

# Development of a Diagnostic PCR Assay Targeting the Mn-Dependent Superoxide Dismutase Gene (*sodA*) for Identification of *Streptococcus gallolyticus*

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**A PCR-based assay to identify *Streptococcus gallolyticus* has been developed. The assay uses an oligonucleotide primer pair targeting a partial sequence of the manganese-dependent superoxide dismutase gene (*sodA*). The assay distinguished members of the *S. gallolyticus* group from other, closely related taxa successfully by yielding a 408-bp specific amplicon.**

*Streptococcus gallolyticus* is often found as a normal member of the gut microflora of various animals (15), while it has been reported to cause mastitis in cattle (8), septicemia in pigeons (3), and meningitis, sepsis, and endocarditis in humans (2, 4, 12). There have also been a number of case reports that suggest an etiological link between underlying infection with this organism and colon cancers in human (1, 5, 9, 10, 11). Most, if not all, of the strains belonging to this species are able to decarboxylate gallate as well as produce tannase, by which a hydrolyzable tannin (i.e., gallotannin) is hydrolyzed to release gallic acid, which is subsequently decarboxylated to pyrogallol (14). The species, which includes strains formerly identified as *S. bovis* biotypes I and II/2, can be distinguished from other, related taxa or biotypes (i.e., *S. equinus*, *S. bovis* biotype II/1) on the basis of the results of DNA-DNA reassociation experiments (14). In a recent taxonomic review, Facklam (6) has suggested that all human isolates of *S. bovis* biotypes I and II/2 are officially identified as *S. gallolyticus*. Nevertheless, the use of the name *S. bovis* biotype I or II/2 instead of *S. gallolyticus* has been a common occurrence in clinical microbiology despite the clearly established status of the latter name (14). This situation seems to have come about in order to avoid confusion among clinical microbiologists. Recently, Poyart et al. (18) demonstrated that a partial sequence of the manganese-dependent superoxide dismutase gene (*sodA*) provides useful information for the differentiation of species of the so-called *S. bovis*-*S. equinus* group. Here we evaluated the use of a PCR-based method for identification of *S. gallolyticus* that uses a primer pair targeting *sodA* by using strains of the *S. bovis*-*S. equinus* group from our culture collection with known DNA-DNA homology status.

Twenty-three streptococcal strains that had been previously

designated *S. gallolyticus* (14) and seven strains that had been assigned by Farrow et al. (7) to *S. equinus* were used in the present study. Whole genomic DNA-DNA homology had been used previously (14) to determine the taxonomic position of

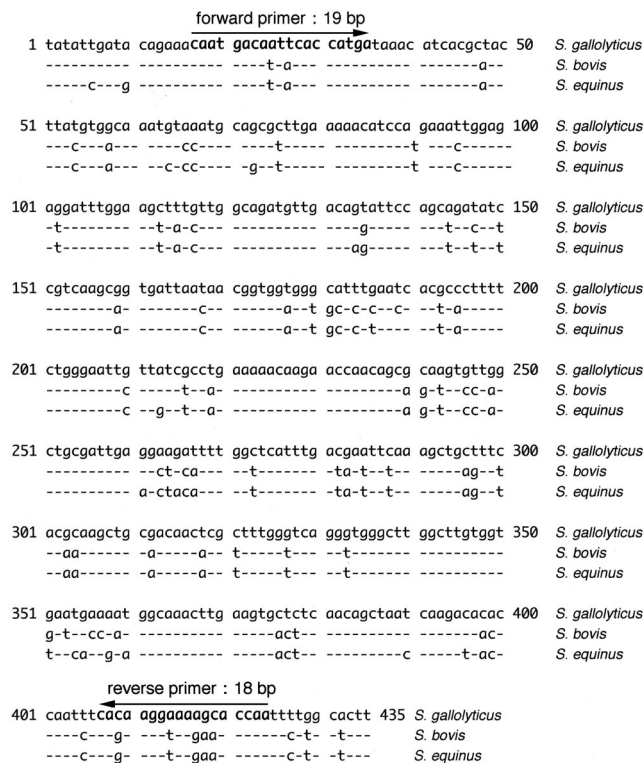


FIG. 1. Alignments of the *sodA* gene DNA sequences used in the present study and retrieved from the GenBank database (*S. gallolyticus* CIP105428<sup>T</sup> [GenBank accession no. AJ297183], *S. bovis* CIP102302 [GenBank accession no. Z95896], and *S. equinus* 102504<sup>T</sup> [GenBank accession no. Z95903]). Boldface letters indicate the positions of the primers in the sequences. The hyphens indicate the same bases as shown in *S. gallolyticus* CIP102302.

