Streptococcus entericus sp. nov., isolated from cattle intestine


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Biochemical, molecular chemical and molecular genetic studies were performed on an unknown Gram-positive, catalase-negative, coccus-shaped organism isolated from the intestine of a cow affected with catarrhal enteritis. The organism was tentatively identified as a streptococcal species based on results of cellular morphological and biochemical tests. 16S rRNA gene sequencing studies confirmed its provisional identification as a member of the genus Streptococcus, but the organism did not correspond to any recognized species of this genus. The nearest phylogenetic relatives of the unknown coccus from a calf were Streptococcus acidominimus and Streptococcus suis. The unknown bacterium, however, was distinguished from these species and other animal streptococci by biochemical tests and electrophoretic analysis of whole-cell proteins. Based on both phenotypic and phylogenetic findings, it is proposed that the unknown bacterium be classified as a novel species of the genus Streptococcus, Streptococcus entericus sp. nov. The type strain is CECT 5353T (= CCUG 44616T).

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

Molecular genetic methods, most notably 16S rRNA gene sequencing, have contributed greatly to improvements in the taxonomy of the genus Streptococcus. In particular, phylogenetic analysis based on comparative 16S rRNA analysis has helped to clarify the intrageneric relationships of the streptococci (Bentley et al., 1991; Kawamura et al., 1995; Hardie & Whiley, 1997) and has aided the recognition of a plethora of novel species from human (e.g. Kawamura et al., 1998; Flint et al., 1999; Collins et al., 2000; Schlegel et al., 2000), animal (e.g. Devriese et al., 1997, 1999; Rurangirwa et al., 2000) and food (e.g. Tsakalidou et al., 1998; Schlegel et al., 2000) sources. Although streptococci can be isolated as part of the normal flora of the alimentary, respiratory and genitourinary tracts, as well as the skin of man and different animals (Kilian, 1998), some species are established pathogens that cause a variety of diseases such as endocarditis, respiratory infections, endocarditis, meningitis, arthritis and mastitis (Chanter, 1997). Streptococcus bovis, Streptococcus intestinalis (junior synonym of Streptococcus alactolyticus) and Streptococcus thoraltensis have been isolated from or are members of the intestinal flora of different animals (Devriese et al., 1997; Kilian, 1998; Vandamme et al., 1999), but their association with pathological processes is not clear. Although there are some reports of intestinal disorders associated with streptococci (Bard et al., 1987; Jergens et al., 1991; Gelshorn et al., 1994; Willard et al., 1998; Svane, 2000), enteric disease associated with this group of organisms remains poorly defined. During the course of a study of a cow affected by catarrhal enteritis, we have isolated a Gram-positive, catalase-negative, coccus-shaped organism that resembles the streptococci. We report the results of a polyphasic taxonomic study on the isolated coccus. Based on the findings presented, a novel streptococcal species, Streptococcus entericus sp. nov., is described.

