

Aerococcus suis sp. nov., isolated from clinical specimens from swine

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Biochemical and molecular genetic studies were performed for five isolates of unknown Gram-positive, catalase-negative, cocci-shaped micro-organisms obtained from clinical samples from pigs. The micro-organisms were tentatively identified as *Aerococcus* species on the basis of the results from cellular morphological and biochemical tests. 16S rRNA gene sequencing studies confirmed the provisional identification of the isolates as members of the genus *Aerococcus*, but the micro-organism did not correspond to any recognized species of this genus. The nearest phylogenetic relatives of these unknown cocci isolated from pigs were *Aerococcus viridans* (95.9% 16S rRNA gene sequence similarity) and *Aerococcus urinaeequi* (95.8%). The unknown bacterium, however, was distinguishable from these two species and from other animal aerococci by using biochemical tests. On the basis of both phenotypic and phylogenetic findings, the isolates represent a novel species of the genus *Aerococcus*, for which the name *Aerococcus suis* sp. nov. is proposed. The type strain is 1821/02^T (= CECT 7139^T = CCUG 52530^T).

The genus *Aerococcus*, when originally described by Williams *et al.* (1953), comprises Gram-positive, micro-aerophilic, catalase-negative cocci arranged in tetrads and clusters. Initially, only one species, *Aerococcus viridans*, was included in this genus. In the last few decades, improved taxonomic methods, such as chemotaxonomic and molecular-based approaches (especially 16S rRNA gene sequencing), have contributed to the reclassification of *Pediococcus urinaeequi* as *Aerococcus urinaeequi* (Felis *et al.*, 2005) and the description of four novel species, namely *Aerococcus urinae*, *Aerococcus sanguinicola*, *Aerococcus christensenii* and *Aerococcus urinaehominis* (Aguirre & Collins, 1992; Collins *et al.*, 1999; Lawson *et al.*, 2001a, b). Aerococci have been isolated from air, vegetation, dust and in the indigenous microbiota of humans and animals. Moreover, some *Aerococcus* species have been involved as infrequent causes of infection in humans. *A. viridans* has been associated with endocarditis, meningitis, urinary tract infections and arthritis (Colman, 1967; Janosek *et al.*, 1980;

Nathavitharana *et al.*, 1983; Taylor & Trueblood, 1985) and *A. urinae* has been documented as an agent of urinary tract infection and endocarditis in immunocompromised patients (Christensen *et al.*, 1991; Kristensen & Nielsen, 1995). However, as far as we know, only one report has shown the isolation of aerococci from clinical specimens from animals (Devriese *et al.*, 1999). In this article, we report the phenotypic and phylogenetic characterization of five strains of an unusual *Aerococcus*-like species isolated from various clinical specimens from pigs.

The bacterial strains were isolated in different years from different clinical specimens from pigs located at different farms. The pigs were kept under intensive management conditions. The bacterial strains (strain no./year of isolation) were isolated from a lung (803/04), a bronchial lymph node (926/04), a joint (2189/02), the gut (519/03) and the brain (1821/02^T) of five different animals with lesions of pneumonia (two strains), arthritis, enteritis and meningitis, respectively. Only isolate 1821/02^T was recovered in pure culture. The other four isolates were obtained in mixed culture with members of the genus *Weeksella* (803/04), *Aerococcus* (926/04), *Haemophilus* (2189/02) and *Escherichia* (519/03). The strains were isolated on Columbia blood agar plates (bioMérieux) and incubated for 24 h at 37 °C under aerobic and anaerobic conditions.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain 1821/02^T is AM230658.

A full version of the neighbour-joining phylogenetic tree shown in Fig. 1 is available as a supplementary figure with the online version of this paper.