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TABLE 1. Strains used in this study and their biochemical and genotypic characteristics

Strain <sup>a</sup>	Source	% Binding with <i>S. gallolyticus</i> ACM 3611 <sup>T</sup> DNA <sup>b</sup>	Phenotypic identity determined by API 20 Strep	Tannase	Gallate decarboxylation	Result of PCR targeting <i>sodA</i>
<i>S. gallolyticus</i> fecal isolates						
ACM 3611 <sup>T</sup>	Koala feces	100	<i>S. bovis</i> biotype I	+	+	+
ACM 3612	Koala feces	74	<i>S. bovis</i> biotype I	+	+	+
ACM 3609	Brush-tail possum feces	98	<i>S. bovis</i> biotype I	+	+	+
ACM 3593	Kangaroo feces	81	<i>S. bovis</i> biotype I	+	+	+
ACM 3850	Ringtail possum feces	72	<i>S. bovis</i> biotype I	+	+	+
ACM 3849	Cow feces	69	<i>S. bovis</i> biotype I	+	+	+
ACM 3551 (= NCDO 2572)	Cow rumen	64	<i>S. bovis</i> biotype I	+	+	+
ACM 3852	Dog feces	79	<i>S. bovis</i> biotype I	+	+	+
ACM 3853	Dog feces	63	<i>S. bovis</i> biotype I	+	+	+
ACM 3848	Horse feces	96	<i>S. bovis</i> biotype I	+	+	+
ACM 3847	Pig feces	83	<i>S. bovis</i> biotype I	+	+	+
ACM 3845	Guinea pig feces	87	<i>S. bovis</i> biotype I	+	+	+
<i>S. gallolyticus</i> clinical isolates						
ACM 3546 (= NCDO 2019)	Cow mastitis lesion	74	<i>S. bovis</i> biotype I	+	+	+
ACM 3547 (= NCDO 2088)	Cow mastitis lesion	94	<i>S. bovis</i> biotype I	+	+	+
ACM 3548 (= NCDO2134)	Cow mastitis lesion	79	<i>S. bovis</i> biotype I	+	+	+
ACM 3552 (= NCDO2631)	Human clinical case	82	<i>S. bovis</i> biotype I	+	+	+
ACM 3874 (= F-1867)	Human clinical case	72	<i>S. bovis</i> biotype I	+	+	+
ACM 3873 (= Vitec 1616)	Human clinical case	82	<i>S. bovis</i> biotype I	+	+	+
ACM 3902 (= CDC 002)	Human clinical case	60	<i>S. bovis</i> biotype I	+	+	+
ACM 3903 (= MG Eath)	Human clinical case	77	<i>S. bovis</i> biotype I	+	+	+
ACM 3870 (= CDC 1723-81)	Human clinical case	60	<i>S. bovis</i> biotype II/2	-	+	+
ACM 3869 (= CDC 2266-81)	Human clinical case	64	<i>S. bovis</i> biotype II/2	-	+	+
ACM 3871 (= CDC 008)	Human clinical case	66	<i>S. bovis</i> biotype II/2	-	+	+
Strains of related taxa						
<i>S. bovis</i>						
ACM 3539 (= NCDO597 <sup>T</sup> )	Cow feces	25	<i>S. bovis</i> biotype II/1	-	-	-
ACM 3540 (= NCDO 598)	Cow feces	26	<i>S. bovis</i> biotype II/1	-	-	-
ACM 3542 (= NCDO 1251)	Sheep rumen	10	<i>S. bovis</i> biotype II/1	-	-	-
ACM 3543 (= NCDO 2128)	Cow feces	20	<i>S. bovis</i> biotype II/1	-	-	-
<i>S. equinus</i>						
ACM 3541 (= NCDO 1037 <sup>T</sup> )	Horse feces	15	<i>S. salivarius</i>	-	-	-
ACM 3544 (= NCDO2445)	Horse feces	10	<i>S. equinus</i>	-	-	-
ACM 3545 (= NCDO 2446)	Horse feces	23	Unidentifiable	-	-	-

