**Streptococcus porci** sp. nov., isolated from swine sources

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Two unidentified Gram-positive, catalase-negative, coccus-shaped organisms were recovered from pigs and subjected to a polyphasic taxonomic analysis. Based on cellular morphology and biochemical criteria, the isolates were tentatively assigned to the genus *Streptococcus*, although the organisms did not appear to correspond to any recognized species. Comparative 16S rRNA gene sequence studies confirmed this identification and showed that the nearest phylogenetic relatives of the unknown cocci were *Streptococcus plurextorum* 1956-02T and *Streptococcus suis* NCTC 10234T (97.9 and 96.0 % 16S rRNA gene sequence similarity, respectively). The new isolates were related most closely to *S. suis* CIP 103217T based on *rpoB* gene sequence analysis (% sequence divergence). DNA–DNA pairing studies showed that one of the unidentified strains (2923-03T) displayed DNA relatedness values of 26.6 and 27.2 % with *S. plurextorum* CECT 7308T and *S. suis* NCTC 10234T, respectively. On the basis of phenotypic and phylogenetic evidence, it is proposed that the unknown isolates from pigs be classified in the genus *Streptococcus* as members of *Streptococcus porci* sp. nov., with the type strain 2923-03T (=CECT 7374T =CCUG 55896T).

Streptococci have been isolated from a wide range of environments and can be found as part of the normal flora of the skin and alimentary, respiratory and genito-urinary tracts of humans and different animals (Kilian, 1998). Some *Streptococcus* species have been implicated as the causes of different diseases in humans and animals, such as endometritis, respiratory infections, endocarditis, meningitis, arthritis and mastitis (Chanter, 1997; Köhler, 2007). During routine microbiological diagnosis from different clinical specimens submitted to the Animal Health Surveillance Centre (VISAVET) of the Universidad Complutense (Madrid, Spain), two unidentified Gram-positive, cocccoid organisms were recovered from the pericardium (strain 2923-03T) and bronchial lymph nodes (strain 2857-03) of two pigs with lesions of pericarditis and pneumonia, respectively. Strains were isolated on Columbia blood agar plates (bioMérieux) incubated for 24 h at 37 °C under both aerobic and anaerobic conditions. On the basis of the phenotypic and phylogenetic results, a novel species of the genus *Streptococcus*, *Streptococcus porci* sp. nov., is proposed.

The new isolates were Gram-stained and assessed for the presence of catalase. Haemolytic reaction was determined on Columbia agar containing 5 % defibrinated sheep blood (bioMérieux) incubated aerobically at 37 °C for 24 and 48 h (Facklam & Elliot, 1995). Determination of growth at 10 and 42 °C, or with 3.0, 4.5 and 6.5 % added NaCl in brain-heart infusion broth (Difco) with the pH adjusted to 7.5, was performed as recommended by Facklam & Elliot (1995). Growth in brain-heart infusion broth (Difco) was assessed at pH 9.6 (Facklam & Elliot, 1995). Lancefield serological group reaction was determined with a commercial SlideX Strepto kit (bioMérieux) by using specific group A, B, C, D, F and G streptococcal latex-agglutinating...
antisera. The isolates were characterized biochemically by using the Rapid ID32 Strep, API 50CH and API ZYM systems (bioMérieux) according to the manufacturer’s instructions. The API 50CH strips using CHB suspension medium were read up to 7 days incubation at 37 °C. The two isolates exhibited almost-identical biochemical characteristics, except for the acidification of L-rhamnose, methyl β-D-xylopyranoside and pullulan (only isolate 2923-03T was positive for these tests). A detailed description of the physiological, biochemical and morphological characteristics of the isolates is given in the species description and in Table 1.

Table 1. Characteristics useful in differentiating Streptococcus porci sp. nov. from other streptococci isolated from pigs

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A phylogenetic analysis was performed by comparative 16S rRNA gene sequence analysis as described previously (Vela et al., 2002). A large, continuous fragment (approx. 1390 bases) of the 16S rRNA gene of the two isolates was obtained bidirectionally. This analysis revealed that the two isolates had the same 16S rRNA gene sequence (100 % similarity). Sequence searches of GenBank using the program FASTA (Pearson, 1994) revealed that the unknown cocci were phylogenetically related most closely to Streptococcus plurextorum 1956-02T and Streptococcus suis NCTC 10234T (97.9 and 96.0 % 16S rRNA gene sequence similarity, respectively). These sequences and those of other representative species with validly published names within the genus Streptococcus were retrieved from GenBank and aligned with the newly determined sequence by using the program DNATools (Rasmussen, 1995). Phylogenetic trees were constructed according to three different methods: the neighbour-joining algorithm (Saitou & Nei, 1987), performed with the programs DNATools and TreeView (Page, 1996), a maximum-likelihood analysis using PHYML software (Guindon & Gascuel, 2003) and the maximum-parsimony method, carried out using the software package MEGA version 3.1 (Kumar et al., 2004). Genetic distances for the neighbour-joining and maximum-likelihood algorithms were calculated by the Kimura two-parameter method (Kimura, 1980), and close-neighbour interchange (search level=2, random additions=100) was applied in maximum-parsimony analysis. Stability of the groupings was estimated by bootstrap analysis (1000 replications). Phylogenetic trees obtained by using the neighbour-joining algorithm (Fig. 1; an extended version of this tree is available in IJSEM Online) presents a full version of the phylogenetic tree, which includes a wider sample of Streptococcus species.