Phylogenetic characterization was performed using 16S rRNA gene sequencing, as described previously (Collins *et al.*, 1999). A large continuous fragment (approx. 1350 bases) of the 16S rRNA gene of one isolate (1821/02^T) and 1000 nt from the sequences of the other four isolates (803/04, 926/04, 519/03 and 2189/02) were obtained bidirectionally. This analysis revealed that the five isolates had the same 16S rRNA gene sequence (100% similarity), thereby demonstrating their high level of genealogical relatedness. Sequence searches of GenBank, performed using the program FASTA (Pearson, 1994), revealed that the unidentified cocci were phylogenetically most closely related to *A. viridans* (95.9% 16S rRNA gene sequence similarity) and *A. urinaeequi* (95.8%). These sequences and those of other related strains with validly published names were retrieved from GenBank and aligned with the newly determined sequences 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); the maximum-likelihood analysis, done using PHYML software (Guindon & Gascuel, 2003); and the maximum-parsimony method, carried out using the software package MEGA (molecular evolutionary genetics analysis), version 3.1 (Kumar *et al.*, 2004). Genetic distances for the neighbour-joining and maximum-likelihood algorithms were calculated by using the Kimura two-parameter model (Kimura, 1980), and close-neighbour-interchange (search level, 2; random additions, 100) was applied in the maximum-parsimony analysis. The stability of the groupings was estimated by means of bootstrap analysis (1000 replications). The phylogenetic tree obtained using the neighbour-joining method (Fig. 1) and the trees constructed using the maximum-likelihood and maximum-parsimony methods (data not shown) approaches all revealed a clear affiliation between the unidentified cocci (as exemplified by strain 1821/02^T) and the genus *Aerococcus*, and placed the novel bacterium as a separate branch within this genus. It is evident from Fig. 1 that strain 1821/02^T is closely related to *A. viridans* and *A. urinaeequi*. Bootstrap resampling revealed the affinity between the unidentified bacterium and the aforementioned species to be statistically significant. This, coupled with 16S rRNA gene sequence divergence values of >4% between the unidentified bacterium and the aforementioned species, suggests that the unidentified bacterium represents a distinct species of the genus *Aerococcus* (Stackebrandt & Goebel, 1994).

The G+C content of the DNA was determined from the mid-point value (T_m) of the thermal denaturation profile (Marmur & Doty, 1962) obtained with a Perkin-Elmer UV-Vis Lambda 20 spectrophotometer at 260 nm; this instrument was programmed for temperature increases of 1.0 °C min⁻¹ using a Peltier temperature programmer (Perkin-Elmer). The T_m was determined by using a graphic method described by Ferragut & Leclerc (1976), and the DNA G+C content was calculated from this temperature by using

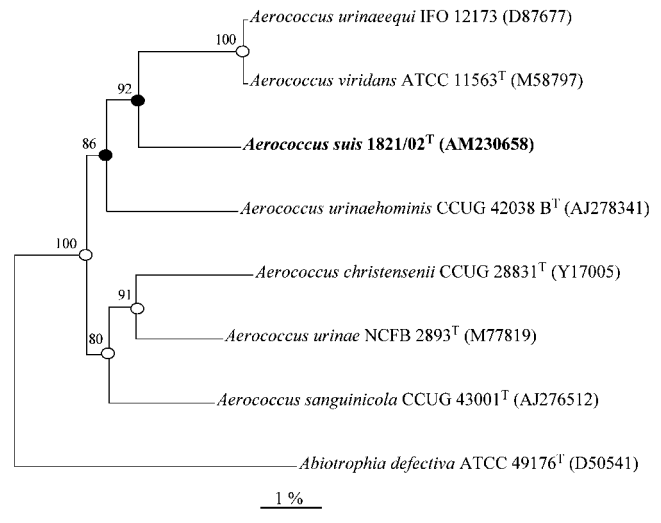


Fig. 1. Unrooted neighbour-joining phylogenetic tree, based on 16S rRNA gene sequence comparison, showing the relationships of strain 1821/02^T and other *Aerococcus* species. Bootstrap percentages (based on 1000 replications) higher than 50% are given at the branching points. Filled circles indicate that the corresponding nodes (groupings) are also obtained in maximum-likelihood trees. Open circles indicate that the corresponding nodes (groupings) are also obtained in maximum-likelihood and parsimony trees. Supplementary Fig. S1 (available in JSEM Online) presents a full version of the phylogenetic tree, which includes a wider sample of genera of Gram-positive, catalase-negative cocci. Bar, 1% sequence divergence.

the equation of Owen & Hill (1979), in 0.1 × SSC buffer (0.15 M NaCl buffered with 0.015 M trisodium citrate, pH 7.0). The T_m of the reference DNA from *Escherichia coli* NCTC 9001 was taken as 74.6 °C in 0.1 × SSC (Owen & Pitcher, 1985). The DNA G+C contents of isolates 1821/02^T and 926/04 are 37 and 35.5 mol%, respectively; percentages were confirmed in three different determinations.

The isolates were biochemically characterized using the API Rapid ID 32 Strep, API 20 Strep, API 50 CH and API ZYM systems (bioMérieux) according to the manufacturer's instructions. The API 50 CH strips were read at up to 7 days incubation at 37 °C. Conventional physiological tests were also performed (Facklam & Elliot, 1995). The five isolates exhibited homogeneous phenotypic and physiological characteristics, except in the tests for β-galactosidase (β-GAL test; only strains 926/04, 519/03, 2189/02 and 1821/02^T were positive) and urea hydrolysis (only strains 519/03, 2189/02 and 1821/02^T gave a positive reaction). A detailed description of the physiological, biochemical and morphological characteristics of the isolates is given in the species description and in Table 1. The unidentified isolates were found to be phenotypically distinct from *A. viridans* and *A. urinaeequi* as well as from the other species of the genus *Aerococcus* (Table 1).

