Streptococcus henryi sp. nov. and Streptococcus caballi sp. nov., isolated from the hindgut of horses with oligofructose-induced laminitis

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Four Gram-positive, catalase-negative, coccoid-shaped isolates were obtained from the caecum and rectum of horses with oligofructose-induced equine laminitis. Phenotypic and phylogenetic studies were performed on these isolates. Initial biochemical profiling assigned two of the isolates to Streptococcus bovis. The other two isolates, however, could not be assigned conclusively on the basis of their biochemical profiles. Gene sequence analysis demonstrated that the four new isolates were related most closely to Streptococcus suis based on the 16S rRNA gene and to Streptococcus orisratti based on the manganese-dependent superoxide dismutase gene (sodA). Sequence divergence values from recognized Streptococcus species based on these two genes were >3 and >13%, respectively, for all four isolates. Phylogenetic and phenotypic analyses demonstrated that the four isolates formed two distinct clonal groups that are suggested to represent two novel species of the genus Streptococcus. The names proposed for these organisms are Streptococcus henryi sp. nov. (type strain 126T = ATCC BAA-1484T = DSM 19005T) and Streptococcus caballi sp. nov. (type strain 151T = ATCC BAA-1485T = DSM 19004T).

Equine laminitis is defined most simply as a failure of attachment between the distal phalanx and the inner hoof wall (Pollitt, 1999) and is considered to be the most serious disease of the equine foot. Laminitis is frequently attributable to dietary intake of excess carbohydrate in the form of lush pasture (45.6% excess) or grain (7.4%) (USDA, 2000), with such exposure resulting in significant population shifts in the equine hindgut microbiota, from one dominated by Gram-negative organisms to one dominated by Gram-positive organisms. By using the oligofructose-induction model of van Eps & Pollitt (2006), streptococci of the Streptococcus bovis/equinus complex have been demonstrated to routinely become established in the caecum as the dominant morphotype by as early as 2 h after oligofructose administration (Milinovich et al., 2007).

Although it is accepted that microbial changes occurring in the equine hindgut represent the initiating event for the foot pathology observed with laminitis, the mechanisms by which this pathology occurs and the factors responsible for triggering them remain to be elucidated (Garner et al., 1975, 1978; Pollitt, 1996). This paper describes the phenotypic and genotypic characteristics of four streptococcal isolates obtained from the hindgut of horses in which laminitis was experimentally induced. We propose that these isolates represent two novel species of the genus Streptococcus.

