Note

Emended description of *Streptococcus ferus* isolated from pigs and rats

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Streptococcus ferus is a mutans-like streptococcus originally isolated from wild rats fed with sugar cane. Taxonomically, this species has not been studied extensively. Ten Gram-positive coccal strains, isolated from the tonsils and nasal conchae of piglets, were identified as *S. ferus* by 16S rDNA sequencing, tRNA-intergenic spacer length polymorphism analysis (tDNA-PCR), whole-cell protein profiling using SDS-PAGE, G+C content determination and extensive biochemical testing. In all these tests, the type strain of *S. ferus* (LMG 16520^T), from a rat, was included. The results of the tests are described and an emended species description is presented.

The upper respiratory tract is a site favoured by streptococci. The oral cavities of many animals are colonized by streptococcal species belonging to the mutans group. The habitat of *Streptococcus mutans* is the human tooth surface (Whiley & Beighton, 1998). *Streptococcus sobrinus* is also a colonizer of the human mouth and can be very cariogenic (Coykendall, 1983). *Streptococcus downei* and *Streptococcus macacae* were isolated from the dental plaque of monkeys (Coykendall, 1977; Beighton *et al.*, 1984).

During an investigation of the tonsillar and nasal flora of piglets with tRNA-intergenic spacer length polymorphism analysis (tDNA-PCR) (Baele *et al.*, 2001a), a group of Gram-positive coccal isolates was isolated and characterized further. Representative strains were studied extensively and were found to belong to the poorly described species *Streptococcus ferus*.

Ten atypical streptococcus-like isolates were isolated on Columbia CNA blood agar (Oxoid) from the tonsils or nasal conchae of pigs. The strains were isolated from three different farms in Belgium. Six isolates originated from the same farm. Three of them were isolated from the nasal

conchae of weaned piglets, one from the tonsils of one of those piglets and two from the tonsils of two other piglets from the same litter. On a second farm, three similar cultures were obtained from the tonsils of weaned piglets. A tenth isolate was received from another laboratory and was isolated on a third farm from the nose of a pig. The following isolates were subjected to further taxonomic study: KOMA 81 (=LMG 19829), KOMA 188 (=LMG 19830), COR 146 (=LMG 19831), KOMA 101, KOMA 115, KOMA 206, KOMA 215, KOMA 229 and KOMA 248, together with the type strain of *S. ferus*, LMG 16520^T (=ATCC 33477^T), isolated from a rat.

DNA extraction, tDNA-PCR, capillary electrophoresis and data analysis were done as described before (Baele *et al.*, 2000). tDNA-PCR has been shown to be discriminative at the species level for all enterococcal and most streptococcal species (Baele *et al.*, 2000, 2001b; De Gheldre *et al.*, 1999). The tDNA-PCR fingerprints of the 10 isolates from the upper respiratory tract of piglets and of the type strain of *S. ferus* were composed of fragments with lengths of 71·5, 81·5, 157, 255·4 and 262·5 bp. A distance matrix was obtained with the in-house software. Cluster analysis of tDNA-PCR fingerprints was done using the UPGMA algorithm with the NEIGHBOR software of PHYLIP (<http://evolution.genetics.washington.edu/phylip.html>) and the dendrogram was visualized using the program TREEVIEW (<http://taxonomy.zoology.gla.ac.uk/rod/treeview.html>). The ten isolates grouped in a single cluster that was clearly separated from other streptococcal species (Fig. 1). A version of the dendrogram including a wider sample of

Abbreviation: tDNA-PCR, tRNA-intergenic spacer length polymorphism analysis.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of *S. ferus* strains LMG 19829 and LMG 16520^T are AF336367 and AY058218.

A dendrogram based on tDNA-PCR fingerprints including a wider sample of *Streptococcus* species is available as supplementary material in IJSEM Online (<http://ijs.sgmjournals.org/>).

Fig. 1. Dendrogram obtained from tDNA-PCR fingerprints, after similarity calculation with in-house software and UPGMA cluster analysis. Bar, 10 % similarity. A version of the dendrogram including a wider sample of *Streptococcus* species is available as supplementary material in IJSEM Online (<http://ijs.sgmjournals.org/>).

