NOTE

Re-evaluation of the taxonomic position of *Streptococcus ferus*Adrian M. Whatmore^{1†} and Robert A. Whiley²

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***Streptococcus ferus* was originally isolated from rats and, although it is often considered a member of the 'mutans group' streptococci on the basis of phenotypic criteria, its phylogenetic position has not been investigated. Here, the relationship of this organism to other streptococcal groups has been determined by DNA–DNA hybridization and by partial sequencing of 16S rDNA and the *sodA* gene. *S. ferus* was found to be distantly related to all currently described streptococci, with no strong evidence in support of its inclusion within any of the currently recognized streptococcal groups.**

Keywords: *Streptococcus ferus*, mutans group

Streptococcus ferus was originally described as a 'mutans-like' streptococcus isolated from the mouths of wild rats living in a sugar-cane field (Coykendall *et al.*, 1974). This description was based on the phenotypic characteristics of the isolates, which were able to ferment mannitol and sorbitol, failed to grow in 6.5% NaCl or at 45 °C and were able to synthesize an adhesive glucan from sucrose. Like *Streptococcus mutans*, the rat isolates possessed the serotype c carbohydrate cell-wall antigen according to the serological scheme of Bratthall (1970) (Coykendall *et al.*, 1976). However, despite a close physiological resemblance to *S. mutans*, no close genetic relationship was found with the latter species or with any previously encountered 'mutans-like' genotype by DNA–DNA base-pairing studies. In addition, the G + C content of these streptococci was determined to be 43–45 mol%, compared with 37.1–37.9 mol% for *S. mutans* (Coykendall, 1977). Taxonomic studies of streptococci designated *S. mutans* had previously revealed the existence of several centres of variation that were subsequently given subspecies status. These included *S. mutans* subspecies *mutans* (serotype c), subspecies *rattus* (serotype b), subspecies *cricketus* (serotype a) and subspecies *sobrinus* (serotypes d and g) (Coykendall,

1974). The mutans-like strains from wild rats were described as a fifth genospecies within the *S. mutans* group of streptococci (Coykendall *et al.*, 1976). A further taxonomic proposal elevated the subspecies of *S. mutans* to species status (*S. mutans*, *Streptococcus rattus*, *Streptococcus cricketus* and *Streptococcus sobrinus*) and, in the same publication, a description of *Streptococcus ferus* was given for the rat isolates (Coykendall, 1977). However, the name *S. ferus* did not appear listed in the Approved Lists of Bacterial Names (Skerman *et al.*, 1980), and a full species description was published later (Coykendall, 1983). Descriptions of additional species of 'mutans streptococci' followed that included *Streptococcus macacae* and *Streptococcus downei* from the dental plaque of monkeys (Beighton *et al.*, 1984; Whiley *et al.*, 1988) and *Streptococcus orisratti*, isolated from the dental plaque of laboratory rats fed a high-sucrose diet (Zhu *et al.*, 2000). Recently, in pursuit of grammatical correctness, the names of two of the 'mutans group' streptococci were corrected: *S. cricketus* and *S. rattus* were corrected to *S. criceti* and *S. ratti* (Trüper & de' Clari, 1997, 1998).

From the initial isolation of strains up to the publication of the species description, *S. ferus* had been studied within the context of the group of cariogenic streptococcal species and was therefore tacitly accepted as a member of the 'mutans species group' or 'complex' (Beighton *et al.*, 1984; Gilmour *et al.*, 1987; Schleifer *et al.*, 1984). However, data obtained using multilocus enzyme electrophoresis to analyse a collection of oral streptococci indicated that *S. ferus* was phylogenetically closer to *Streptococcus sanguinis* and

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The EMBL accession numbers for the sequences determined in this study are AJ420196 (*S. ferus* HD3 16S rDNA), AJ420197 (*S. ferus* 851^T 16S rDNA), AJ420198 (*S. criceti* ATCC 19642^T 16S rDNA), AJ420199 (*S. macacae* NCTC 11558^T 16S rDNA), AJ420200 (*S. downei* NCTC 11391^T 16S rDNA), AJ420201 (*S. ratti* ATCC 19645^T 16S rDNA), AJ420202 (*S. ferus* 851^T *sodA*) and AJ420203 (*S. ferus* HD3 *sodA*).

