The Tannin-Degrading Species *Streptococcus gallolyticus* and *Streptococcus caprinus* Are Subjective Synonyms

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The tannin-degrading species *Streptococcus gallolyticus* and *Streptococcus caprinus* have been shown to be subjective synonyms on the basis of their levels of 16S rRNA sequence similarity (98.3%) and DNA-DNA homology (>70%) and the phenotypes of their type strains. *S. gallolyticus* has nomenclatural priority according to Rule 24h(2) of the *International Code of Nomenclature of Bacteria*.

Recently, two tannin-degrading *Streptococcus* species have been described. Osawa et al. isolated strains of streptococci capable of degrading tannin-protein complexes from the feces of various animals (11–15). These strains, which ferment mannitol, have conventionally been classified as *Streptococcus bovis* biotype I strains and have been distinguished from biotype II strains, which do not ferment mannitol. Farrow et al. (5) demonstrated on the basis of DNA relatedness data that biotype I strains were genotypically homogeneous and distinct from biotype II strains, which include the type strains of *Streptococcus bovis* and *Streptococcus equinus*. Similar observations were made by Knight and Shlaes (9) and Coykendall and Gustafson (3) for isolates of clinical origin. Osawa and Walsh (16) demonstrated that many *S. bovis* biotype I strains produce an enzyme, tannase, which hydrolyzes tannins to release gallic acid, which is then decarboxylated to pyrogallol by gallate decarboxylase activities, which had not been determined previously.

Concurrently with our studies, Brooker et al. (2) described a novel tannin-resistant species, *Streptococcus caprinus*, which was isolated from the rumen of a feral goat in Australia. The three strains studied were shown to form a coherent DNA homology group distinct from *S. bovis* and to be distinct from *S. equinus* and *S. bovis* on the basis of rRNA sequence similarity and phenotype data.

In order to determine the taxonomic relationship between *S. gallolyticus* and *S. caprinus*, we compared the type strains by studying their rRNA sequence similarity, DNA-DNA homologies, and phenotypes, including their tannase and galactose decarboxylase activities, which had not been determined previously for *S. caprinus*.

Extraction of genomic DNA and amplification of the 16S tRNA gene were performed as described by Dorsch and Stackebrandt (4). The PCR products were purified by using a MicroSpin type S-300 purification column (Pharmacia Biotech) as described by the manufacturer. A PRISM Ready Reaction DyeDeoxy terminator cycle sequencing kit (Applied Biosystems, Foster City, Calif.) was used to directly sequence the PCR products with an Applied Biosystems model 373A automatic DNA sequencer. The 16S ribosomal DNA (rDNA) sequence obtained was aligned manually with the sequence of *S. caprinus* 2.3T (= ACM 3969T) (accession no. Y10868) and representative bacterial 16S rDNA sequences obtained from the Ribosomal Database Project (10). Positions at which length and sequence variations made alignment uncertain were omitted. Pairwise levels of evolutionary similarity and distances (8) were computed with the DNADIST program in the PHYLIP, version 3.4, software package (6). A phylogenetic tree (data not shown) that was constructed by using the neighbor-joining method of Saitou and Nei (18) showed that *S. gallolyticus* and *S. caprinus* clustered together in a phylogenetic group with *S. bovis*, *S. equinus*, and *Streptococcus alactolyticus*. The latter three species were also previously shown to form a coherent phylogenetic group in an analysis that included 31 species of the genus *Streptococcus* (1).

The rRNA sequences of *S. gallolyticus* and *S. caprinus* exhibited 98.3% sequence similarity in an analysis of 1,266 nucleotide positions.

DNA homology experiments with the type strains of *S. gallolyticus*, *S. caprinus*, and *S. equinus* were performed by using the SI nuclease method as previously described (13). Table 1 shows that the levels of homology between the DNAs of *S. gallolyticus* and *S. caprinus* are 67 to 74%. Levels of DNA-DNA homology of >70% indicate that strains belong to the same species (20). The results also confirmed that the type strains of *S. gallolyticus* and *S. caprinus* are genotypically distinct from *S. equinus*.

*S. caprinus* ACM 3969T was shown to have the tannase activity and the galactose decarboxylase activity characteristic of *S. gallolyticus* (13) by the methods of Osawa and Walsh (16) and Osawa et al. (17), respectively. The biochemical characteristics of the type strains of *S. gallolyticus* and *S. caprinus* were determined by the API 20 STREP strip method (API System, Montalieu, Verceil, France). The results showed that both strains were positive for the Voges-Proskauer reaction, esculin hydrolysis, leucine arylamidase activity, and acid production from mannitol, lactose, trehalose, starch, and glycogen. The strains were negative for hippurate hydrolysis, pyrrolidonylarylamidase, -glucuronidase, -galactosidase, alkaline phosphatase, and arginine dihydrolase activities, and acid production from ribose, L-arabinose, sorbitol, and inulin. Neither strain produced beta-hemolysis of sheep blood cells. The two type strains differed in two characteristics. The *S. gallolyticus* strain was positive for -galactosidase activity and acid production from raffinose, while the *S. caprinus* strain was negative for these characteristics. Brooker et al. (2) found that *S. caprinus* utilized raffinose as a growth substrate.

Overall, the phylogenetic, genotypic, and phenotypic results...
indicate that *S. gallolyticus* ACM 3611\(^T\) and *S. caprinus* ACM 3969\(^T\) belong to the same species. Both species were described outside the International Journal of Systematic Bacteriology, and the names were validated in 1996 on the same validation list (7). This validation list indicates that *S. gallolyticus* has higher priority than *S. caprinus* based on the order of receipt of their effective publications for validation. Under Rule 24b(2) of the International Code of Nomenclature of Bacteria (19), *S. gallolyticus* therefore has nomenclatural priority.

**Nucleotide sequence accession number.** The nucleotide sequence of *S. gallolyticus* ACM 3611\(^T\) determined in this study has been deposited in the EMBL Data Library (Cambridge, United Kingdom) under accession no. X94337.

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### REFERENCES