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Streptococcus pseudoporcinus sp. nov., a Novel Species Isolated from the Genitourinary Tract of Women

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Streptococcus **strains from animal and human sources identified biochemically as** ***Streptococcus porcinus*** **were investigated by 16S rRNA gene sequencing. The nine human strains isolated between 1997 and 2005 formed a single cluster with more than 2.1% dissimilarity with** ***S. porcinus*** **strains from animal sources. A novel species,** ***Streptococcus pseudoporcinus*** **sp. nov., is proposed.**

The genus *Streptococcus* is a large group with currently more than 60 species, many of which were described in the last decade (6). The recent use of molecular characterization techniques may in part explain the sudden increase in the number of species, and one can assume that this trend will continue.

The species *Streptococcus porcinus* was described in 1984 (2) and is commonly associated with various pathological infections in swine (2, 12). Isolation of *S. porcinus* from humans has rarely been reported and is mostly from the female genitourinary tract (3, 5, 9). In fact, because of its biochemical similarities with *Streptococcus agalactiae* (commonly associated with female genitourinary tract infection or colonization) and serological cross-reactivity with group B streptococcal reagent (5, 11), human infections or colonization by *S. porcinus* may have been underestimated. In the province of Québec, Canada, nine strains isolated from human clinical specimens were identified as *Streptococcus porcinus* by conventional physiological tests described previously (5, 10).

These strains, obtained from three Montreal hospitals, were isolated between 1997 and 2005 from the genitourinary tracts of nine female patients (21 to 49 years old, mean = 30) originating from different countries. Table 1 shows phenotypic characteristics of the nine human strains as well as seven animal strains isolated from swine between 1995 and 2005 in the province of Québec. Past reports (3, 11) described all *S. porcinus* strains of human origin as being acetoin (Voges-Proskauer [VP]) positive and pyrrolidonyl arylamidase (PYR) positive or weakly positive, whereas group B streptococci were reported to be VP and PYR negative. In the present investigation, human strains were positive for VP and PYR in 56% and 33% of the cases, respectively, compared to 100% and 57% for animal strains.

Recently, we subjected these 16 strains to 16S rRNA gene sequencing for molecular characterization and identification purposes. In addition, the 16S rRNA gene sequences of 12 *Streptococcus* ATCC type strains were sequenced and included in the study. For sequencing purposes, bacteria were disrupted with zirconium/silica beads by using a bead beater (Biospec Inc.). Genomic DNA was extracted using a QIAamp DNA mini kit (QIAGEN Inc.). The 16S rRNA gene was amplified by PCR using primers Ai (AGRGTTYGATYCTGGCTCAGGAYG) and rJ (GGTTACCTTGTTACGACTT) (4, 8). The 1,500-bp amplified fragment was purified by using the Minelute filtration system (QIAGEN Inc.) and sequenced on both strands using the Ai, rJ, D, E, and rE primers (7).

DNA sequences were determined with an ABI 3100 sequencer using a BigDye sequencing kit (Applied Biosystems). The sequences were subjected to a BLAST analysis and aligned with the ClustalW program. Phylogenetic analysis was performed using the Lasergene software V6.1 (DNAstar).

The phylogenetic tree confirms that all of the isolates are related to the genus *Streptococcus*. The nine human strains exhibit 100% similarity/0% dissimilarity and form a single cluster that exhibits more than 2.1% dissimilarity with the other *Streptococcus* species. The animal strains (including the type strain ATCC 43138) form another cluster that exhibits between 2.1 and 2.4% dissimilarity with the nine human strains (Fig. 1).

Table 2 presents sequence dissimilarities between 16S rRNA gene sequences of one representative strain of human isolates (LQ 940-04^T) and the closest type strains of *Streptococcus* species. The lowest scores were for *S. porcinus* (2.5%), *Streptococcus iniae* (2.7%), and *Streptococcus uberis* (2.7%). Considering the 1.0% value the limit in species definition (1),

TABLE 1. Phenotypic characteristics of human and animal strains of *Streptococcus* biochemically identified as *S. porcinus*

Test	% of strains of indicated type with positive results	
	Human (n = 9)	Animal (n = 7)
Hydrolysis of hippurate	0	0
VP reaction	56	100
PYR test	30	57
Leucine aminopeptidase test	100	100
Acid production from:		
Mannitol	100	100
Sorbitol	100	100

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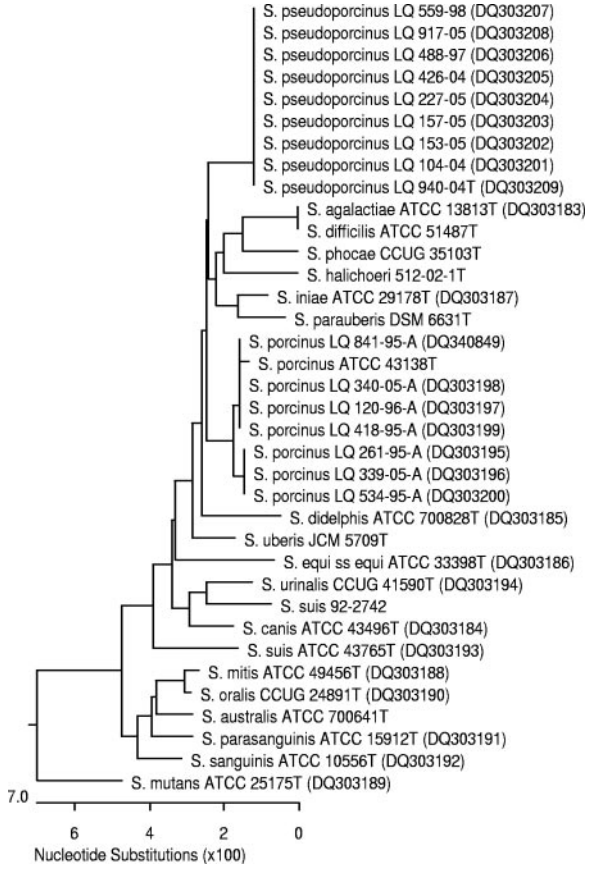