Table 1. DNA base-pairing experiments: hybridization with radioactive reference DNA from *S. ferus* strain 8S1^T

Strain	Hybridization with <i>S. ferus</i> 8S1 ^T (%)
<i>S. ferus</i> 8S1 ^T (= ATCC 33477 ^T)	100.0
<i>S. ferus</i> HD3	90.0
'Mutans group'	
<i>S. mutans</i> NCTC 10449 ^T (= ATCC 25175 ^T)	4.0
<i>S. sobrinus</i> SL-1 ^T (= ATCC 33478 ^T)	9.0
<i>S. criceti</i> HS6 ^T (= ATCC 19642 ^T)	7.0
<i>S. ratti</i> FA-1 ^T (= ATCC 19645 ^T = NCTC 10920 ^T)	9.0
<i>S. macacae</i> 25-1 ^T (= NCTC 11558 ^T)	1.0
<i>S. downei</i> MFe28 ^T (= NCTC 11391 ^T)	7.0
'Sanguinis group'	
<i>S. sanguinis</i> NCTC 7863 ^T	7.0
'Mitis group'	
<i>S. oralis</i> LVG1 ^T (= NCTC 11427 ^T)	2.0
<i>S. gordonii</i> HF90A (= ATCC 12396)	3.0
<i>S. gordonii</i> NCTC 3165	5.0
<i>S. mitis</i> NS51 ^T (= NCTC 12261 ^T)	6.0
'Anginosus group'	
<i>S. anginosus</i> NCTC 10713 ^T	2.0
'Salivarius group'	
<i>S. salivarius</i> NCTC 8618 ^T	7.0

closely related species (Gilmour *et al.*, 1987), i.e. the 'mitis group' of Kawamura *et al.* (1995). Comparative analysis of 16S rRNA gene (rDNA) sequences of streptococci confirmed the presence of several species groups within the genus *Streptococcus* that were designated 'pyogenic', 'anginosus', 'mitis', 'salivarius', 'bovis' and 'mutans' (Kawamura *et al.*, 1995). However, *S. ferus* was not included in those studies and its phylogenetic position currently remains unknown. Here, we present further analysis of the taxonomic position of *S. ferus* within the streptococci based on data obtained from 16S rDNA and manganese-dependent superoxide dismutase (*sodA*) sequence comparisons and DNA base-pairing experiments between it and other streptococci.

The type and reference strains listed in Table 1 were used in DNA base-pairing experiments. DNA base pairing was carried out using the S1 nuclease/trichloroacetic acid procedure as described previously (Whiley *et al.*, 1989). Briefly, DNA was prepared from approximately 3 g wet weight of cells grown anaerobically at 37 °C in brain/heart infusion broth (Oxoid)

supplemented with 0.5 % (w/v) glucose, by the method of Garvie (1976). DNA was labelled with [1',2',5'-³H]dCTP (Amersham) prepared by nick translation. DNA reassociation experiments were performed in triplicate in 300 µl 0.42 M NaCl containing 30 µg sheared (sonicated), denatured, unlabelled DNA and 0.02 µg sheared, denatured, labelled DNA under optimum (60 °C) conditions. DNA reassociation between *S. ferus* strains 8S1^T (labelled DNA from this strain was used as the index DNA in all experiments) and HD3 was 90 %. Levels of less than 10 % reassociation were obtained between *S. ferus* strain 8S1^T and all other type and reference strains used in the study (Table 1). These data confirm and extend those of previous authors who also examined representatives of the streptococcal species groups by this approach and found no close relatives of *S. ferus* (Schleifer & Kilpper-Bälz, 1987).

In order to facilitate phylogenetic analysis, a PCR product corresponding to the majority of the 16S rDNA was amplified by PCR from chromosomal DNA of *S. ferus* strains HD3 and 8S1^T, prepared as described previously (Whatmore *et al.*, 1999), using primers 16Sup and 16Sdown (Whatmore *et al.*, 2001). The sequence of a large portion of the 16S rDNA was obtained by using a series of internal sequencing primers and the Beckman CEQ2000 sequencing system. The sole 16S rDNA sequences of four other members of the currently described 'mutans group' (*S. criceti*, *S. downei*, *S. ratti* and *S. macacae*) available in GenBank were those originally described by Bentley *et al.* (1991), obtained by reverse transcription. As these sequences contained a large number of anomalous residues, sequences of the 16S rDNA from these four organisms were obtained by the same approach.