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Pig strain LMG 19829 and the type strain LMG 16520^T from a rat were subjected to 16S rDNA sequence analysis. The 16S rDNA gene was amplified using primers $\alpha\beta$ -NOT (5'-TCAAACCTAGGACCGAGTC) and ω_{MB} (5'-TACCTTGTTACTTCACCCCA) and the *Taq* Mastermix (Qiagen). Sequencing was performed using the BigDye Terminator sequencing kit with primers pD, Gamma*, 3 and O* and an ABI PRISM 310 Genetic Analyzer (Coenye *et al.*, 1999). Phylogenetic analysis was performed using the software Bionumerics (Applied Maths) after including the consensus sequence in an alignment of small ribosomal subunit sequences collected from GenBank. Multiple alignment was calculated using an open gap penalty of 100 % and a unit gap penalty of 0 %. A similarity matrix was created by homology calculation with a gap penalty of 0 % and after discarding unknown bases. The resulting tree was constructed using the neighbour-joining method. The two strains represented a separate phylogenetic branch in the *S. mutans* group (Fig. 2), which contains several other 'oral' streptococci (Bentley *et al.*, 1991). Similarities of

Fig. 2. 16S rDNA phylogenetic tree showing the relationships among *S. ferus* strains, related species of the *S. mutans* group and species representing different lineages within the genus *Streptococcus*. The tree was constructed by neighbour-joining analysis of a distance matrix from a multiple-sequence alignment. *Lactococcus lactis* subsp. *cremoris* was used as the outgroup and bootstrap values are indicated at the branch-points (100 trees resampled). Accession numbers are given.

94 and 93.9 %, respectively, were obtained with the closest relatives *S. mutans* and *S. macacae*.

For determination of the DNA base composition, strains LMG 19829 and LMG 19830 were grown for 24–48 h on brain/heart infusion agar (Difco) and incubated at 37 °C in a microaerobic atmosphere containing approximately 5 % O₂, 10 % CO₂ and 85 % N₂. High-molecular-mass native DNA was extracted from 0.75–1.25 g wet weight of cells using the protocol described by Pitcher *et al.* (1989) with the following modifications: the washed cell pellet was resuspended and lysed in a buffer (10 mM Tris/HCl, 100 mM EDTA, pH 8.0) containing RNase (200 µg ml⁻¹; Sigma), mutanolysin (100 U ml⁻¹; Sigma) and lysozyme (25 mg ml⁻¹; Serva) for 1 h at 37 °C. Before addition of GES reagent, proteinase K (200 µg ml⁻¹; Merck) was added and the mixture was incubated for 15 min. DNA was enzymically degraded into nucleosides and separated by HPLC as described previously (Vancanneyt *et al.*, 2001). The G+C contents of strains LMG 19829 and LMG 19830 were respectively 43.0 and 42.7 mol%.

Isolates LMG 19829, LMG 19830 and LMG 19831 and LMG 16520^T were included in SDS-PAGE analysis of whole-cell proteins. Cells were cultivated as indicated for determination of DNA base compositions. Whole-cell protein extracts were prepared and PAGE was performed as described before (Pot *et al.*, 1994). Registration of the protein patterns,

Fig. 3. Protein profiles of *S. ferus* strains and related taxa and corresponding dendrogram derived from the UPGMA cluster analysis of correlation coefficients, r (expressed as percentages). Molecular mass markers (lane labelled M) are (from left to right): lysozyme, 14 500; trypsin inhibitor, 20 100; trypsinogen, 24 000; carbonic anhydrase, 29 000; glyceraldehyde-3-phosphate dehydrogenase, 36 000; egg albumin, 45 000; bovine albumin, 66 000; and β -galactosidase, 116 000 Da.

normalization of the densitometric traces, pattern storage, grouping of the strains using Pearson's product-moment correlation coefficient (r) and UPGMA cluster analysis were performed as described by Pot *et al.* (1994) using the software GelCompar (Applied Maths). This yielded highly similar patterns, confirming that the isolates represent a single species. The profiles were different from all patterns of lactic acid bacteria in the database (data not shown), confirming their separate species status. The patterns of the three strains studied and their phylogenetically closest neighbours are shown in Fig. 3.