Laminitis was induced in two horses by administration of a bolus dose of oligofructose, as described previously by van Eps & Pollitt (2006). Preliminary 16S rRNA gene sequence analysis of four isolates recovered on incubated equine caecal fluid agar (Milinovich et al., 2006) revealed them to represent novel streptococci, and these isolates were characterized further. Two of the four isolates, 120 and 126T, were recovered from the caecum of a caecally fistulated horse at 32 and 40 h after oligofructose induction, respectively. Isolates 148 and 151T were
recovered from faeces collected per rectum at 16 and 24 h after oligofructose administration, respectively (Milinovich et al., 2006). The new isolates were Gram-stained, assessed for the presence of catalase and subjected to Lancefield grouping by using group A-, B-, C-, D-, F- and G-specific streptococcal latex agglutinating antisera (Oxoid). The ability of the isolates to tolerate the presence of 6.5 % NaCl, 0.016 % potassium tellurite and 40 % bile was assessed (Swan, 1954), and biochemical profiling was performed on all four isolates by using the commercially available API 20 Strep and Rapid ID32 Strep kits (bioMérieux). The phylogenetic position of the four isolates was determined through sequence analysis of the 16S rRNA and manganese-dependent superoxide dismutase (sodA) genes. Amplification of the 16S rRNA gene was performed by using primers 27f (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492r (5'-TACGGYTACCTTGGTACGACTT-3') (Lane, 1991) and the cycle parameters described by Burrell et al. (1998). Amplification of the sodA gene was performed by using the cycle parameters and primers sodA-F (5'-TRCGATCATGAYAARCCACCAT-3') and sodA-R (5'-ARRTARTAMGCRGTYCCCARACRTC-3') of Hoshino et al. (2005). All PCRs described were performed by using high-fidelity DNA polymerase (iProof high-fidelity DNA polymerase; Bio-Rad). PCR products were purified by using a QiAquick PCR purification kit (Qiagen) and sequencing was performed with a BigDye Terminator sequencing kit (Applied Biosystems). In accordance with the biochemical profiles, 16S rRNA and sodA gene sequence analysis identified isolates 120 and 151T as clones distinct from isolates 148 and 151T. Isolates 120 and 126T were determined by BLAST analysis to have highest sequence similarity to the type strain of S. suis (96.4 %) based on the 16S rRNA gene and to that of Streptococcus orisratti (86.6 %) based on the sodA gene. Isolates 148 and 151T were determined to be related most closely to the type strain of S. suis (96.8 %) based on the 16S rRNA gene and that of S. orisratti (83.4 %) based on the sodA gene. Sequence divergence between the two groups of isolates was 4.1 % for the 16S rRNA gene and 14.7 % for the sodA gene. The DNA G + C content was assayed for isolates 126T and 151T at the DSMZ by using the HPLC method of Mesbah et al. (1989) and was determined to be 38.7 and 46.8 mol %, respectively.

Phylogenetic analyses were performed as described by Bjornsson et al. (2002). In brief, one parsimonious and three distance analyses were performed on a neighbour-joining tree to generate bootstrap values, and the summary of these four analyses (with bootstrap values of over 70 %) is illustrated in a 16S rRNA gene sequence-based phylogenetic tree (Fig. 1; an extended version of this tree is available as Supplementary Fig. S1 in IJSEM Online). The topology of this phylogenetic tree, together with comparative 16S rRNA gene sequence analysis, gave divergence values of >3 % between the new isolates and their nearest recognized relatives (Stackebrandt & Goebel, 1994). These data support the recognition of these two streptococcal groups of isolates as representing two novel and distinct species. sodA gene sequence analysis, which has been demonstrated to be a useful means of differentiating streptococci on phylogenetic grounds (Poyart et al., 1998, 2002), demonstrated divergence values of >13 % between the new isolates and their nearest recognized relatives (not shown), further supporting this finding. A BLAST analysis revealed that the closest relative to the two groups of isolates was the type strain of S. suis, which is supported by phylogenetic analysis (Fig. 1), illustrating that S. suis is the closest relative based on branch lengths and phylogenetic positioning. 16S rRNA gene sequence similarity matrix analyses (data not shown) also revealed that the two groups of isolates showed 95.7 % similarity to each other, further supporting the hypothesis that these represent two distinct and novel streptococcal species. Tests which are useful for differentiating strains 126T and 151T from closely related species are shown in Table 1. On the basis of the data presented, we conclude that the four novel strains represent two novel species of the genus Streptococcus, for which the names Streptococcus henryi sp. nov. and Streptococcus caballi sp. nov. are proposed.

**Description of Streptococcus henryi sp. nov.**

*Streptococcus henryi* [hen.ry’i. N.L. gen. n. *henryi* named in honour of Dr Dick Peter Henry (AM) (1924–2003), a highly respected Australian veterinarian and microbiologist].

Cells are Gram-positive, non-motile ovoids, typically occurring in pairs or short chains. When grown anaerobically for 24 h on sheep blood agar, colonies are round, flat, shiny, 2 mm in diameter, off-white in colour and exhibit...
-haemolysis. Cells are facultatively anaerobic and catalase-negative. Cells react to streptococcal group D antisera and are able to grow in the presence of 40 % bile and 0.016 % potassium tellurite, but do not grow in the presence of 6.5 % NaCl. In API 20 Strep and Rapid ID32 Strep kits, cells are able to produce acid from starch, glycogen, inulin, lactose, galactosidase, and mannosidase.

