INTERNATIONAL JOURNAL OF SYSTEMATIC BACTERIOLOGY, Apr. 1995, p. 406–408 Vol. 45, No. 2
0020-7713/95/$04.00+0

Determination of 16S rRNA Sequences of *Streptococcus mitis* and *Streptococcus gordonii* and Phylogenetic Relationships among Members of the Genus *Streptococcus*

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We determined the 16S rRNA sequences of the type strains of *Streptococcus mitis* and *Streptococcus gordonii* and calculated the phylogenetic distances between those organisms and other members of the genus *Streptococcus*. The viridans group streptococci were separated into five phylogenetic groups; we named these groups the anginosus group, the mitis group, the salivarius group, the bovis group, and the mutans group. *S. mitis* and *S. gordonii* clustered in the mitis group together with *Streptococcus pneumoniae*, *Streptococcus oralis*, *Streptococcus sanguis*, and *Streptococcus parasanguis* at levels of sequence homology of more than 96%. Within this group, *S. mitis*, *S. oralis*, and *S. pneumoniae* exhibited more than 99% sequence homology with each other, although the DNA-DNA similarity values for their total chromosome DNAs were less than 60%.

In the past 10 years, several new members have been added to the viridans streptococcus group (3, 11, 18–20). *Streptococcus oralis*, *Streptococcus parasanguis*, and *Streptococcus gordonii* are the major new species that have been described. These organisms are often isolated from human clinical specimens and thus are clinically important species. However, workers at clinical laboratories have found that it is difficult to identify viridans group streptococci because of the lack of decisive phenotypic characteristics.

Some of the confusion concerning identification of these bacteria came from the type strain of *Streptococcus mitis*, strain NCTC 3165. This type strain had traits different from the traits described for the species. Therefore, Coykendall et al. (5) proposed that *S. mitis* NCTC 3165 should be rejected as the type strain, and in Opinion 66 (10) strain NCTC 12261 was designated the neotype strain of *S. mitis*. The rejected type strain is now identified as an *S. gordonii* strain, and we have confirmed this identification by DNA-DNA hybridization (unpublished data). In a previous study, we developed quantitative microplate DNA-DNA hybridization methods to identify streptococci (6, 7) and found that many strains which were identified as *S. mitis*, *S. oralis*, and *Streptococcus sanguis* by phenotypic methods were misidentified. Another problem arose when we applied quantitative DNA-DNA hybridization methods to the identification of viridans group streptococci. Many strains identified biochemically as *S. mitis* were difficult to differentiate from *S. oralis* and *Streptococcus pneumoniae* even by the hybridization method because clinical strains

