Streptococcus pharyngis sp. nov., a novel streptococcal species isolated from the respiratory tract of wild rabbits

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Four isolates of an unknown Gram-stain-positive, catalase-negative coccus-shaped organism, isolated from the pharynx of four wild rabbits, were characterized by phenotypic and molecular genetic methods. The micro-organisms were tentatively assigned to the genus Streptococcus based on cellular morphological and biochemical criteria, although the organisms did not appear to correspond to any species with a validly published name. Comparative 16S rRNA gene sequencing confirmed their identification as members of the genus Streptococcus, being most closely related phylogenetically to Streptococcus porcorum 682-03T (96.9 % 16S rRNA gene sequence similarity). Analysis of rpoB and sodA gene sequences showed divergence values between the novel species and S. porcorum 682-03T (the closest phylogenetic relative determined from 16S rRNA gene sequences) of 18.1 and 23.9 %, respectively. The novel bacterial isolate could be distinguished from the type strain of S. porcorum by several biochemical characteristics, such as the production of glycyl-tryptophan arylamidase and α-chymotrypsin, and the non-acidification of different sugars. Based on both phenotypic and phylogenetic findings, it is proposed that the unknown bacterium be assigned to a novel species of the genus Streptococcus, and named Streptococcus pharyngis sp. nov. The type strain is DICM10-00796BT (=CECT 8754T=CCUG 66496T).

Wild European rabbits (Oryctolagus cuniculus) have ecological and economic importance in the Mediterranean area, as they represent an economically important hunting activity and sustain a large number of predator species, hence contributing to the preservation of the diversity of the Mediterranean ecosystem (Delibes-Mateos et al., 2008; Gálvez et al., 2009). However, knowledge of the bacterial species isolated from wild rabbits is very limited. In this study, we report the phenotypic and phylogenetic features of four Streptococcus-like organisms (DICM10-00793A, DICM10-00795A, DICM10-00796B1 and DICM10-00803A) isolated from pharyngeal swab samples taken from four apparently healthy wild rabbits hunted on the same day and at the same location. Samples were collected, transported under refrigeration to the laboratory and processed for bacteriological analysis within 48 h. Rabbits were located in a plot inside an industrial park (429589.5 E 4593922.2 N). Samples were cultured on Columbia-CNA agar plates (bioMérieux) that were incubated at 37 °C for 24 h under aerobic and anaerobic [with 4–10 % CO2 using a GasPak Plus system (BBL)] conditions. Based on the phenotypic and phylogenetic findings, it is proposed that the unknown bacterium be assigned to a novel species of the genus Streptococcus is proposed.

Phylogenetic analysis was performed by comparative 16S rRNA gene sequence analysis as described previously (Vela et al., 2002). A large continuous fragment (approximately 1420 bases) of the 16S rRNA gene of the four isolates (DICM10-00793A, DICM10-00795A, DICM10-00796B and DICM10-00803A) was determined from PCR-amplified products, derived from universal primers.
**Streptococcus pharyngis sp. nov.**

Fig. 1. Neighbour-joining phylogenetic tree inferred from comparison of partial 16S rRNA gene sequences of strains of *Streptococcus pharyngis* sp. nov. and related members of the genus *Streptococcus*. *Enterococcus faecalis* ATCC 19433\(^T\) was used as an out-group (not shown). Filled circles indicate that the corresponding nodes (groupings) were also obtained in the maximum-parsimony tree. Open circles indicate that the corresponding nodes (groupings) were also obtained in the maximum-likelihood and maximum-parsimony trees. Bootstrap values (expressed as a percentage of 1000 replications) higher than 70 % are given at branch points. The different branches were supported by the results of the other two algorithms. Bar, 1 % sequence divergence.

Additional gene sequence analyses were carried out to clarify the phylogenetic affinities of the novel isolates. Partial sequences of the *rpoB* (701 bp) and *sodA* (397 bp) genes were amplified using the primer pairs d1 and d2 (Poyart *et al.*, 1998) and StreptoF and StreptoR (Drancourt *et al.*, 2004), respectively, and sequenced as described previously (Glazunova *et al.*, 2006). Comparative sequence analysis revealed 99.2–100 and 98.2–100 % sequence similarities between the isolates for the *sodA* and *rpoB* gene sequences, respectively. Isolate DCM10-00796B\(^T\) exhibited highest sequence similarities with *Streptococcus dysgalactiae* CIP 102914\(^T\) (85.3 %) and *Streptococcus cuniculi* NED12-00049-6B\(^T\) (76.3 %) based on the *rpoB* and *sodA* genes, respectively. Levels of *rpoB* and *sodA* gene sequence divergence between DCM10-00796B\(^T\) and *S. porcorum* 682-03\(^T\) were 18.1 and 23.9 %, respectively (sequence similarities of 81.9 and 76.1 %, respectively). These divergence values are higher than the mean interspecies divergence values obtained with these genes between pairs of species of the genus *Streptococcus* (Glazunova *et al.*, 2009). Isolate DCM10-00796B\(^T\) formed a separate branch from other species of the genus *Streptococcus* in the phylogenetic trees inferred from the *rpoB* (Fig. S1, available in the online Supplementary Material) and *sodA* (Fig. S2) genes. All these data support separate species status for the unidentified streptococci from wild rabbits.