The strains formed small (measuring up to 0.5 mm), dry, adherent, corroding and pitting colonies that were non-haemolytic. These characteristics resembled those of other taxa of the *S. mutans* group. A detailed phenotypic description of the species is given below. Table 1 summarizes the characteristics that can be used for the differentiation of the species from phylogenetically related species. It should be noted that the tests indicated in the original description of *S. ferus* (Coykendall, 1977) and in related papers (Freedman *et al.*, 1982; Coykendall, 1983) as well as in reviews (Hardie & Whiley, 1995) cannot be used. Notably, failure to produce acid from raffinose, as described by Coykendall (1977, 1983), is not a distinguishing characteristic, because the test was found to be positive for all strains from pigs.

Table 1. Characteristics that differentiate *S. ferus* from related species

Data for reference taxa were based on Hardie & Whiley (1995). Traits are scored as: D, strain-dependent; D+, usually positive; ND, not determined.

Biochemical trait	<i>S. ferus</i>	<i>S. mutans</i>	<i>S. macacae</i>
Acid from:			
Melibiose	D	D+	—
Glycogen	+	—	ND
Inulin	D	+	—
Starch	+	—	—

Emended description of *Streptococcus ferus* (ex Coykendall 1977) Coykendall 1983

Cells are Gram-positive, relatively small, lanceolate coccobacilli that occur singly or in pairs or short chains. They are non-sporulating and catalase-negative. Colonies on blood agar are small, dry, white and adhering, pitting and corroding the agar surface. Growth is slightly enhanced in the presence of 5 % CO₂ and is strongly inhibited at 25 °C and to a lesser extent, at 30 °C. Growth is equally good at 42 and 37 °C. Cells precipitate in brain/heart infusion broth and strains are unable to grow in the presence of 6.5 % NaCl. Growth on Edwards' medium occurs but no browning or blackening of the medium is observed. On Slanetz–Bartley agar (Oxoid), containing sodium azide, isolates grow well and form white colonies. They are resistant to bile, as tested by inoculating organisms onto aesculin/bile agar (Difco). No Lancefield carbohydrate antigens (Streptococcal grouping kit, Oxoid) are detected. Positive for amylase production, as tested by spot inoculation onto Columbia agar without added blood and flooding the plates with Gram's iodine after overnight incubation.

Acid is produced (API 50 CH, bioMérieux) from *N*-acetylglucosamine, aesculin, amygdalin, cellobiose, D-fructose, galactose, D-glucose, glycogen, β -gentiobiose, lactose, D-mannose, maltose, maltotriose, sucrose, salicin, starch and trehalose. Variable acidification of D-tagatose, arbutin, inulin, mannitol, melibiose, D-raffinose and sorbitol. No acid is produced from adonitol, D- or L-arabinose, D- or L-arabitol, dulcitol, erythritol, D- or L-fucose, gluconate, glycerol, inositol, 2- or 5-ketogluconate, D-lyxose, melezitose, methyl β -glycoside, methyl α -D-mannoside, methyl α -D-glycoside, rhamnose, ribose, L-sorbose, D-turanose, xylitol or D- or L-xylose.

S. ferus strains are positive in tests (API 20 STREP, bioMérieux; BBL Crystal Gram-positive ID kits, Becton Dickinson) for aesculin, leucine arylamidase, enzymic hydrolysis of 4-methylumbelliferyl (4MU) β -D-glucoside, L-valine 7-amido-4-methylcoumarin (AMC), L-phenylalanine AMC, 4MU α -D-glucoside, L-tryptophan AMC, methyl α - and β -glucoside, *p*-nitrophenyl (pNP) β -D-glucoside, pNP

β -D-cellobioside, proline and leucine *p*-nitroanilide, pNP phosphate and pNP α -D-maltoside. They are usually positive in tests for acetoin, L-arginine AMC and L-isoleucine AMC and variable for alkaline phosphatase and α -galactosidase. Negative for hippurate, pyrrolidonyl arylamidase, β -glucuronidase, β -galactosidase and hydrolysis of arginine (in API 20 Strep), L-pyroglutamic acid AMC, 4MU *N*-acetyl β -D-glucosaminide, 4MU phosphate and 4MU β -D-glucuronide. An adhesive glucan is produced from sucrose.

The DNA G+C content is 43 mol% and the characteristic tDNA-PCR fingerprint is composed of fragments of 71.5, 81.5, 157, 255.4 and 262.5 bp, as determined by fluorescent capillary electrophoresis.

The type strain, strain LMG 16520^T (=ATCC 33477^T), was isolated from a rat.

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