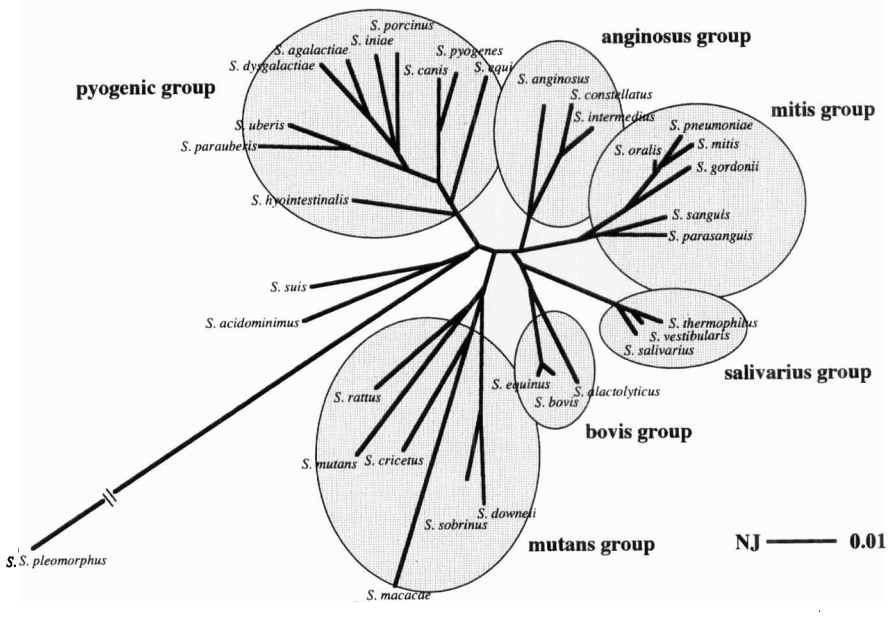

FIG. 1. Phylogenetic relationships among 34 *Streptococcus* species. Distances were calculated by the neighbor-joining (NJ) method. *S. pleomorphus* was located far from other species, so its distance is indicated with an ellipsis; its true distance from the junction was 0.16944.

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TABLE 1. Levels of 16S rRNA sequence homology among *S. mitis*, *S. gordonii*, and other *Streptococcus* species

Group	Species	% Homology with:	
		S. mitis	*S. gordonii*
Mitis	*S. mitis*	100.00	
	S. gordonii	97.63	100.00
	S. pneumoniae	99.01	97.24
	S. oralis	99.39	98.16
	S. sanguis	96.71	97.01
	S. parasanguis	96.78	96.25
Anginosus	*S. anginosus*	94.41	94.64
	S. constellatus	95.86	95.48
	S. intermedius	95.56	96.17
Salivarius	*S. salivarius*	95.10	95.86
	S. thermophilus	94.49	95.41
	S. vestibularis	94.78	95.70
Bovis	*S. bovis*	94.49	95.10
	S. equinus	94.72	95.18
	S. alactolyticus	95.02	95.25
Mutans	*S. mutans*	93.42	94.64
	S. rattus	93.57	94.10
	S. cricetus	92.73	93.87
	S. downeii	93.41	93.64
	S. sobrinus	93.80	94.18
	S. macacae	90.72	91.49
Pyogenic	*S. pyogenes*	94.49	94.87
	S. agalactiae	94.79	94.03
	S. canis	94.26	94.87
	S. dysgalactiae	94.86	94.10
	S. equi	93.57	93.95
	S. iniae	95.02	94.18
	S. porcinus	94.63	93.64
	S. uberis	93.65	94.26
	S. parauberis	93.79	93.41
	S. hyointestinalis	94.56	94.87
None[a]	*S. acidominimus*	94.33	94.72
	S. suis	94.26	94.64
	S. pleomorphus	82.46	82.30

[a] No group name is proposed for these three species.

strongly hybridized to both *S. mitis* and *S. oralis* and sometimes to *S. pneumoniae*. To solve this problem, we decided to determine the 16S rRNA sequence of the type strain of the viridans streptococcus group in order to identify members of this group by sequencing. Fortunately, Collins and other workers have published 16S rRNA sequences of most of the members of the genus *Streptococcus* (2, 17, 18, 21). However, the sequences of two type strains, *S. mitis* NCTC 12261 and *S. gordonii* NCTC 7865, have not been determined previously, and thus we were not able to identify viridans group streptococci on the basis of sequence data.

Type strains NCTC 12261 and NCTC 7865 were purchased directly from the National Collection of Type Cultures, and their 16S rRNA genes were amplified as described previously (8, 13). The sequences were determined by using the dye primer method and an ABI automatic sequencer (model 373A; Applied Biosystems, Foster City, Calif.). The sequence of each 16S rRNA from position 8 to position 1392 (*Escherichia coli* numbering) was determined. The sequences of the other members of the genus *Streptococcus* used for alignment and for calculating levels of homology were obtained from the GenBank and EMBL databases. The ODEN program set of the DNA Data Bank of Japan was used to align the sequences, and phylogenetic distances were calculated by using the neighbor-joining method (15).

A phylogenetic tree for 34 species of the genus *Streptococcus* is shown in Fig. 1, and the levels of homology for *S. mitis*, *S. gordonii*, and other species are shown in Table 1.

