***Aerococcus sanguicola* sp. nov., isolated from a human clinical source**

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Phenotypic and phylogenetic studies were performed on an unknown, Gram-positive, catalase-negative coccus isolated from human blood. Comparative 16S rRNA gene sequencing demonstrated that the organism represents a new subspecies within the genus *Aerococcus*. The unknown bacterium was readily distinguished from the three currently recognized *Aerococcus* species, *Aerococcus christensenii*, *Aerococcus urinae* and *Aerococcus viridans*, by biochemical tests and electrophoretic analysis of whole-cell proteins. On the basis of phylogenetic and phenotypic evidence, it is proposed that this unknown bacterium from blood be classified as *Aerococcus sanguicola* sp. nov. The type strain of *Aerococcus sanguicola* is CCUG 43001^T (= CIP 106533^T).

Keywords: *Aerococcus sanguicola*, 16S rRNA, taxonomy, phylogeny

INTRODUCTION

The genus *Aerococcus* was created by Williams *et al.* (1953) to accommodate some Gram-positive, micro-aerophilic, catalase-negative organisms that were clearly distinguishable from streptococci. Initially, the genus contained a single species, *Aerococcus viridans* (Williams *et al.*, 1953). *Aerococcus viridans* occurs in a wide range of habitats (e.g. air, soil) and has been reported sporadically to be associated with human clinical infections (Colman, 1967; Janosek *et al.*, 1980; Nathavitharana *et al.*, 1983; Taylor & Trueblood, 1985). In recent years, two additional members of the genus *Aerococcus* have been described, *Aerococcus urinae* (Aguirre & Collins, 1992) and *Aerococcus christensenii* (Collins *et al.*, 1999b). *A. urinae* has been isolated from urine of elderly persons suffering from urinary tract infections and from the blood of patients with endocarditis and urosepticaemia (Christensen *et al.*, 1991, 1995), whereas *A. christensenii* has been recovered from the human vagina (Collins *et al.*, 1999b). During the past decade, 16S rRNA gene sequencing has proved to be an exceptionally powerful tool for the characterization of taxonomically problematic Gram-positive, catalase-negative cocci from human sources. This molecular genetic approach, used in concert with improved phenotypic methods (e.g.

PAGE analysis of whole-cell proteins), has not only resulted in improved classification schemes for these organisms, but has facilitated the recognition of a plethora of new genera and species, e.g. *Dolosigranulum* (Aguirre *et al.*, 1993), *Facklamia* (Collins *et al.*, 1997), *Globicatella* (Collins *et al.*, 1992) and *Ignavigranum* (Collins *et al.*, 1999a). In the course of this continuing study of Gram-positive, catalase-negative organisms from human sources, we have characterized a strain of a hitherto unknown *Aerococcus*-like bacterium from blood. On the basis of the results of a polyphasic taxonomic study, we describe a fourth species of the genus *Aerococcus*, *Aerococcus sanguicola* sp. nov.

METHODS

Bacterial strain and biochemical characterization. Strain CCUG 43001^T was isolated from a human blood culture; it was co-isolated with a Gram-positive coccus identified as *Staphylococcus epidermidis*. The strain was characterized biochemically by using the API rapid ID32 Strep and API ZYM systems according to the manufacturer's instructions (API bioMérieux). Conventional physiological tests were also conducted as described by Facklam & Elliott (1995). The type strains and other reference strains of *A. viridans*, *A. christensenii* and *A. urinae* were also tested. All tests were performed in duplicate.

SDS-PAGE of whole-cell proteins. SDS-PAGE analysis of whole-cell proteins was carried out as described by Pot *et al.* (1994) and Vandamme *et al.* (1998). For densitometric

The GenBank accession number for the 16S rRNA gene sequence of strain CCUG 43001^T is AJ276512.

