**Streptococcus troglodytidis** sp. nov., isolated from a foot abscess of a chimpanzee (*Pan troglodytes*)

Michael Zhang,1† Lifang Yan,1 Guan Zhu,2 Michael Holifield,3 Donna Todd3 and Shuping Zhang2

1Mississippi Veterinary Research and Diagnostic Laboratory, College of Veterinary Medicine, Mississippi State University, Pearl, MS 39208, USA
2Department of Veterinary Pathobiology, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX 77843, USA
3The Jackson Zoological Park, Jackson, MS 39209, USA

A facultative anaerobic, non-motile, non-spore-forming, Gram-positive-staining, coccus-shaped bacterium was isolated from an abscess on the right foot of a chimpanzee (*Pan troglodytes*). The colonies were β-haemolytic. Catalase and oxidase activities were negative. The Lancefield group B antigen was expressed. On the basis of morphological and biochemical characteristics, the bacterium was tentatively identified as a streptococcal species. 16S rRNA gene sequence analysis indicated that the bacterium shared 96.7%, 96.4%, 96.1%, 95.8% and 95.7% sequence similarities with *Streptococcus gordonii*, *S. cristanus*, *S. intermedius*, *S. anginosus* and *S. constellatus*, respectively. Phylogenetic analyses based on the sequences of the 16S rRNA gene and housekeeping genes encoding d-alanine : d-alanine ligase (ddl), the β-subunit of RNA polymerase (rpoB) and manganese-dependent superoxide dismutase (sodA) revealed that the bacterium represented a novel species closely related to, albeit different from, *S. gordonii*, *S. cristanus* and the anginosus streptococci. The name *Streptococcus troglodytidis* sp. nov. is proposed. The type strain is M09-11185T (=ATCC BAA-2337T=KCTC 33006T).

Based on 16S rRNA gene sequences, the genus *Streptococcus* is currently divided into six groups including anginosus, bovis, mitis, mutans, pyogenic and salivarius (Kawamura et al., 1995). The anginosus group consists of three closely related species, *Streptococcus anginosus*, *S. intermedius* and *S. constellatus* (Whiley & Beighton, 1991). All three species in this group have been associated with suppurative infections of various organs (Jacobs et al., 2000; Whiley et al., 1992). The anginosus group is unique in that members may produce α-, β- or γ-haemolysis and possess one of the four Lancefield’s antigens, F, C, A or G (Whiley et al., 1990, 1992). The mitis group currently comprises a well-recognized pathogen named *S. pneumoniae* and twelve other commensal species, including *S. australis*, *S. cristatus*, *S. gordonii*, *S. infantis*, *S. mitis*, *S. oligofermentans*, *S. oralis*, *S. parasanguinis* (a corrected version of the original name *parasanguis*), *S. peroris*, *S. pseudopneumoniae*, *S. sanguinis* and *S. sinensis* (Hoshino et al., 2005). Many mitis streptococci have been implicated in odontogenic infections and subacute bacterial endocarditis (Tunkel & Sepkowitz, 2002). Unlike the members of the anginosus group, none of the mitis streptococci produces β-haemolysis (Gaustad, 1985; Facklam, 2002). Some members of the mitis group may express Lancefield’s antigens A, H, or W (Gaustad, 1985; Facklam, 2002). Laboratory identification of these streptococci is traditionally based on their physiological and biochemical traits (Flynn & Ruoff, 1995; Kikuchi et al., 1995; Limia et al., 2000; Whiley et al., 1992). In general, the two groups and most species within each group can be satisfactorily differentiated by the use of biochemical identification strips, such as the Rapid ID 32 Strep system (Flynn & Ruoff, 1995; Kikuchi et al., 1995). For more accurate species classification, 16S rRNA gene sequencing and phylogenetic reconstruction have recently become the techniques of choice (Hoshino et al., 2005; Limia et al., 2000; Summanen et al., 2009). In this report, we describe the phenotypic and phylogenetic characterization of a novel β-haemolytic, Gram-positive-staining, catalase-negative, coccus-shaped bacterium isolated from a non-human primate, the chimpanzee (*Pan troglodytes*). 

†Present address: Texas Veterinary Medical Diagnostic Laboratory, 1 Sippel Rd., College Station, TX 77843, USA

Abbreviations: BI, Bayesian inference; BP, bootstrap proportion; ML, maximum-likelihood.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA, *ddl*, gdh, rpoB and sodA gene sequences of strain M09-11185T are JF414111, JF414112, JF414113, JF414114 and JF414115, respectively.

A supplementary table and a supplementary figure are available with the online version of this paper.
A 14-year-old male chimpanzee in the Jackson Zoological Park (MS, USA) developed an abscess on its right foot. The abscess content was cultured on trypticase soy agar with 5% sheep blood (TSA II), MacConkey Agar (MAC), and CDC anaerobe 5% sheep blood agar with phenylethyl alcohol (PEA) (Thermo Fisher Scientific Remel Products). The aerobic cultures were incubated at 37 °C in an atmosphere. The anaerobic cultures were incubated at 37 °C in an AnaeroPack jar with an AnaeroPouch (Thermo Fisher Scientific Remel Products). After 24 h incubation, the colonies were surrounded by a wide zone of complete haemolysis.

