NOTE

***Aerococcus urinaehominis* sp. nov., isolated from human urine**Paul A. Lawson,¹ Enevold Falsen,² Maria Ohlén² and Matthew D. Collins¹Author for correspondence: Matthew D. Collins. Tel: +44 118 935 7000. Fax: +44 118 935 7222.
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Phenotypic and phylogenetic studies were performed on an unknown Gram-positive catalase-negative coccus isolated from human urine. 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. Based on phylogenetic and phenotypic evidence, it is proposed that the unknown bacterium from urine be classified as *Aerococcus urinaehominis* sp. nov. The type strain of *Aerococcus urinaehominis* is CCUG 42038b^T (= CIP 106675^T).

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

The aerococci consist of Gram-positive, facultatively anaerobic, catalase-negative organisms in which cells divide on two planes at right angles, resulting in tetrad and cluster arrangements. For many years the genus *Aerococcus* comprised the single species *Aerococcus viridans* (Williams *et al.*, 1953). *A. viridans* has been isolated from a broad range of habitats such as air, vegetation, dust, soil and meat-curing brines, and has also been associated, albeit rarely, with human infections (Facklam & Elliott, 1995). Despite its presence in such diverse sources, the taxonomic kinship of strains has been consistently demonstrated. In recent years, however, *Aerococcus*-like organisms which are different from *A. viridans* have been recovered from clinical specimens (e.g. Christensen *et al.*, 1989, 1991, 1995) and two additional members of the *Aerococcus* genus, *Aerococcus urinae* (Aguirre & Collins, 1992) and *Aerococcus christensenii* (Collins *et al.*, 1999) have been described. *A. urinae* has been isolated from urine of elderly persons suffering from urinary tract infections and from blood of patients with endocarditis and urosepticaemia (Christensen *et al.*, 1991, 1995; Facklam & Elliott, 1995), whereas *A. christensenii* has been recovered from the human vagina (Collins *et al.*, 1999). In the course of a continuing study of problematic Gram-positive catalase-negative organisms from human sources, we have characterized a hitherto un-

known *Aerococcus*-like bacterium from urine that does not correspond to any of the currently described species of the genus.

Strain CCUG 42038b^T was isolated from a human urine culture. The unidentified organism was cultured on Columbia agar supplemented with 5% defibrinated horse blood (Oxoid, Unipath) at 37 °C, in air plus 5% CO₂. The strain was biochemically characterized by using the API rapid ID32 Strep, API CORYNE and API ZYM systems according to the manufacturer's instructions (API bioMérieux). The type strains and other culture collection reference strains of *A. viridans*, *A. christensenii* and *A. urinae* were also tested. All biochemical tests were performed in duplicate. SDS-PAGE analysis of whole-cell proteins was carried out as described by Pot *et al.* (1994) and Vandamme *et al.* (1998). For densitometric analysis, normalization and interpretation of protein patterns, the GCW 3.0 software package (Applied Maths, Kortrijk, Belgium) was used. The similarity between all pairs of traces was expressed by the Pearson product moment correlation coefficient converted for convenience to a percentage similarity. Phylogenetic analysis was performed by comparative 16S rRNA gene sequence analyses. 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. The PCR product was directly sequenced using a Taq Dye-Deoxy Terminator Cycle Sequencing kit (Applied Biosystems) and an automatic DNA sequencer (model

The GenBank accession number for the 16S rRNA gene sequence of strain CCUG 42038b^T is AJ278341.

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 (Felsenstein, 1989).

The isolate recovered from human urine consisted of Gram-positive, non-spore-forming cocci that occurred as single cells, in pairs, tetrads or in small groups. The isolate was facultatively anaerobic and catalase- and oxidase-negative. When grown on Columbia horse blood agar for 24 h, colonies were small (< 1 mm diam.), entire and grey in colour. α -Haemolysis was observed on blood agar. Acid was produced from glucose, maltose, methyl β -D-glucopyranoside, D-ribose and sucrose but not from D-arabitol, L-arabinose, cyclodextrin, glycogen, lactose, pullulan, sorbitol, tagatose, mannitol, melibiose, melezitose, D-raffinose, trehalose or D-xylose. The isolate gave positive reactions for acid phosphatase, esterase C-4 (weak reaction), β -glucuronidase and pyrazinamidase. Negative reactions were observed for *N*-acetyl- β -glucosaminidase, alanine phenylalanine proline arylamidase, arginine dihydrolase, chymotrypsin, cysteine arylamidase, ester lipase C8, α -fucosidase, α -galactosidase, β -galactosidase, α -glucosidase, glycine tryptophan arylamidase, lipase C14, leucine arylamidase, β -mannosidase, phosphoaminidase, pyrrolidonyl arylamidase, pyroglutamic acid arylamidase, trypsin, urease and valine arylamidase. Results for the production of β -glucosidase differed between the API systems employed. Using the API rapid ID Strep kit, the production of this enzyme was not detected, but with the API ZYM system, this enzyme was produced. Similarly, the production of alkaline phosphatase was found to be negative with the API rapid ID Strep kit but weakly positive with the API CORYNE and ZYM systems. The isolate hydrolysed aesculin and hippurate but not gelatin. The bacterium did not reduce nitrate to nitrite and gave a negative Voges-Proskauer test. On the basis of its cellular morphological and biochemical characteristics the unknown isolate resembled the genus *Aerococcus*. To further assess the phenotypic resemblance of the unknown isolate with aerococci and some other catalase-negative coccus-shaped taxa, SDS-PAGE analysis of whole-cell proteins was performed. PAGE analysis clearly showed the unidentified organism represents a taxon distinct from all other reference Gram-positive catalase-nega-

