. vestibularis* is 99.6%. Such high values of homology are due to minor differences between the 16S rRNA gene sequences of *S. thermophilus* and *S. salivarius* (four substituted nucleotides of the total 1540 bp and between the 16S rRNA gene sequences of *S. thermophilus* and *S. vestibularis* (six substitutions and one mononucleotide deletion) (Fig. 2)).

The results of the phylogenetic analysis of the typical representatives of the genus *Streptococcus* (Fig. 1) are in agreement with the results of the study of the 16S–23S rRNA internal transcribed spacers (ITSs) of *S. thermophilus* and closely related streptococci

(*S. macedonicus*, *S. salivarius*, and *S. bovis*) [7]. In spite of the fact that these four streptococci belong to different phylogenetic groups (the *salivarius* and *bovis* rRNA groups), their nucleotide sequences are highly homologous. In the degree of similarity of their ITS regions, the four species under study form two clusters, one containing the species *S. macedonicus* and *S. bovis* and the other containing the species *S. thermophilus* and *S. salivarius* [7]. The nucleotide sequences of the ITS regions of *S. thermophilus* and *S. salivarius* are highly conservative and differ only in one mononucleotide insertion (in *S. thermophilus*) of a total of 285 bp [8].

Most bacteria of the genus *Streptococcus* are pathogenic to humans and animals [4, 9] and can cause such infectious diseases as strangles in horses, mastitis in cattle, scarlet fever and tonsillitis in humans, as well as mixed infections (abscess, nephritis, polyarthritis, and sepsis). The species *S. agalactiae* causes sepsis and meningitis in newborns, *S. mutans* causes dental caries, *S. gordonii* causes oral sepsis, and *S. pyogenes* causes skin diseases. The members of the *salivarius* group differ in their habitats and pathogenicity. *S. thermophilus* is a lactic acid bacterium, various strains of which are used as starters in the production of fermented dairy products. *S. salivarius* inhabits the oral cavity of humans and animals, causing dental diseases [10]. *S. vestibularis* also inhabits the oral cavity of humans; however, this bacterium is not infectious [11]. The close phylogenetic relationship of the streptococci from the *salivarius* group poses the problem of their reliable differentiation and identification, which can easily be approached by determining the nucleotide sequences of their 16S rRNA genes and 16S–23S rRNA ITS regions.

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