Two bacterial isolates derived from the aerobic (M09-11185T) and anaerobic (M09-11185AN) cultures were subjected to phenotypic and molecular characterization. Both isolates were facultatively anaerobic and required CO2 for optimal growth. Anaerobic conditions slightly enhanced the growth of the isolates. The isolates were non-motile, non-sporulating, and gave a negative result for catalase and oxidase activities. Gram stain revealed Gram-positive round to ovoid cells (0.5–1.0 μm in diameter), opaque and slightly convex with entire edges. After 48 h incubation, the colonies were surrounded by a wide zone of complete haemolysis.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-Haemolysis</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Lancefield’s antigen</td>
<td>B</td>
<td>F</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Hydrolysis of:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aesculin</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>2-Naphthyl phosphate</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Acid from:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inulin</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Lactose</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Raffinose</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Production of:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>β-Galactosidase</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>α-Glucosidase</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>β-Glucosidase</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>N-Acetyl-β-Glucosaminidase</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
</tr>
</tbody>
</table>

To clarify the inconclusive biochemical identification results, 16S rRNA gene sequencing was performed. The nearly complete 16S rRNA gene was amplified by PCR as described previously (Nikkari et al., 2002). The PCR amplicon was cloned into the pCR2.1 vector and the resulting plasmids were introduced into Escherichia coli TOP10F’ according to the manufacturer’s instructions (Invitrogen). Two clones derived from each bacterial isolate were sequenced from both directions using the following primers M13 forward (5’-CTAACTACGTGCCAGCAGCC-3’), M09-11185R1 (5’-TGTAACACGACCGCCAGT-3’), M13 reverse (5’-CAGGAACAGCTATGAC-3’), and M09-11185F1 (5’-CTAACTACGTGCCAGCCAGC-3’). Multiple sequence alignments using the MUSCLE program (Unix version 3.8) showed that the 16S rRNA gene sequences of the two isolates were identical. A GenBank BLAST (http://www.ncbi.nlm.nih.gov/BLAST/) search using the 1515 bp 16S rRNA gene sequence of strain M09-11185T revealed 96–97% sequence similarity to various anginosus and mitis streptococci. Pairwise sequence comparisons between the
Phylogenetic analysis was performed based on the 16S rRNA gene sequences (1424 nt positions) of strain M09-11185T and anoginos and mitis streptococci, including S. anginosus strains ATCC 33397T, ChDC-YA12 and F0211, S. australis ATCC 700641T, S. constellatus strains ATCC 27823T and 15, S. cristatus ATCC 51100T, S. gordonii strains ATCC 10558T, ATCC 33399 (former type strain of S. mutis) and Challis, S. infantis ATCC 700779T, S. intermedius strains ATCC 27335T, 535 and 1877, S. mitis strain B6, S. oralis strain ATCC 35037T, S. parasanguinis clone 3V4 (16S rRNA gene amplified from biological samples), S. pneumoniae strain ATCC 49619 and clone 4V4 (16S rRNA gene amplified from biological samples), S. sanguinis strain ChDC-B203 and S. sinensis strain HKU4T (Fig. 1). The GenBank accession numbers are indicated in Fig. 1. Bayesian inference (BI) analysis was performed using a parallel version of MrBayes (v3.1.2) for a total of 107 generations of searches (Huelsenbeck & Ronquist, 2003). A general-time reversible (GTR) model of nucleotide substitutions was used and the among-site rate heterogeneity considered a 4-rate gamma distribution ($\Gamma(n)$) and the fraction of invariance ($F_{inv}$) (i.e. GTR+$\Gamma(n)$+$F_{inv}$). Phylogenetic reconstruction was also performed using a maximum-likelihood (ML) method using TreeFinder program (Jobb et al., 2004). The same model of nucleotide substitutions and consideration of heterogeneity (GTR+$\Gamma(n)$+$F_{inv}$) was used for computing the best ML tree and bootstrap probability (BP) support values derived from 100 replicates. In the phylogenetic tree inferred from the 16S rRNA gene sequences, strain M09-11185T fell between S. gordonii and S. cristatus within the mitis group (Fig. 1). However, the phylogenetic relationships between strain M09-11185T and S. gordonii and S. cristatus were not well resolved by ML analysis, as the nodes were not supported by BP values under the 50 % majority rule.