tive cocci examined (data not shown). In particular, the unknown organism showed no specific phenotypic resemblance to any of the currently described *Aerococcus* species. The nearest neighbour to the clinical isolate corresponded to *Vagococcus salmoninarum*, which joined the unknown organism at a level of approximately 65%. To determine the phylogenetic affinities of the unknown isolate, its 16S rRNA gene was amplified by PCR and sequenced. Sequence database searches showed the unknown bacterium was phylogenetically most closely related to aerococci (92.6–94.0% 16S rRNA sequence similarity). A tree constructed using the neighbour-joining method showing the phylogenetic position of the unknown human bacterium amongst some related Gram-positive catalase-negative taxa is depicted in Fig. 1. The treeing analysis clearly showed the unknown bacterium represents a new subline within the *Aerococcus* genus. While the bacterium shared a branching node with *A. viridans*, bootstrap analysis showed this relationship was not statistically significant (recovered in 50% resamplings). The bootstrap value for the overall *Aerococcus* clade, however, was statistically significant (100% of reconstructed trees) (see Fig. 1).

The unknown Gram-positive coccus recovered from human urine was found to be morphologically and biochemically consistent with its assignment to the genus *Aerococcus* although it differed from the three established species of this genus. Comparative 16S rRNA sequencing unequivocally demonstrated that the unknown bacterium corresponds to a hitherto unrecognized subline within the *Aerococcus* clade. Furthermore, 16S rRNA sequence divergence values of > 6% show the organism merits classification as a distinct species. Thus based on the presented phylogenetic evidence and its distinctive biochemical characteristics, we consider the unidentified organism from human urine be assigned to the genus *Aerococcus*, as *Aerococcus urinaehominis* sp. nov. Tests that serve to distinguish *A. urinaehominis* from other members of the genus *Aerococcus* are given in Table 1. To date, all three species of the genus *Aerococcus* have been recovered from human sources. In particular, *A. urinae* is found in a constant but low percentage of urine specimens (0.8%) but nearly all patients present clinical signs of acute infection. Although *A. viridans* has been shown to be responsible for a number of clinical conditions, a large proportion of strains isolated from clinical samples fail to exhibit any clinical manifestations (Christensen *et al.*, 1997; Colman, 1967; Janosek *et al.*, 1980; Facklam & Elliott, 1995; Nathavitharana *et al.*, 1983; Taylor & Trueblood, 1985). The newly described species, *A. urinaehominis*, also originated from a human urine sample but no further clinical details are available on the organism and its pathogenic potential remains unknown. Nevertheless, we consider the formal description of this species will facilitate its identification in clinical laboratories thereby enabling its prevalence and association, if any, with human disease to be established.

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

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

Biochemical tests were determined using API CORYNE, API rapid ID32S and API ZYM systems. v, Variable.

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

Description of *Aerococcus urinaehominis* sp. nov.

Aerococcus urinaehominis (u.ri'nae.ho'mi.nis. L. fem. n. *urina* urine; L. gen. n. *hominis* of man; M. L. gen. n. *urinaehominis* pertaining to human urine from which the bacterium was first isolated).

Gram-positive, non-spore-forming cocci occurring as single cells, in pairs, tetrads or in small groups. It is facultatively anaerobic and catalase- and oxidase-negative. When grown on Columbia horse blood agar for 24 h at 37 °C colonies are small (< 1 mm diameter), entire, non-pigmented and α -haemolytic. Using commercial API systems acid is produced from glucose, maltose, methyl β -D-glucopyranoside, D-ribose and sucrose but not from D-arabitol, L-arabinose, cyclodextrin, glycogen, lactose, pullulan, sorbitol, tagatose, mannitol, melibiose, melezitose, D-raffinose, tagatose, trehalose or D-xylose. Positive reactions are observed for acid phosphatase, esterase C-4 (weak reaction), β -glucuronidase and pyrazinamidase. Negative reactions are observed for *N*-acetyl- β -glucosaminidase, alanine phenylalanine proline arylamidase, arginine dihydro-

lase, chymotrypsin, cysteine arylamidase, ester lipase C8, α -fucosidase, α -galactosidase, β -galactosidase, α -glucosidase, glycine tryptophan arylamidase, lipase C14, leucine arylamidase, α -mannosidase, β -mannosidase, phosphoaminidase, pyroglutamic acid arylamidase, pyrrolidonyl arylamidase, trypsin, urease and valine arylamidase. Alkaline phosphatase and β -glucosidase may or may not be produced. Aesculin and hippurate are hydrolysed but gelatin is not. Nitrate is not reduced to nitrite and acetoin is not produced. Isolated from human urine. Habitat is not known. The type strain is CCUG 42038b^T (= CIP 106675^T).

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