NOTE

***Streptococcus ovis* sp. nov., isolated from sheep**

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Seven strains of an unknown Gram-positive catalase-negative chain-forming coccus-shaped organism isolated from clinical specimens from sheep were characterized by phenotypic and molecular taxonomic methods. Comparative 16S rRNA gene sequencing studies demonstrated that the bacterium represents a new sub-line within the genus *Streptococcus*. The unknown bacterium was readily distinguished from recognized streptococcal species by biochemical tests and electrophoretic analysis of whole-cell proteins. Based on phylogenetic and phenotypic evidence, it is proposed that the unknown bacterium be classified as *Streptococcus ovis* sp. nov. The type strain of *Streptococcus ovis* is CCUG 39485^T (= LMG 19174^T).

Keywords: *Streptococcus ovis* sp. nov., taxonomy, phylogeny, 16S rRNA

The genus *Streptococcus* encompasses a broad range of Gram-positive catalase-negative chain-forming coccus-shaped organisms. Currently over 40 species are recognized, many of which cause and/or are associated with disease in man and animals. In recent years, molecular genetic analyses based on 16S rRNA have provided new insights into the phylogenetic interrelationships of these organisms (Bentley *et al.*, 1991) and provided a powerful means for characterizing new diversity. In particular, the cumulative nature of the rRNA sequence data combined with improved phenotypic methodologies (e.g. PAGE analysis of whole-cell proteins) and data have led to the recognition of several new species within *Streptococcus* and related genera (e.g. Williams & Collins, 1990; Whiley *et al.*, 1990; Kawamura *et al.*, 1998; Devriese *et al.*, 1997, 1999; Flint *et al.*, 1999). Despite the large number of described streptococcal species, there is a growing awareness that many others remain to be discovered, particularly from human and animal sources. In this article, we report the polyphasic characterization of seven streptococcal-like isolates originating from clinical specimens from sheep. Based on the findings of this study, another new streptococcal species, *Streptococcus ovis*, is described.

Strain S369/98/1 (CCUG 39485^T) was isolated in mixed culture with *Fusobacterium necrophorum* and an

Actinomyces sp. from a superficial abscess on skin of a 12-year-old ewe. Strain S577/98/1 was isolated from a chest wall abscess of a 1-year-old sheep along with *Actinomyces hyovaginalis*, *F. necrophorum*, *Bacteroides fragilis* and *Prevotella melaninogenica*. Strain S392/97/1 (CCUG 41614) was recovered from a fatal navel infection of a 1-d-old lamb along with *Escherichia coli* and an *Actinomyces* sp., whereas strain S95/98/4 (CCUG 42595) was isolated from a fatal lung abscess of a 10-month-old lamb together with *Arcanobacterium pyogenes*, *F. necrophorum*, *Mannheimia haemolytica* and *Streptococcus sanguinis*. Strain S1121/98/4 (CCUG 42596) originated from a mouth abscess resulting from a dosing gun injury of a 3-month-old lamb along with *Arcanobacterium pyogenes*, *Pasteurella trehalosi* and a coagulase-negative *Staphylococcus* sp. Isolate CCUG 42212 originated from a case of ovine pneumonia and strain CCUG 28672 from sheep arthritis. The unidentified strains were cultured aerobically on Columbia agar (Difco) supplemented with 5% horse blood at 37 °C. The organisms were biochemically characterized by using the API rapid ID32S and API ZYM systems according to the manufacturer's instructions (API bioMérieux). PAGE analysis of whole-cell proteins was performed as described by Pot *et al.* (1994). For densitometric analysis, normalization and interpretation of protein patterns, the GelCompar GCW 3.0 software package (Applied Maths, Kortrijk, Belgium) was used. The determined profiles were compared with an extensive database maintained by the Culture

The GenBank accession number for the 16S rRNA gene sequence of *Streptococcus ovis* CCUG 39485^T is Y17358.

Fig. 1. Unrooted tree showing the phylogenetic relationships of *Streptococcus ovis* sp. nov. and some other reference streptococcal species. The tree, constructed using the neighbour-joining method, was based on a comparison of approximately 1320 nucleotides. Bootstrap values, expressed as a percentage of 200 replications, are given at branching points. Bar, 1%.

Collection of the University of Göteborg, Sweden. The G+C content of DNA of a representative isolate was determined by thermal denaturation as described by Garvie (1978). For phylogenetic analysis, 16S rRNA genes were amplified by PCR and directly sequenced using a *Taq* Dye-Deoxy Terminator Cycle Sequencing kit (Applied Biosystems) and an automatic DNA sequencer (model 373A; Applied Biosystems). A phylogenetic tree was constructed according to the neighbour-joining method and the stability of the groupings was estimated by bootstrap analysis (200 replications) (Felsenstein, 1989).