To further characterize the isolates, the sequences of four housekeeping genes encoding d-alanine ligase (ddl), glutamate dehydrogenase (gdh), the $\beta$-subunit of RNA polymerase (rpoB) and manganese-dependent superoxide dismutase (sodA) were determined as described by Hoshino et al. (2005). The partial sequences of ddl (292 bp), gdh (392 bp), rpoB (517 bp) and sodA (365 bp) of strain M09-11185T were most similar to those of S. mitis (78.4 %), S. oralis (80.4 %), S. anginosus (87.2 %) and S. constellatus (87.1 %), respectively. Because some Streptococcus species do not have the gdh gene, concatenated sequences of the ddl, rpoB and sodA genes were used for phylogenetic analysis. The GenBank accession numbers of nucleotide sequences are provided in Table S1 (available in IJSEM Online). BI and ML trees were inferred using the same models as described for the 16S rRNA gene sequence trees, except that the BI analysis was performed with 104 generations of searches. The housekeeping gene tree placed.

### Fig. 1. Phylogenetic tree inferred from the 16S rRNA gene sequences of nearest neighbour taxa. Both BI and ML methods using the same (GTR+$\Gamma(n)$+$F_{inv}$) model yielded the same tree topologies. Numbers at the nodes are posterior probability values followed by BP support values as a percentage. Nodes marked with ‘x’ are not well supported by BP values under the 50 % majority rule. GenBank accession numbers are shown in parentheses. The asterisk indicates that S. gordonii ATCC 33399 was originally deposited as the type strain of S. mitis. 1, 16S rRNA genes amplified from biological samples. Bar, 0.03 substitutions per nucleotide position.
Strain M09-11185\textsuperscript{T} at the base of the anginosus cluster and between the anginosus and mitis groups, which were fully or nearly fully supported by both posterior probability (PP) and BP values (Fig. S1). The use of concatenated sequences is known to have advantages in not only providing more informative positions for phylogeny, but also reducing potential long-branch attraction artefacts (Gribaldo & Philippe, 2002; Philippe et al., 2005; Templeton et al., 2010). Trees based on concatenated sequences of housekeeping genes allow unequivocal differentiation of streptococci (Hoshino et al., 2005; Kiratisin et al., 2005). In the present study, the phylogenetic inference based on the concatenated housekeeping gene sequences suggested a closer evolutionary affiliation with anginosus streptococci than with \textit{S. gordonii} and \textit{S. cristatus}. The difference in the phylogenetic position in 16S rRNA gene tree versus the housekeeping gene tree may reflect the different evolutionary rates of the 16S rRNA gene and the housekeeping genes. It could also be a result of horizontal genetic transfer between streptococcal species as proposed by Schouls et al. (2003). Further analysis based on additional isolates and more genes will eventually resolve the evolutionary position of the novel species as represented by strain M09-11185\textsuperscript{T}.

In summary, phenotypic and phylogenetic characterizations suggested that the isolates from the foot abscess represent a novel species of the genus \textit{Streptococcus} that is closely related to, but distinct from, \textit{S. gordonii}, \textit{S. cristatus} and \textit{S. anginosus}. To signify the host species infected by the bacterium, the name \textit{Streptococcus troglodytidis} sp. nov. is proposed for this novel species.

**Description of \textit{Streptococcus troglodytidis} sp. nov.**

\textit{Streptococcus troglodytidis} (tro.glo.dyi.tis. L. gen. adj. troglodytis of or belonging to the Troglobytes, here isolated from an infection in a chimpanzee, \textit{Pan troglodytes}).

Cells are Gram-positive-staining, non-motile, non-spore-forming cocci (0.5–1 \textmu m). Colonies on blood agar are small (0.5–1.0 mm), opaque, convex and entire with a large zone of complete haemolysis after 48 h incubation at 37 °C. Cells are facultatively anaerobic and require 5–10% CO\textsubscript{2} for optimal growth. Cells react with Lancefield’s group B antisera. Tests for catalase and oxidase activities are negative. Arginine, aseculin and 2-naphthyl phosphate are hydrolysed, but hippurate is not. Inulin, lactose and trehalose are acidified. Mannitol, raffinose, ribose and sorbitol are not acidified. Gives a positive result in tests for \(\beta\)-glucosidase, but a negative result for \(\alpha\)-galactosidase, \(\beta\)-galactosidase, \(\alpha\)-glucosidase and \(N\)-acetyl-\(\beta\)-glucosaminidase. Voges–Proskauer reaction is positive. Cells are susceptible to most antimicrobial agents.

The type strain, M09-11185\textsuperscript{T} (=ATCC BAA-2337\textsuperscript{T}=KCTC 33006\textsuperscript{T}=ABB-12D01\textsuperscript{T}), was isolated from an abscess from a chimpanzee.

**Acknowledgements**

The authors would like to thank Dr Ian Tizard for critical review of this manuscript. This work was supported partially by Mississippi Veterinary Research & Diagnostic Laboratory, College of Veterinary Medicine, Mississippi State University and Department of Veterinary Pathobiology, College of Veterinary Medicine & Biomedical Sciences, Texas A&M University.

**References**