A diarrhoeic process affecting thirty, 7- to 10-day-old Holstein–Friesian calves of the same farm was investigated. Catarrhal enteritis was the clinical sign observed after post-mortem examination of one diseased animal. Gram stain of faeces and the mucous membrane of the jejunum revealed the presence of large numbers of Gram-positive, ovoid-shaped bacteria. A coccus-
Two Gram-positive, coccus-shaped isolates, which formed short chains, were isolated from faeces and jejunum mucous membrane. The two isolates exhibited identical physiological, serological and biochemical characteristics and were assumed to represent a single clinical strain, as they were isolated from the same animal. The unidentified organisms were facultatively anaerobic, catalase-negative bacteria producing un pigmented, circular colonies, 1 mm in diameter after 24 h incubation at 37 °C on blood agar. They were α-haemolytic. The isolates grew at pH 9.6 and at 30 and 37 °C, but not in 6.5% (w/v) NaCl broth or at 10 or 45 °C. They were bile-/aesculin-negative. Using commercial API systems, they produced acid from starch, cyclodextrin, glycogen, lactose, maltose, trehalose, sucrose and methyl β-D-glucopyranoside, but failed to produce acid from D-arabitol, L-arabinose, inulin, mannitol, melibiose, melezitose, pullulan, ribose, raffinose, sorbitol or tagatose. Activity was observed for β-glucosidase, leucine arylamidase, β-galactosidase, alanine-phenyllalanine-proline arylamidase and glycyltryptophan arylamidase, but not for alkaline phosphatase, β-glucuronylase, α-galactosidase, pyroglycemic acid arylamidase, N-acetyl-β-glucosaminidase or β-mannosidase. The isolates hydrolysed aesculin but not arginine, hippurate or urea. They were Voges–Proskauer-negative and vancomycin-sensitive. The morphological and biochemical characteristics of the unknown clinical isolates were consistent with those of the genus Streptococcus, but they did not correspond to any of the currently defined species of this genus. The numerical profile with API 20 Strep was 4040413, corresponding to a doubtful discrimination between S. bovis biotype I and Streptococcus suis biotype I. API Rapid ID32 Strep gave a profile 60036421111, corresponding to an unacceptably identification.

To investigate the phylogenetic affinities of the two unknown isolates, comparative 16S rRNA gene sequencing was performed. Approximately 600 bases (including variable regions V1, V2 and V3) were compared for the two isolates and were found to be identical, thereby strengthening the view that they represent a single strain. The almost complete (> 1400 bases) 16S rRNA gene sequence of the bacterium (as exemplified by CCUG 44616T) was determined and subjected to a comparative phylogenetic analysis. Sequence searches of GenBank revealed that the unknown coccus was phylogenetically most closely related to the genus Streptococcus (data not shown). Treeing analysis confirmed this affinity and a dendrogram depicting the phylogenetic relationships of the unidentified coccus within the genus Streptococcus is shown in Fig. 1. The coccus formed a distinct subline with Streptococcus acidominimus (94.8% sequence similarity) and S. suis (95% sequence similarity) as its closest phylogenetic relatives. Bootstrap resampling, however, showed that the unknown coccus did not share a statistically significant association with either of these taxa. In order to investigate further the affinity of the unidentified coccus with the aforementioned species and other streptococci, PAGE analysis of whole-cell proteins was performed. The protein analysis showed that the unknown organism was different from all described streptococcal species (data not shown). The coccus formed a long line and displayed a loose affinity (correlation level <70%) with Streptococcus hyovaginalis and Streptococcus plurimamma- lium. S. acidominimus and S. suis displayed lower similarities to the unknown organism (correlation level of 50%).

It is clear from the polyphasic taxonomic study that the unidentified catalase-negative coccus represents a...
previously unrecognized streptococcal species. Phylogenetically, the bacterium from a calf forms a distinct subline within the genus and exhibits a loose association with \textit{S. acidominimus} and \textit{S. suis}. 16S rRNA sequence divergence values of > 4% between the novel isolate and \textit{S. acidominimus} and \textit{S. suis}, however, demonstrate unequivocally that it represents a distinct species (Stackebrandt & Goebel, 1994). PAGE analysis

\begin{table}
\centering
\caption{Characteristics useful in differentiating \textit{Streptococcus entericus} sp. nov. from genotypically related species and other Lancefield group D streptococci}
\begin{tabular}{llllllllllllll}
\hline
Characteristic & 1 & 2 & 3 & 4 & 5 & 6 & 7 & 8 & 9 & 10\textsuperscript{*} & 11 & 12 & 13 \\
\hline
Production of: & & & & & & & & & & & & & \\
\textalpha{}-Galactosidase & - & + & + & + & + & (v)\textsuperscript{†} & - & + & + & v - (+) & + & - & v \\
Production of acid from: & & & & & & & & & & & & & \\
Lactose & + & v + & + & v - (+) & - & + & + & + & - & + & - & v \\
\hline
\end{tabular}
\textsuperscript{*} \textit{S. galolyticus} includes the strains formerly identified as \textit{S. bovis} biotype I (Osawa et al., 1995).
\textsuperscript{†} Different results were reported by Schlegel et al. (2000), as given in parentheses.
\textsuperscript{‡} Given as variable (most strains negative) by Vandamme et al. (1999).
\end{table}