S. mitis and *S. gordonii* formed one cluster together with *S. pneumoniae*, *S. oralis*, *S. sanguis*, and *S. parasanguis*; (we named this group the mitis group). Within this group, *S. mitis*, *S. oralis*, and *S. pneumoniae* exhibited more than 99% sequence homology with each other. Thus, these three species are closely related. Recently, Stackebrandt and Goebel (16) found that 16S rRNA sequence analysis can be used to determine the phylogenetic relationships of prokaryotic species when the levels of sequence homology are less than 97% and that DNA-DNA hybridization experiments are necessary to confirm the taxonomic positions when homology values are greater than 97%. The DNA-DNA similarity values for all of the species belonging to the mitis group are shown in Table 2. All of the members of the mitis group exhibited less than 60% DNA similarity with each other; thus, our data clearly demonstrated that all of these species are distinct taxa. *S. oralis* and *S. mitis* exhibited less than 55% DNA similarity with each other as determined by quantitative DNA-DNA hybridization, even though they exhibited 99.39% sequence homology. While *S. gordonii* and *S. sanguis* exhibited almost the same level of DNA similarity, they exhibited only 97.01% sequence homology. *S. gordonii* was described by Kilian et al. (11) as a new species that was distinct from *S. sanguis*. These authors also reported that *S. gordonii* was more closely related to *S. sanguis* than to *S. oralis* as determined by DNA similarity data. Our hybridization data showed almost the same results. However, our sequence data showed that *S. gordonii* is more closely related to *S. oralis*, *S. mitis*, and *S. pneumoniae* than to *S. sanguis* and *S. parasanguis*. In this case, whether the data came from 16S rRNA

TABLE 2. Levels of DNA-DNA hybridization between strains belonging to the mitis group

Strain	% DNA-DNA hybridization with[a]:					
	S. mitis NCTC 10234	*S. gordonii* ATCC 10558	*S. oralis* NCTC 11427	*S. sanguis* ATCC 10556	*S. pneumoniae* NCTC 7465	*S. parasanguis* ATCC 15912
S. mitis NCTC 10234	100					
S. gordonii ATCC 10558	9–31	100				
S. oralis NCTC 11427	44–55 (30–38)	13–38 (20–40)	100			
S. sanguis ATCC 10556	16–24 (28)	34–57 (40–60)	23–30 (20–40)	100		
S. pneumoniae NCTC 7465	30–46	0–7	10–19	1–11	100	
S. parasanguis ATCC 15912	22–52	11–24	14–36	20–37	14–37	100

[a] Data from a previous study (1). The data in parentheses are data from reference 11.

sequence homology studies or from DNA-DNA hybridization similarity studies made a difference.

We divided the genus *Streptococcus* into six major clusters (the pyogenic group, the anginosus group, the mitis group, the salivarius group, the bovis group, and the mutans group), which included 31 species (Fig. 1). *Streptococcus suis* and *Streptococcus acidominimus* were not related to either the viridans group or the pyogenic group.

Four strictly anaerobic streptococcal species were described in *Bergey's manual of Systematic Bacteriology* (9). Three of these species, *Streptococcus morbillorum*, *Streptococcus parvulus*, and *Streptococcus hansenii*, have been transferred to the genera *Gemella* (12), *Atopobium* (4), and *Ruminococcus* (8), respectively. Thus, *Streptococcus pleomorphus* is now the only anaerobic member of the genus. Ludwig et al. (14) reported that *S. pleomorphus* was not closely related to streptococci but was more closely related to certain clostridia. In our study we found that *S. pleomorphus* exhibited less than 85% sequence homology with *S. mitis* or *S. gordonii* (Table 1) or any other member of the genus *Streptococcus* (data not shown). Our neighbor-joining data (Fig. 1) also revealed that *S. pleomorphus* was not related to any member of the genus *Streptococcus*. Our data supported the observation of Ludwig et al., and we concluded that *S. pleomorphus* should be removed from the genus *Streptococcus*.

Nucleotide sequence accession number. The 16S rRNA sequences of *S. mitis* and *S. gordonii* have been deposited in the DNA Data Bank of Japan under accession numbers D38482 and D38483, respectively.

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