Streptococcus marimammalium sp. nov., isolated from seals

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Two strains of an unidentified, Gram-positive, catalase-negative, chain-forming, coccus-shaped organism recovered from seals were characterized using phenotypic and molecular taxonomic methods. Based on morphological and biochemical criteria the strains were tentatively identified as streptococci but they did not appear to correspond to any recognized species of the genus Streptococcus. Comparative 16S rRNA gene sequencing studies showed that the strains were closely related to each other and confirmed their placement in the genus Streptococcus. Sequence divergence values of > 5% with reference streptococcal species demonstrated the organisms from seals represent a novel species. SDS-PAGE analysis of whole-cell proteins confirmed that the two organisms were closely related to each other but were different from all currently defined streptococcal species. Based on biochemical criteria, molecular chemical and molecular genetic evidence, it is proposed that the unknown isolates from seals be assigned to a novel species of the genus Streptococcus, Streptococcus marimammalium sp. nov. The type strain is M54/01/1T (=CCUG 48494T = CIP 108309T).

The genus Streptococcus embraces a phenotypically diverse group of catalase-negative, Gram-positive, coccus-shaped organisms. In recent years the genus has undergone considerable expansion with a plethora of novel species described, especially from human and animal sources (Facklam, 2002). Although the genus now accommodates more than 60 species, relatively few of these streptococcal species have been recovered from marine animals. Indeed only three species, Streptococcus phocae (Skaar et al., 1994) and Streptococcus halichoeri (Lawson et al., 2004) from seals and Streptococcus iniae recovered from freshwater dolphins and subsequently found in aquacultures of fish and from humans (Pier & Madin, 1976; Weinstein et al., 1997), have originated from marine mammals. During the course of a study of taxonomically problematic, catalase-negative, Gram-positive cocci from marine mammals we have characterized two novel Streptococcus-like isolates from a common seal and a grey seal. Based on the results of a polyphasic taxonomic study, we describe a novel species, Streptococcus marimammalium sp. nov., from marine mammals.

Two bacterial isolates were recovered from different species of seal. Strain M54/01/1T was recovered from a grey seal (Halichoerus grypus) and strain M529/97/1 (=CCUG 48934) was isolated from the lung of a common seal (Phoca vitulina) following post-mortem in Inverness, Scotland. The unidentified strains were cultured on Columbia agar base supplemented with 5% sheep blood at 37°C under capnophilic conditions. Organisms were characterized biochemically using the API Rapid ID 32Strep, API 20Strep and API ZYM systems according to the manufacturer’s instructions (API bioMérieux). Lancefield serological grouping was determined by using the Streptex (Remel Europe) test system. To assess the overall phenotypic resemblance of the new isolates and related species, a comparative analysis of whole-cell protein profiles by SDS-PAGE was performed. PAGE analysis of whole-cell proteins was performed as described by Pot et al. (1994) and Vandamme et al. (1998). For densitometric analysis, normalization and interpretation of protein patterns, the GCW 3.0 software package (Applied Maths) was used. Similarity between all pairs of traces was expressed by using the Pearson product moment correlation coefficient converted for convenience to a percentage similarity. The DNA G+C content of strain M54/01/1T was determined by HPLC according to the method of Mesbah et al. (1989). For phylogenetic analysis, 16S rRNA genes were amplified by PCR and sequenced.
directly using a Taq dye-Deoxy terminator cycle sequencing kit (Applied Biosystems) and an automatic DNA sequencer (model 373A; Applied Biosystems). The closest known relatives of the new isolates were determined by performing database searches. Closely related sequences were retrieved from EMBL and aligned with the newly determined sequences using the program DNATools (Rasmussen, 1995). The resulting multiple sequence alignment had approximately 100 bases at the 5’ end of the rRNA omitted from further analysis, because of alignment uncertainties due to the highly variable region V1, using the program GeneDoc (Nicholas et al., 1997). A phylogenetic tree was reconstructed using the neighbour-joining method (Saitou & Nei, 1987) with the programs DNATools and TreeView (Page, 1996), and the stability of the groupings was estimated by bootstrap analysis (1000 replications).

The almost-complete gene sequences (> 1400 bases) of the two strains were determined; pair-wise analysis revealed 100 % sequence similarity, indicating that the organisms were genetically highly related. Searches of the GenBank database revealed streptococci to be the nearest phylogenetic relatives of the unidentified isolates. Phylogenetic analysis confirmed the association of the unidentified seal bacterium with the genus Streptococcus, with the unknown bacterium forming a distinct subline. A tree based on a reduced data set showing the nearest phylogenetic relatives of the unknown bacterium is depicted in Fig. 1. The DNA G+C content of a representative strain (M54/01/1T) of the unknown bacterium was determined to be 38 mol%.