Fig. 1. Similarity dendrogram based on whole-cell protein patterns of *Aerococcus sanguicola* sp. nov. and some related species. For convenience, levels of correlation are expressed as percentages of similarity.

analysis, normalization and interpretation of protein patterns, the GCW 3.0 software package (Applied Maths) was used. The similarity between all pairs of traces was expressed by the Pearson product-moment correlation coefficient, converted to percentage similarity for convenience.

Determination of 16S rRNA gene sequences and phylogenetic analysis. A phylogenetic analysis was performed by comparative 16S rRNA gene sequence analysis. A large fragment of the 16S rRNA gene (corresponding to positions 30–1521 of the *Escherichia coli* 16S rRNA gene) was amplified by PCR using conserved primers close to the 3′- and 5′-ends of the gene, as described previously (Hutson *et al.*, 1993). The PCR product was sequenced directly using a *Taq* DyeDeoxy terminator cycle sequencing kit (Applied Biosystems) and an automatic DNA sequencer (model 373A; Applied Biosystems). The closest known relatives of the new isolate were determined by performing database searches. These sequences and those of other known related strains were retrieved from the EMBL or Ribosomal Database Project data libraries and aligned with the newly determined sequence using the program CLUSTAL X (Jeanmougin *et al.*, 1998). The resulting multiple sequence alignment was corrected and approximately 100 bases at the

5′-end of the rRNA were omitted from further analyses because of alignment ambiguities. Pairwise evolutionary distances were computed from a continuous stretch of 1320 bases, using the correction of Jukes & Cantor (1969). A phylogenetic tree was constructed, according to the neighbour-joining method, with the program NEIGHBOR (Felsenstein, 1989). The stability of the groupings was estimated by bootstrap analysis (500 replications) using the programs SEQBOOT, DNADIST, NEIGHBOR and CONSENSE (Felsenstein, 1989).

RESULTS AND DISCUSSION

The isolate recovered from human blood consisted of Gram-positive, non-spore-forming cocci that occurred as single cells, in pairs, in tetrads or in small groups. The isolate was facultatively anaerobic and catalase- and oxidase-negative. When grown on Columbia horse-blood agar, colonies were small (<1 mm in diameter), entire and non-pigmented. The bacterium was α -haemolytic when grown on blood agar, grew in 6.5% NaCl and gave a positive reaction for the bile-aesculin test. Acid was produced from maltose,

Fig. 2. Unrooted tree showing the phylogenetic relationships of *A. sanguicola* sp. nov. and some related Gram-positive bacteria. The tree, constructed by the neighbour-joining method, was based on a comparison of approximately 1320 nucleotides. Bootstrap values, each expressed as a percentage of 500 replications, are given at the branching points. Bar, 1 % sequence divergence.

sucrose and trehalose but not from D-arabitol, L-arabinose, cyclodextrin, glycogen, lactose, pullulan, sorbitol, tagatose, mannitol, melibiose, melezitose, methyl β -D-glucopyranoside, D-raffinose, D-ribose or D-xylose. The isolate gave positive reactions for acid phosphatase, arginine dihydrolase, cysteine arylamidase, esterase C-4 (weak reaction), β -glucuronidase, leucine arylamidase, pyroglutamic acid arylamidase and valine arylamidase. Negative reactions were observed for *N*-acetyl- β -glucosaminidase, alanine-phenylalanine-proline arylamidase, chymotrypsin, ester lipase C8, α -fructosidase, α -galactosidase, β -galactosidase, α -glucosidase, β -glucosidase, glycine-tryptophan arylamidase, lipase C14, α -mannosidase, β -mannosidase, phosphoamidase, trypsin and urease. The isolate hydrolysed hippurate and gave a negative Voges-Proskauer test. On the basis of its cellular morphological and biochemical characteristics, the unknown isolate resembled members of the genus *Aerococcus*, in particular *A. viridans*. However, the

In order to determine the phylogenetic affinities of the unknown isolate, its 16S rRNA gene was amplified by PCR and sequenced. Sequence database searches

Table 1 Tests useful in differentiating *A. sanguicola* sp. nov. from *A. christensenii*, *A. urinae* and *A. viridans*

Biochemical test results were determined by using API rapid ID32S and API ZYM systems. v, Variable.