<sup>a</sup> ACM, Australian Collection of Microorganisms, Department of Microbiology, University of Queensland, St. Lucia, Queensland, Australia; NCDO, National Collection of Dairy Organisms, Reading, United Kingdom; CDC, Centers for Disease Control and Prevention strains; Vitec 1616, MG Eath, and F-1867, gifts from R. G. Knight, Department of Veterans Affairs Medical Center 10701, East London, United Kingdom.

<sup>b</sup> As described in reference 20.

these strains, in which the relative percent DNA binding of each strain with *S. gallolyticus* ACM 3611<sup>T</sup> was measured (Table 1). The *S. gallolyticus* strains consisted of 12 fecal or rumen isolates from various animals and 11 clinical isolates from cases of cow mastitis and human septicemia or endocarditis. It should be noted that the seven *S. equinus* strains included five strains originally received as *S. bovis*. All strains were cultured at 37°C on Columbia blood agar (Oxoid Ltd., Basingstoke, United Kingdom) with 5% defibrinated horse blood prior to use. The strains were characterized for their biochemical properties with a commercial identification kit (API 20 Strep; bioMérieux, Lyon, France). Tannase and gallic acid decarboxylating activities of strains were determined by visual methods described elsewhere (16, 17).

The *sodA* gene sequences of *S. gallolyticus* CIP105428<sup>T</sup> (GenBank accession no. AJ297183), *S. bovis* CIP102302 (GenBank accession no. Z95896), and *S. equinus* 102504<sup>T</sup> (GenBank accession no. Z95903) from the GenBank database were aligned and scanned for variable regions that could provide a suitable primer set specific to *S. gallolyticus* (Fig. 1). On the basis of this analysis, forward primer SgsodA-F (5'-CAATGA CAATCACCATGA-3'; positions 17 to 34) and reverse primer SgsodA-R (5'-TTGGTGCTTTTCCTTGTG-3'; posi-

tions 424 to 407) were designed as shown in Fig. 1. With this primer set, PCR amplification was performed in a total volume of 20 µl. Whole genomic DNA from each isolate was prepared by the method of Marmur (13). Two microliters of each genomic DNA preparation (10 ng of DNA/µl of Tris-EDTA) was added to the PCR master mixture, which consisted of 2 µl of 10× PCR buffer (Mg<sup>2+</sup> free; Promega Corporation, Madison, Wis.), 2 µl of 25 mM MgCl<sub>2</sub>, 0.4 µl of a 10 mM deoxynucleoside triphosphate mixture, 0.5 µl of each primer (10 pmol/µl), and 0.1 µl (0.5 U) of *Taq* DNA polymerase (Promega), with the remaining volume consisting of distilled water. A GeneAmp PCR System 2700 thermal cycler (Applied Biosystems, Foster City, Calif.) was used for PCR amplification consisting of an initial denaturation at 94°C for 5 min; 30 cycles of denaturation at 94°C for 60 s, annealing at 54°C for 30 s, and extension at 72°C for 30 s; and a final extension at 72°C for 7 min. Five microliters of the PCR products was electrophoresed on 2% agarose gels, stained with ethidium bromide (0.25 µg/ml), and photographed under UV light.

The PCR yielded an amplicon of 408 bp from all 23 *S. gallolyticus* strains, while other *S. bovis-equinus* strains did not (Table 1), suggesting that the assay is a useful molecular tool for the identification of *S. gallolyticus*. Further work with more

strains of *S. gallolyticus* and other, related species is in progress in order to confirm the reliability of the PCR-based method as a diagnostic tool. With recently accumulating evidence of its taxonomic distinction and its etiological significance, we propose that the name *S. gallolyticus* be adopted for use by clinical microbiologists.

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