Comparative 16S rRNA gene sequence analysis unequivocally demonstrated that the unidentified, catalase-negative, coccus-shaped organisms from seals represent a hitherto unknown streptococcal species. Phylogenetically, the unknown bacterium forms a distinct subline within the genus. Although the bacterium (as exemplified by strain M54/01/1T) displayed a loose association with a small subcluster of species (embracing Streptococcus entericus, Streptococcus suis and Streptococcus acidominimus), bootstrap resampling showed that it did not possess a statistically significant association with any recognized species. This strain had a 16S rRNA sequence similarity of 94 % with its nearest neighbour S. entericus, an organism isolated from cattle (Vela et al., 2002). Similar levels of sequence divergence (5–7 %) were also found between the unknown seal bacterium and its other close phylogenetic relatives (Streptococcus hyovaginalis, 93-7 %; Streptococcus pluranimalium, 93-2 %; Streptococcus thoraltensis, 93-9 %; Streptococcus ovis, 93-2 %; Streptococcus minor, 93 %; S. suis, 93-6 %; S. acidominimus, 94-3 %). Divergence values of 3 % or greater are considered to be strong evidence that organisms are not related at the species level. Support for the distinctiveness of the unknown seal bacterium was also evident from phenotypic analyses. In particular, whole-cell protein profiling, a powerful technique for comparing closely related organisms and which correlates well with results from DNA–DNA hybridization experiments (Vandamme et al., 1996), showed the organisms from seals were phenotypically quite separate from all other streptococcal species. The unidentified seal bacterium also produced very distinct biochemical profiles (API Rapid ID 32Strep numerical profile 4/5 0/4 0 1 2 0/4 0 0/0 0 0/2 and API 20Strep profile 0 0 0/2 0/1 4 0 0), which served to distinguish it from all other described streptococcal species. Therefore, based on both phenotypic and phylogenetic criteria, we suggest that the unknown bacterium from seals merits assignment to a novel species within the genus Streptococcus, for which the name Streptococcus marimammalium sp. nov. is proposed. Tests that are useful in distinguishing S. marimammalium from other closely related streptococcal species are shown in Table 1.

Description of Streptococcus marimammalium sp. nov.

Streptococcus marimammalium (ma.ri.mam.ma’li.um. L. neut. n. mare the sea; N.L. neut. gen. pl. n. mammalium

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**Fig. 1.** Unrooted tree based on 16S rRNA gene sequence analysis showing the phylogenetic relationships of *Streptococcus marimammalium* sp. nov. Bar, 1 % sequence divergence.

**Table 1.** Tests that are useful in distinguishing *Streptococcus marimammalium* sp. nov. from its closest phylogenetic relatives and some other streptococci from sea mammals

Species: 1, *Streptococcus marimammalium* sp. nov.; 2, *S. entericus*; 3, *S. acidominimus*; 4, *S. suis*; 5, *S. iniae*; 6, *S. phocae*; 7, *S. ovis*; 8, *S. hyovaginalis*; 9, *S. thoraltensis*. Information on Lancefield antigens and haemolytic reactions is from Facklam (2002). +, Positive; −, negative; V, variable; ND, not determined. Biochemical results were obtained from the API Rapid ID 32Strep test system from Collins et al. (2001), Devriese et al. (1997), Vela et al. (2002) and the present study.

<table>
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<th>1</th>
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<th>6</th>
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<td>R, S, T</td>
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<td>ND</td>
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<td>–</td>
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<td>–</td>
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<td>–</td>
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<td>+</td>
<td>+</td>
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<td>–</td>
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<td>V</td>
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<td>–</td>
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<td>V</td>
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<td>Food</td>
<td>Swine, human</td>
<td>Dolphins, fish, human</td>
<td>Seals, cetaceans</td>
<td>Sheep</td>
<td>Swine</td>
<td>Swine</td>
</tr>
</tbody>
</table>

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of mammals; N.L. gen. pl. n. *marimammalium* of marine mammals).

Cells stain Gram-positive and are coccii which occur in pairs or short chains. Non-spor-forming. Colonies are 0.2–0.3 mm in diameter after 24 h incubation on sheep blood agar, grey–white in colour and non-haemolytic on first isolation, becoming β-haemolytic after 3 days. No growth occurs on MacConkey agar with or without salt. Lancefield serological group C. Facultatively anaerobic and catalase-negative. Using API test kits, acid is produced from lactose but not from L-arabinose, D-arabitol, cyclodextrin, glycogen, inulin, mannotol, melibiose, melezitose, methyl β-D-glucopyranoside, pullulan, raffinose, ribose, sorbitol, starch, sucrose, D-tagatose or trehalose; acid may or may not be produced from maltose. Alanine phenylalanine proline arylamidase, acid phosphatase (weak), chymotrypsin, leucine arylamidase and valine arylamidase are produced but activity for cysteine arylamidase, χ-fucosidase, χ-galactosidase, β-glucosidase, χ-glucuronidase, lipase C14, χ-mannosidase, β-mannosidase, N-acetyl-β-glucosaminidase, pyroglutamic acid arylamidase and trypsin are not detected. Activity for arginine dihydrolase, alkaline phosphatase, β-galactosidase, urease and glycyll tryptophan arylamidase is variable. Aesculin and hippurate are not hydrolysed. Acetoin is not produced.

The type strain, M54/01/1 T (=CCUG 48494 T = CIP 108309 T), was isolated from a grey seal. Its DNA G+C content is 38 mol%.

**Acknowledgements**

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**References**