**Table 1.** Tests useful in distinguishing strains 126\(^T\) and 151\(^T\) from closely related species of the genus *Streptococcus*

<table>
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<tr>
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<td>Acetoin (Voges–Proskauer)</td>
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<td>α-Galactosidase</td>
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<td>Methyl β-D-glucopyranoside</td>
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<td>Trehalose</td>
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Fig. 1. Phylogenetic tree showing the positions of strains 126\(^T\) and 151\(^T\) and their closest relatives based on 16S rRNA gene sequences. All four of the new isolates were fully sequenced; however, only one representative of each of the novel species described is illustrated in this phylogenetic tree as the two isolates for each species had identical 16S rRNA gene sequences. Closed circles indicate that bootstrap values were >70 % (of 1000 trees) in all four distance and parsimonious analyses, whereas open circles indicate that bootstrap values were >70 % (based on 1000 trees) in three of the four distance and parsimonious analyses. Bar, 10 substitutions per 100 nucleotides. An extended version of this tree is available as Supplementary Fig. S1 in IJSEM Online.
maltose, mannitol, melibiose, methyl β-D-glucopyranoside, pullulan, sucrose and trehalose, but not from D-arabinose, L-arabinose, cyclodextrin, melezitose, raffinose, ribose, sorbitol or tagatose. Cells do not produce acetoin (Voges-Proskauer-negative) or hydrolyse arginine or hippurate, but do hydrolyse ascinulin. Positive for alanyl-phenylalanine-proline arylamidase, ß-galactosidase, ß-galactosidase, ß-glucosidase and leucine aminopeptidase, but negative for N-acetyl-β-glucosaminidase, alkaline phosphatase, ß-glucuronidase, glycyl tryptophan arylamidase, ß-mannosidase, pyrrolidonyl arylamidase and urease. The DNA G+C content of the type strain is 38.7 mol%.

The type strain, 126T (=ATCC BAA-1484T =DSM 19005T), was isolated from the cecum of a horse with oligofructose-induced laminitis.

**Description of Streptococcus caballi sp. nov.**

*S. caballi* (ca’bal’li. L. gen. n. caballi of an inferior riding- or pack-horse; named in recognition of the domestic horse, *Equus caballus*, which, of the seven species of Equidae, suffers most from laminitis).

Cells are Gram-positive, non-motile ovoids, typically occurring in pairs or chains commonly over 20 cells long. Anaerobic growth for 24 h on sheep blood agar produces colonies with a tendency to imbde into sheep blood agar. Cells are facultatively anaerobic and catalase-negative. Cells do not react with streptococcal catalase. Cells are able to grow in the presence of 40% bile and 0.016% potassium tellurite, but not 6.5% NaCl. In API 20 Strept and Rapid ID32 Strep kits, cells are able to produce acid from starch, glycogen, inulin, maltose, melibiose, methyl β-D-glucopyranoside, pullulan, raffinose, sucrose and trehalose, but not from D-arabinose, L-arabinose, cyclodextrin, lactose, mannitol, melezitose, ribose, sorbitol or tagatose. Cells do not hydrolyse arginine or hippurate, but do hydrolyse ascinulin and are able to produce acetoin (Voges-Proskauer-positive). Positive for alanyl-phenylalanine-proline arylamidase, ß-galactosidase, ß-glucosidase and leucine aminopeptidase, but negative for N-acetyl-β-glucosaminidase, alkaline phosphatase, ß-galactosidase, ß-glucuronidase, glycyl tryptophan arylamidase, ß-mannosidase, pyrrolidonyl arylamidase and urease. The DNA G+C content of the type strain is 46.8 mol%.

The type strain, 151T (=ATCC BAA-1485T =DSM 19004T), was isolated from the rectum of a horse with oligofructose-induced laminitis.

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