Table 1. Characteristics that differentiate *Aerococcus suis* sp. nov. from other *Aerococcus* species

Species: 1, *A. suis* sp. nov.; 2, *A. viridans*; 3, *A. urinae*; 4, *A. sanguinicola*; 5, *A. christensenii*; 6, *A. urinaehominis*; 7, *A. urinaeequi*. Data for *Aerococcus* species were obtained from Garvie (1986), Collins *et al.* (1999), Lawson *et al.* (2001a, b), Facklam *et al.* (2003) and Felis *et al.* (2005). Symbols: +, positive reaction; -, negative reaction; v, variable reaction; ND, not determined.

Characteristic	1*	2	3	4	5	6	7
Hydrolysis of:							
Arginine	+	-	-	+†	-	-	-
Hippurate	-	v	+	+	+	+	ND
Aesculin	-	+	v	+	-	+	ND
Production of:							
Pyroglutamic acid arylamidase	-	+	-	+	-	-	-
β -Glucuronidase	-	v	+	+	-	+	ND
Acid production from:							
Maltose	-	+‡	-	+	-	+	+
Mannitol	-	v	+	-	-	-	v
Ribose	+§	v	v	-	-	+	ND
Sucrose	-	+	+	+	-	+	+
Trehalose	-	+	-	+	-	-	+

*Species *Aerococcus suis* sp. nov. gave a negative reaction for leucine aminopeptidase, this being a characteristic that differentiates it from *A. christensenii*.

†Negative reaction according to Facklam *et al.* (2003).

‡Variable reaction according to Facklam *et al.* (2003).

§Acidification after 7 days.

||Positive reaction according to Facklam *et al.* (2003).

Therefore, on the basis of both phylogenetic and phenotypic criteria it is evident that the unidentified cocci merit classification as a novel species of the genus *Aerococcus*, for which the name *Aerococcus suis* sp. nov. is proposed. Tests that are useful in differentiating *A. suis* from the other *Aerococcus* species are shown in Table 1. Only one isolate of *A. suis* was isolated in pure culture from the brain of a pig with meningitis, which precludes any conclusions about the possible pathogenicity of this novel species for pigs.

Description of *Aerococcus suis* sp. nov.

Aerococcus suis (su'is. L. fem. n. *sus*, *suis* pig, hog; L. gen. n. *suis* of the hog).

Gram-positive cocci, arranged as single cells, in pairs, in tetrads or in small groups. Non-motile. Facultatively anaerobic and oxidase-negative. A positive catalase reaction is clearly evident when cells are cultivated on blood agar, but cells grown on blood-free medium are catalase-negative. Colonies are non-pigmented, circular and <1 mm in diameter after 24 h on blood agar, and produce an α -haemolytic reaction. Growth occurs at 37 °C, at pH 9.6 and in broth containing 6.5% NaCl. Aesculin and hippurate are not hydrolysed. Nitrate is not reduced and acetoin is

not produced. Hydrolysis of urea is variable. Acid is produced from 5-ketogluconate after 48 h and from ribose and D-tagatose after 7 days. Acid is not produced from mannitol, sorbitol, lactose, trehalose, glucose, fructose, mannose, maltose, sorbose, galactose, rhamnose, raffinose, sucrose, cellobiose, gentiobiose, inulin, arabinose, xylose, turanose, lyxose, fucose, arabitol, adonitol, xylitol, dulcitol, inositol, aesculin, salicin, *N*-acetylglucosamine, amygdalin, arbutin, cyclodextrin, glycogen, pullulan, melibiose, melezitose, methyl β -D-xylopyranoside, methyl α -D-mannopyranoside, methyl β -D-glucopyranoside, glycerol, erythritol or 2-ketogluconate. Activities for arginine dihydrolase and β -galactosidase (β -GAL test), esterase C4, ester lipase C8, acid phosphatase, naphthol-AS-BI-phosphohydrolase (weak reaction) and alkaline phosphatase (weak reaction) are detected. β -Glucosidase, β -galactosidase (β -GAR test), β -glucuronidase, α -galactosidase, alanyl-phenylalanyl-proline arylamidase, pyroglutamic acid arylamidase, *N*-acetyl- β -glucosaminidase, lipase C14, leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, chymotrypsin, α -glucosidase, α -mannosidase, α -fucosidase and glycyl tryptophan arylamidase are not produced. The DNA G+C content of the type strain is 37 mol%.

The type strain, 1821/02^T (= CECT 7139^T = CCUG 52530^T), was isolated from the brain of a pig with meningitis.

Acknowledgements

A. I. V. has a fellowship from the Ramon y Cajal Program (Spanish Ministry of Science and Technology/UCM). This work was partially funded by project AGL2003-08848-C02-01/GAN of the Spanish Ministry of Science and Technology.

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