The 16S rDNA sequences of the two *S. ferus* strains differed by only one nucleotide over the 1444 bp examined from the two strains. However, in agreement with DNA-DNA hybridization analysis, pairwise analysis of 16S rDNA sequence data (trimmed to 1266 bp to allow comparison with existing database sequences) indicates that *S. ferus* is not closely related to any other previously characterized streptococci. Indeed, the most closely related streptococcal sequences show no more than 95 % identity to *S. ferus* (range 91.92–95.02 % identity) over the region compared in this study. In addition, there is no clear relationship with any of the currently recognized streptococcal groups, with very similar identities seen to members of a number of the currently recognized streptococcal groups. The highest percentage identities found were to members of the pyogenic group (*Streptococcus canis*, 95.02 %; *Streptococcus iniae*, 94.87 %; *Streptococcus urinalis*, 94.63 %; *Streptococcus pyogenes*, 94.63 %), the mutans group (*S. ratti*, 94.63 %) and the anginosus group (*S. anginosus*, 94.45 %). Although there is no obviously closer relationship between *S. ferus* and any particular species group, overall, on the basis of 16S rDNA sequence pairwise similarity scores, the results of phylogenetic analysis

Fig. 1. Unrooted phylogenetic tree showing relationships of *S. ferus* based on partial 16S rDNA sequences (between 1231 and 1267 bp). Analysis was performed using the MEGA package. Distance matrices were calculated using the Jukes–Cantor correction and the pairwise-deletion option and the tree was constructed using the neighbour-joining method. The percentage bootstrap confidence levels of internal branches were calculated from 500 resamplings of the original data, with only bootstrap values exceeding 50 % shown. Bar, 0.01 nucleotide substitutions per site.

do show *S. ferus* grouping with many of the organisms considered members of the ‘mutans group’ (Fig. 1). However, this grouping is not robust and, in contrast to the other streptococcal groups, for which bootstrap analysis provided reasonable support, the low bootstrap levels obtained for the ‘mutans’ streptococci provide no support for this grouping as a whole. During the course of this work, a partial 16S rDNA sequence submitted to GenBank (AJ336367) was found to be 100% identical to *S. ferus* HD3 over the sequence in common between the two sequences. This sequence was obtained from an organism (KOMA81) isolated from the nose and tonsils of pigs and identified as *S. ferus*. The virtual identity of this sequence with the *S. ferus* type strain confirms the identity of this organism and suggests that *S. ferus* may be widely distributed among vertebrates.

In order to attempt to clarify the phylogenetic position of *S. ferus*, sequencing of a second gene, *sodA*, was undertaken. This gene was chosen because a large number of sequences from streptococcal species type strains have recently been described by Poyart *et al.* (1998), who proposed using this gene in the characterization of strains that could not be speciated on the basis of their conventional phenotypic reactions. A partial *sodA* sequence was amplified by PCR using primers d1 and d2 (Poyart *et al.*, 1998) and sequence was obtained by sequencing from both ends of the PCR product using the same primers. Sequence comparisons were performed using 381 bp of *sodA* sequence obtained from both *S. ferus* strains: the *sodA* sequence from these isolates was identical. As with the 16S rDNA data, the results from pairwise sequence comparisons of *sodA* indicate that *S. ferus* is distantly

Fig. 2. Unrooted phylogenetic tree showing relationships of *S. ferus* based on *sodA* gene sequences (381 bp). See Fig. 1 for details of tree construction. Bar, 0.1 nucleotide substitutions per site.

related to other streptococci from which this gene has been characterized (range 70.34–76.90% identity). The most closely related sequence is from a member of the 'mitis group' (*S. gordonii*), and there is no evidence for close relatedness with any other members of the 'mutans group' streptococci (range 70.34–74.54% identity). Phylogenetic analysis (Fig. 2) reproduces the conclusions of Poyart *et al.* (1998), showing broad similarity to the 16S rDNA tree, but with incongruent positions of both *Streptococcus agalactiae* and *S. mutans*. This analysis confirms that *S. ferus* does not fall within any of the recognized streptococcal groups on the basis of *sodA* sequencing. Although *S. ferus* *sodA* is distant from sequences of all other species, it is notable that the findings of Gilmour *et al.* (1987), based on multi-locus enzyme electrophoresis, indicated *S. ferus* to be a closer relative of the 'mitis group' than the 'mutans' streptococci. Clearly, the *sodA* gene is a

good candidate gene target allowing identification of *S. ferus* isolates and these authors concur with Poyart *et al.* (1998) and Kawamura *et al.* (1999) in promoting *sodA* as a basis for the identification of streptococcal species.

In conclusion, neither DNA–DNA hybridization studies nor analysis of the sequences of two distinct housekeeping genes (16S rDNA and *sodA*) provides strong support for the inclusion of *S. ferus* within the relatively loose association of streptococcal species that is referred to as the 'mutans group'. *S. ferus* appears distantly related to all currently recognized streptococci and further clarification of its phylogenetic relationships to other streptococci awaits thorough analysis of alternative gene targets and the identification of more closely related organisms that may facilitate phylogenetic placement.

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