\textit{Streptococcus entericus} sp. nov.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{tree.png}
\caption{Unrooted tree showing the phylogenetic relationships of \textit{Streptococcus entericus} sp. nov. and some other reference streptococcal species. The tree, constructed using the neighbour-joining method, was based on a comparison of approx. 1320 nucleotides. Bootstrap values, expressed as percentages of 500 replications, are given at branching points. Bar, 1% sequence divergence.}
\end{figure}
of whole-cell proteins and biochemical profiling also showed that the isolate was distinct from all recognized streptococcal species. It is pertinent to note that the novel coccus-shaped organism gave a positive reaction for Lancefield group D. No other Lancefield group was detected. The unknown isolate is phenotypically distinct from S. bovis/Streptococcus equinus and S. suis, however, as well as from other Lancefield group D streptococci (Table 1). The Lancefield group D antigen can also be found in enterococci, but the novel isolate could be readily distinguished from enterococci by its lack of resistance to bile, its negative pyrrolidonylarylamidase reaction and its failure to grow at 10 °C and 6.5% NaCl, enterococci being positive for these tests (Facklam & Elliott, 1995). Therefore, based on both phylogenetic and phenotypic criteria, it is evident that the unidentified coccus merits classification as a novel species of the genus Streptococcus, for which the name Streptococcus entericus sp. nov. is proposed.

Tests that are useful in differentiating S. entericus from its nearest phylogenetic relatives and from some other animal streptococci with which it may be confused are shown in Table 1. Although it is not possible to conclude that S. entericus was involved in the pathological process, its isolation from faeces and intestinal mucosa of a calf with catarrhal enteritis indicates that it is possibly clinically significant. The formal description of S. entericus and the availability of tests to facilitate its identification will aid clinical laboratories in recognizing this species in the future and improve knowledge of its distribution and possible association with disease.

Description of Streptococcus entericus sp. nov.

Streptococcus entericus (en.te’ri.cus. N.L. adj. entericus from Gr. n. enteron gut, pertaining to the gut).

Cells are Gram-positive cocci arranged in short chains. Non-motile. Facultatively anaerobic, catalase- and oxidase-negative. Colonies are non-pigmented, circular and 1 mm in diameter after 24 h on blood agar and produce an α-haemolytic reaction. Reacts with Lancefield group D antisera. Growth occurs at 37 °C but not at 10 or 45 °C. Growth occurs at pH 9·6 but not in broth containing 6·5% NaCl. Bile/esculin test is negative. Sensitive to vancomycin. Using the commercial API Rapid ID32 Streptococcus and API 20 Streptococcus systems, acid is produced from starch, cyclohextrin, glycerogen, lactose, maltose, trehalose, sucrose and methyl β-d-glucopyranoside but not from D-arabinol, L-arabinose, inulin, mannitol, melibiose, melezitose, pullulan, ribose, raffinose, sorbitol or tagatose. β-Glucosidase, leucine arylamidase, β-galactosidase, alanine-phenylalanine-proline arylamidase and glycy1-tryptophan arylamidase are detected. No activity is detected for β-glucuronidase, α-galactosidase, alkaline phosphatase, pyroglutamic acid arylamidase, N-acetyl-β-glucosaminidase or β-mannosidase. Aesculin is hydrolysed but arginine, hippurate and urea are not. Aceto produced. Isolated from faeces and jejunum of a calf with enteritis. Habitat is not known.

The type strain of Streptococcus entericus is CECT 5353T (= CCUG 44616T).

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