DNA–DNA hybridization experiments were carried out between the two isolates (strains 2923-03T and 2857-03) and between strain 2923-03T and its nearest phylogenetic neighbours, S. plurextorum CECT 7308T and S. suis NCTC 10234T. Genomic DNA was isolated by using a French pressure cell (Thermo Spectronic) and was purified by chromatography on hydroxyapatite as described by Cashion et al. (1977). DNA–DNA hybridization was carried out in 2× SSC at 68 °C by the Identification Service of the DSMZ (Braunschweig, Germany), using the method described by De Ley et al. (1970) under consideration of the modifications described by Huß et al. (1983), with a model Cary 100 Bio UV/VIS spectrophotometer equipped with a Peltier-thermostatted 6×6 multicell changer and a temperature controller with in situ temperature probe (Varian). The DNA–DNA hybridization study showed 81.7 % DNA relatedness between the two new isolates, demonstrating that they are members of the same species (Wayne et al., 1987). DNA–DNA reassociation values between strain 2923-03T and S. plurextorum CECT 7308T and S. suis NCTC 10234T were 26.6 and 27.2 %, respectively, clearly confirming that the new isolates constitute a separate species (Wayne et al., 1987).

The G+C content of the DNA of one representative isolate (strain 2923-03T) was determined at the DSMZ by using the HPLC method of Mesbah et al. (1989). The DNA G+C content of strain 2923-03T was 41.5 mol%.

Based on phylogenetic and phenotypic criteria, it is clear that the unidentified catalase-negative cocci merit clas-
sification as members of a novel species of the genus Streptococcus, for which the name Streptococcus porci sp. nov. is proposed. The two isolates were recovered from different animals and in different farms located in different provinces of Spain. Therefore, it is very unlikely that the isolates could have any epidemiological clonality. Tests that are useful in differentiating S. porci from those streptococcal species that have been isolated from pigs or have clinical relevance for this animal species are shown in Table 1. Only one isolate of S. porci was isolated in pure culture from the heart of a pig with pericarditis, which precludes any conclusions about the possible pathogenic role for pigs of this novel species of Streptococcus.

**Description of Streptococcus porci sp. nov.**

*Streptococcus porci* (por’ci. L. gen. n. porci of a pig). Cells are Gram-positive, non-spore-forming cocci, 0.3–0.5 μm in diameter, occurring in pairs or short chains of three to eight cells. Colonies on blood agar are small, circular and non-pigmented, 0.75–1.0 mm in diameter and non-haemolytic at 37 °C. Cells are facultatively anaerobic, catalase-negative and non-motile. Cells react to streptococcal group B antisera and are able to grow at 37 °C and at pH 9.6, but do not grow at 10 or 42 °C and at the presence of 6.5 % NaCl. With the API 50CH and Rapid ID32 Strep kits (bioMérieux), cells are able to produce acid from D-lactose, D-glucose, D-fructose, salicin, maltose, melibiose, D-galactose, D-xylene, D-mannose, N-acetylgalcosamine, trehalose, starch, cellobirose, arbutin, raffinose and sucrose, but not from glycerol, erythritol, D-arabinose, D-ribose, L-xylene, D-adenitol, L-arabitol, D-arabitol, gentiobiose, D-mannitol, inositol, dulcitol, melezitose, inulin, methyl α-D-mannopyranoside, methyl β-D-glucopyranoside, methyl β-D-glucuronoside, L-sorbose, turanose, D-lyxose, xylitol, D-fucose, L-fucose, amygdalin, sorbitol, aesculin, 2-ketoglucurate, 5-ketogluconate, cyclodextrin or tagatose. Cells are able to produce acid from glycogen and L-arabinose using the API 50CH system. The type strain produces acid from L-rhamnose, methyl β-D-xlyopyranoside (API 50CH) and pullulan (Rapid ID32 Strep). β-Galactosidase, α-glucosidase, leucine arylamidase (API ZYM), α-galactosidase, β-glucosidase (API ZYM and Rapid ID32 Strep), alanine-phenylalanine-proline arylamidase and glycel-tryptophan arylamidase (Rapid ID32 Strep) are detected. No activity is detected for N-acetyl-β-glucosaminidase, naphthol-NS-phosphohydrolase, α-mannosidase, α-fucosidase, esterase C4, esterase lipase C8, lipase C14, valine arylamidase, cystine arylamidase, trypsin, α-phosphatase, β-cholesterol, β-glucosidase (API ZYM), alkaline phosphatase, β-gluconidase (API ZYM and Rapid ID32 Strep), β-mannosidase or pyrogallic acid arylamidase (Rapid ID32 Strep). Arginine, hippurate and urea are not hydrolysed (Rapid ID32 Strep). Acetoin is not produced (Rapid ID32 Strep). The DNA G+C content of the type strain is 41.5 mol%.

The type strain, 2923-03 T (＝CCUG 55896 T), was isolated from the heart of a pig with pericarditis. Full range of habitat is not known.

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