The four novel isolates were Gram-stained and assessed for the presence of catalase. The haemyolytic reaction was determined on Columbia agar containing 5 % defibrinated sheep blood (bioMérieux) incubated aerobically at 37 °C for 24 and 48 h (Facklam & Elliott, 1995). Determination of growth at 4, 15, 22, 30, 37 and 42 °C was performed in brain heart infusion broth (Difco) with the pH adjusted to 7.5 (Facklam & Elliott, 1995). The ability of the isolates to tolerate the presence of 3.5, 4.5 and 6.5 % (w/v) NaCl was assessed as recommended by Facklam & Elliott (1995). The isolates were biochemically characterized using the Rapid ID32 STREP and API ZYM systems (bio-Mérieux) according to the manufacturer’s instructions. The new isolates exhibited almost identical biochemical characteristics, except for the acidification of trehalose (isolate DCM10-00803A was positive). The novel isolates could be distinguished from *S. porcorum* (the closest
related species based on 16S rRNA gene similarities) by several phenotypic characteristics such as acid production from lactose, methyl β-δ-glucopyranoside, maltose and sucrose (S. porcorum is positive), and the production of glycy1-tryptophan arylamidase and α-chymotrypsin (S. porcorum is negative). The phenotypic characteristics that differentiate the novel strains from S. cuniculi, which was isolated from nasal and tonsil samples of rabbits (Vela et al., 2014), are shown in Table 1. Because determination of the Lancefield group antigen is still an important routine identification technique, the serological group reaction of the isolates was determined with a commercial SlideX Strept kit (bioMérieux) by using specific group A, B, C, D, F and G streptococcal latex agglutinating antisera. Based on the phylogenetic, genotypic and phenotypic data presented, we consider DICM10-00796B<sup>T</sup> to be the type strain of a novel species of the genus Streptococcus, for which the name Streptococcus pharyngis sp. nov. is proposed. Given that four isolates were obtained from apparently healthy animals, it is not possible to reach any conclusions about possible non-pathogenic roles for this novel species of Streptococcus in wild rabbits.

### Description of Streptococcus pharyngis sp. nov.

**Streptococcus pharyngis** (pha.ryn’gis. Gr. n. pharynx throat; Gr. gen. n. pharyngis of the throat).

Cells are Gram-stain-positive, non-spore-forming cocci, 0.5–0.75 mm in diameter, occurring in pairs or chains commonly over 7–12 cells long. Colonies on blood agar are small, circular and non-pigmented, 0.25–0.5 mm in diameter and α-haemolytic at 37 °C after 24 h. Cells are facultatively anaerobic, catalase-negative and non-motile. Reacts with Lancefield group G antisera. Growth occurs at 30, 37 and 42 °C, but not at 4, 15 or 22 °C. Growth does not occur in broth containing 3.5, 4.5 or 6.5 % (w/v) NaCl. Cells are not able to produce acid from ribose, mannitol, sorbitol, trehalose, raffinose, sucrose, L-arabinose, D-arabitol, cyclodextrin, glycosgen, pullulan, maltose, lactose, melibiose, melezitose, methyl β-δ-glucopyranoside or tagatose. Leucine arylamidase, esterase C4, lipase C14 (weak reaction), naphthol-AS-BI-phosphohydrolase (weak reaction), acid phosphatase, α-chymotrypsin and glycyl-tryptophan arylamidase activities are detected. No activity is detected for alanine–phenylalanine–proline arylamidase, α-mannosidase, α-fucosidase, esterase lipase C8, valine arylamidase, cystine arylamidase, trypsin, β-glucuronidase, N-acetyl-β-glucosaminidase, β-galactosidase, α-galactosidase, pyrogulamic acid arylamidase or β-mannosidase. Arginine, hippurate and urea are not hydrolysed and acetoin is not produced.

The type strain, DICM10-00796B<sup>T</sup> (=CECT 8754<sup>T</sup> = CCUG 66496<sup>T</sup>), was isolated from the pharynx of a wild rabbit. Three additional strains of the species (DICM10-00793A, DICM10-00795A and DICM10-00803A) were also isolated from the pharynx of wild rabbits.

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### References