Cells of the unknown isolates from ovine clinical specimens were ovoid in shape, stained Gram-positive and occurred singly, in pairs and in short chains. They were catalase-negative and oxidase-negative and produced α -haemolytic colonies on sheep and horse blood agar. Using commercial API systems, all seven

isolates produced acid from glucose, glycogen, lactose, maltose, mannitol, raffinose, trehalose, sorbitol and sucrose. The organisms failed to produce acid from D-arabitol, L-arabinose, melibiose, melezitose, methyl β -D-glucopyranoside, N-acetyl- β -glucosamine, pullulan or ribose. The isolates displayed alanyl-phenylalanine-proline arylamidase, esterase C-4, α -glucosidase, β -glucosidase, phosphoamidase and leucine arylamidase activities. No activity was detected for alkaline phosphatase, chymotrypsin, trypsin, ester lipase C8, lipase C14, α -fucosidase, β -galactosidase, β -glucuronidase, α -mannosidase, β -mannosidase, pyroglutamic acid arylamidase or urease. Variable reactions were obtained for acid phosphatase, arginine dihydrolase, cystine arylamidase, glycyl-tryptophan arylamidase and α -galactosidase. None of the strains hydrolysed hippurate and all were Voges-Proskauer negative. The cellular morphology and general biochemical reactions of the isolates were consistent with their assignment to

the genus *Streptococcus*. The phenotypic similarities of the ovine strains were further examined by PAGE analysis of whole-cell proteins. Four representative isolates were subjected to protein profiling and were found to closely resemble each other, forming a distinct cluster (within-group similarity of greater than 88%) which was separate from all currently recognized streptococcal reference species (data not shown). *Streptococcus suis* was the nearest relative of the unknown sheep bacterium based on whole-cell protein patterns, joining the latter at approximately 80% similarity. To assess the phylogenetic position of the unknown coccus-shaped organisms, their 16S rRNA gene sequences were determined and subjected to a comparative analysis. Over 1400 bases were determined for each strain and pair-wise analysis revealed 99.8–100% sequence similarity, demonstrating their high genotypic relatedness. Sequence searches of GenBank and RDP Libraries revealed that the unknown bacterium (as exemplified by strain CCUG 39485^T) was phylogenetically most closely associated with streptococcal species (data not shown). A tree constructed by neighbour-joining depicting the phylogenetic affinity of the unknown coccus within the genus *Streptococcus* is shown in Fig. 1. The unknown bacterium formed a distinct sub-line and did not display a particularly close affinity with any described species.

It is evident from the polyphasic taxonomic study that the unidentified organisms from clinical specimens from sheep represent an unknown member of the genus *Streptococcus*. Biochemical profiling and PAGE analysis of whole-cell protein patterns demonstrate that the bacterium is phenotypically distinct from all described members of this genus. In addition, 16S rRNA gene sequence divergence values of greater than 5% between the unknown coccus-shaped organisms and currently recognized streptococcal species unequivocally demonstrate that the bacterium represents a novel taxon at the species level. Therefore, based on the described findings, we consider that the bacterium from ovine sources merits classification as a new species of the genus *Streptococcus*, for which the name *Streptococcus ovis* sp. nov. is proposed. The novel species from sheep is biochemically unique and can be distinguished from all described streptococcal species. The species which phenotypically most closely resembles *Streptococcus ovis* is *Streptococcus suis*. However, *Streptococcus ovis* can be readily distinguished from the latter species in forming acid from mannitol and sorbitol and in not producing β -glucuronidase. In contrast, *Streptococcus suis* does not produce acid from these substrates and is β -glucuronidase-positive.

Description of *Streptococcus ovis* sp. nov.

Streptococcus ovis (o'vis. L. gen. n. *ovis* of the sheep).

Cells are Gram-positive, non-spore-forming cocci that occur singly, in pairs or in short chains. Colonies are

0.5–1.0 mm diameter after 24 h and are α -haemolytic on sheep and horse blood. Aesculin-positive colonies on Edwards medium. Facultatively anaerobic and catalase- and oxidase-negative. Using commercial API systems, strains produce acid from glucose, glycogen, lactose, maltose, mannitol, raffinose, trehalose, sorbitol and sucrose. Most strains produce acid from D-tagatose but most fail to ferment cyclodextrin. Acid is not produced from D-arabitol, L-arabinose, melibiose, melezitose, methyl β -D-glucopyranoside, pullulan or ribose. Alanine-phenylalanine-proline arylamidase, α -glucosidase, β -glucosidase, esterase C-4 (weak reaction), leucine arylamidase and phosphoamidase (weak reaction) activities are detected. No activity is detected for alkaline phosphatase, chymotrypsin, ester lipase C8, lipase C14, α -fucosidase, β -galactosidase, β -glucuronidase, α -mannosidase, β -mannosidase, N-acetyl- β -glucosaminidase, pyroglutamic acid arylamidase, trypsin or urease. Variable reactions are obtained for acid phosphatase, arginine dihydrolase, α -galactosidase, cystine arylamidase, valine arylamidase and glycyl-tryptophan arylamidase. Hippurate hydrolysis and Voges-Proskauer tests are negative. The G+C content of DNA is 38 mol%. Isolated from clinical specimens from sheep. Habitat is not known. The type strain is CCUG 39485^T (= LMG 19174^T).

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