Test	<i>A. christensenii</i>	<i>A. sanguicola</i>	<i>A. urinae</i>	<i>A. viridans</i>
Production of acid from:				
Lactose	—	—	—	+
Maltose	—	+	—	+
Mannitol	—	—	+	v
Ribose	—	—	v	v
Sucrose	—	+	+	+
Trehalose	—	+	—	+
Production of:				
Arginine dihydrolase	—	+	—	—
β -Glucuronidase	—	+	+	v
Pyroglutamic acid arylamidase	—	+	—	+

showed that the unknown bacterium was phylogenetically most closely related to aerococci (95.1–95.4% 16S rRNA sequence similarity). Much lower levels of sequence relatedness (< 92% similarity) were shown with respect to other reference Gram-positive taxa (data not shown). A tree constructed by the neighbour-joining method, showing the phylogenetic position of the unknown human bacterium with respect to some related Gram-positive, catalase-negative taxa, is shown in Fig. 2. The treeing analysis clearly showed that the unknown bacterium represents a new subline within the *Aerococcus* clade. Bootstrap resampling showed that this relationship to the *Aerococcus* group of organisms was statistically significant (Fig. 2).

The morphological and biochemical properties of the unknown Gram-positive coccus recovered from human blood were found to be consistent with its assignment to the genus *Aerococcus*, although it did not correspond to any of the three established species of this genus. Comparative 16S rRNA sequencing demonstrated unequivocally that the unknown bacterium corresponds to a hitherto unrecognized subline within the *Aerococcus* clade. Furthermore, 16S rRNA sequence divergence values of > 4.5% show that the organism merits classification as a distinct species. Thus, on the basis of the phylogenetic evidence presented and its distinctive biochemical characteristics, we consider that the unidentified organism from human blood should be assigned to the genus *Aerococcus* as *Aerococcus sanguicola* sp. nov. The characteristics that are useful in distinguishing *A. sanguicola* from the other species of the genus *Aerococcus* are shown in Table 1.

Description of *Aerococcus sanguicola* sp. nov.

Aerococcus sanguicola (san.gui'co.la. L. n. *sanguis* blood; L. subst. *cola* dweller; M.L. n. *sanguicola* blood-dweller, pertaining to the initial isolation source).

Cells consist of Gram-positive, non-spore-forming cocci that occur singly, in pairs, in tetrads or in small groups. Facultatively anaerobic and catalase- and oxidase-negative. Grows in 6.5% NaCl. Colonies are small (< 1 mm in diameter), entire and non-pigmented when grown on Columbia horse-blood agar. α -Haemolytic and produces a positive reaction in the bile-aesculin test. When commercial API systems are used, acid is produced from maltose, sucrose and trehalose but not from D-arabitol, L-arabinose, cyclodextrin, glycogen, lactose, pullulan, sorbitol, tagatose, mannitol, melibiose, melezitose, methyl β -D-glucopyranoside, D-raffinose, D-ribose or D-xylose. Positive reactions for acid phosphatase, arginine dihydrolase, cysteine arylamidase, esterase C-4 (weak reaction), β -glucuronidase, leucine arylamidase, pyroglutamic acid arylamidase and valine arylamidase. Negative reactions are observed for *N*-acetyl- β -glucosaminidase, alanine-phenylalanine-proline arylamidase, chymotrypsin, ester lipase C8, α -fructosidase, α -galactosidase, β -galactosidase, α -glucosidase, β -glucosidase, glycine-tryptophan arylamidase, lipase C14, α -mannosidase, β -mannosidase, phosphoamidase, trypsin and urease. Hippurate is hydrolysed and acetoin is not produced. Isolated from human blood. Habitat unknown. The type strain is CCUG 43001^T (= CIP 106533^T).

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