FIG. 1. Phylogenetic tree, based on comparative analysis of the 16S rRNA gene sequences, showing the relationships of *Streptococcus pseudoporcinus* sp. nov. with other *Streptococcus* species. GenBank accession numbers are shown in parentheses.

the nine human strains can be considered representative of a new species, namely, *Streptococcus pseudoporcinus* sp. nov., in reference to the similarity of its biochemical profile to that of *S. porcinus*. Strain LQ 940-04^T was chosen as the type strain.

In their study conducted on 25 human and 16 nonhuman strains (mostly swine), Duarte et al. (3) reported high similarities of whole-cell protein profiles among *S. porcinus* strains from different sources. However, using the randomly amplified polymorphic DNA-PCR and pulsed-field gel electrophoresis analyses, the authors identified two main clusters, I and II. The human isolates were included in cluster I, whereas the nonhuman ones were included in cluster II. The authors determined the existence of a few clonal groups of *S. porcinus*, adapted to the human host.

Our findings are in agreement with those of Duarte et al. (3), but our study reveals that some human clinical isolates may in fact belong to the new species *Streptococcus pseudoporcinus*. However, 16S sequencing is a necessary tool to differentiate *S. porcinus* from *S. pseudoporcinus*. The nine human strains of *Streptococcus* isolated from the genitourinary tract of women in the province of Québec and identified biochemically as *Streptococcus porcinus* were ultimately identified as *Streptococcus pseudoporcinus* by 16S rRNA gene sequencing. To our knowledge, we present the first molecular characterization by 16S rRNA gene sequencing of biochemically identified *S. porcinus*.

Description of *Streptococcus pseudoporcinus* sp. nov. Cells of *Streptococcus pseudoporcinus* sp. nov. are spherical to ovoid, gram positive, nonmotile, and generally arranged in short chains. On blood agar, its colonies are generally small and smooth, with entire margins, and β hemolytic, showing a large zone of complete hemolysis. There is slight to normal growth at 10°C, but mostly no growth at 45°C, in 7 days. Growth in 6.5% NaCl broth is obtained in 24 to 48 h. Catalase and benzidine tests are negative. No extracellular polysaccharide is produced. Fermentative metabolism is used. Acid is usually produced from glucose, glycerol, maltose, mannitol, ribose, salicin, sorbitol, sucrose, and trehalose but not from lactose or melibiose. Arginine and esculin, but not starch, bile esculin, urea, or hippurate, are usually hydrolyzed. Leucine, aminopeptidase test results are positive. VP reaction is variable. PYR is often negative. The species does not react with Lancefield group A, B, C, D, F, or G antisera (Streptex by Murex). A vancomycin screen (30-μg disks; Oxoid) indicated sensitivity.

Nucleotide sequence accession numbers. The 16S rRNA gene sequences obtained in this study were submitted to GenBank, and the obtained accession numbers are shown in Fig. 1. All of the accession numbers in Fig. 1 are newly established in our study.

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TABLE 2. Similarities and dissimilarities among 16S rRNA sequences of *Streptococcus* species type strains

Species	% Similarity or dissimilarity to indicated species									
	S. iniae	*S. didelphis*	*S. canis*	*S. agalactiae*	*S. uberis*	*S. difficilis*	*S. parauberis*	*S. halichoeri*	*S. porcinus*	LQ 940-04^T
S. iniae		95.6	96.4	96.5	96.7	96.5	97.7	96.1	96.3	97.2
S. didelphis	4.3		95.2	95.3	96.5	95.3	95.7	95.5	96.4	96.4
S. canis	3.5	5.0		96.0	97.2	96.0	96.4	96.2	96.4	96.1
S. agalactiae	3.3	4.9	4.2		95.4	100.0	96.0	95.9	96.2	96.5
S. uberis	3.1	3.6	2.9	4.8		95.4	97.0	95.1	97.1	97.4
S. difficilis	3.3	4.9	4.2	0.0	4.8		96.0	95.9	96.2	96.5
S. parauberis	2.2	4.5	3.7	4.1	3.0	4.1		95.6	96.5	96.5
S. halichoeri	3.7	4.5	3.9	4.2	5.0	4.2	4.5		96.3	96.2
S. porcinus	3.6	3.7	3.7	3.9	3.0	3.9	3.6	3.7		97.5
LQ 940-04^T	2.7	3.7	4.0	3.6	2.7	3.6	3.6	3.8	2.5	

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