NOTE***Aerococcus christensenii* sp. nov., from the human vagina**

Matthew D. Collins,¹ Mar Rodriguez Jovita,¹ Roger A. Hutson,¹
Maria Ohlén² and Enevold Falsen²

Author for correspondence: Matthew D. Collins. Tel: +44 118 935 7000. Fax: +44 118 926 7917.
e-mail: david.collins@bbsrc.ac.uk

¹ Department of Food
Science and Technology,
University of Reading,
Reading RG6 6AP, UK

² Culture Collection,
Department of Clinical
Bacteriology, University of
Göteborg, S-41346
Göteborg, Sweden

Phenotypic and phylogenetic studies were performed on two strains of a hitherto undescribed *Aerococcus*-like organism isolated from the human vagina. Comparative 16S rRNA gene sequencing studies demonstrated that the unknown strains constitute a new subline within the genus *Aerococcus*. The unknown bacterium was readily distinguished from the two currently recognized *Aerococcus* species, *Aerococcus viridans* and *Aerococcus urinae*, by biochemical tests and electrophoretic analysis of whole-cell proteins. On the basis of phylogenetic and phenotypic evidence, it is proposed that the unknown bacterium be classified as *Aerococcus christensenii* sp. nov. The type strain of *A. christensenii* is CCUG 28831^T.

Keywords: *Aerococcus christensenii* sp. nov., phylogeny, taxonomy, 16S rRNA

The genus *Aerococcus* until recently contained a single species, *Aerococcus viridans* (Williams *et al.*, 1953). This species is found in a wide range of environments and, although considered saprophytic, has been shown to cause disease in lobsters and to be associated, albeit rarely, with human infections, e.g. subacute bacterial endocarditis (Janosek *et al.*, 1980; Parker & Ball, 1976), urinary tract infections (Colman, 1967) and meningitis (Nathavitharana *et al.*, 1983). Recently, Christensen *et al.* (1989, 1991) reported the isolation of some *Aerococcus*-like organisms from human clinical specimens that were distinct from *A. viridans*. These *Aerococcus*-like organisms were assigned to the genus *Aerococcus*, as *Aerococcus urinae*, by Aguirre & Collins (1992). *A. urinae* has been isolated from urine specimens of elderly persons suffering from urinary tract infections and from blood cultures taken from patients with endocarditis and urosepticaemia (Christensen *et al.*, 1989, 1991, 1995). In the course of a study of *Aerococcus*-like organisms from human sources, we have used 16S rRNA gene sequencing to characterize two strains of a hitherto unknown *Aerococcus*-like bacterium. On the basis of the results of a polyphasic taxonomic study, a new species, *Aerococcus christensenii* sp. nov., is described.

Two strains, designated UWO1 (=CCUG 28826) and

UWO6^T (=CCUG 28831^T), were submitted to the Culture Collection of the University of Göteborg by L. K. Rabe and S. L. Hillier (University of Washington, Seattle, USA) for identification. Both strains were isolated from vaginal sources and tentatively identified as *Streptococcus acidominimus*. The organisms were cultured on Columbia agar (Difco) supplemented with 5% horse blood at 37 °C, in air plus 5% CO₂. The strains were characterized biochemically by using the API Rapid ID32S system according to the manufacturer's instructions (API bioMérieux). PAGE analysis of whole-cell proteins was performed as described by Pot *et al.* (1994). For densitometric analysis, normalization and interpretation of protein patterns, the GELCOMP GCW 3.0 software package (Applied Maths) was used. The cell wall murein structure and G+C content of DNA of strain CCUG 28831^T was determined as described by Schleifer & Kandler (1972) and Garvie (1978), respectively. The 16S rRNA genes of the isolates were amplified by PCR and directly sequenced by using a *Taq* Dye-Deoxy terminator cycle sequencing kit (Applied Biosystems) and an automatic DNA sequencer (model 373A, Applied Biosystems). The closest known relatives of the new isolates were determined by performing sequence database searches. These sequences and those of other known related strains were retrieved from the GenBank or Ribosomal Database Project libraries and aligned with the newly determined sequences by using the program PILEUP (Devereux *et al.*, 1984). The resulting multiple sequence alignment was corrected

The GenBank accession numbers for the 16S rRNA gene sequences of strains CCUG 28826 and CCUG 28831^T are Y17318 and Y17005.

Fig. 1. Similarity dendrogram based on whole-cell protein patterns of *A. christensenii* sp. nov. and related species. Levels of correlation are expressed as percentages of similarity for convenience.

manually and a distance matrix was calculated by using the programs PRETTY and DNADIST (with the Kimura two-correction parameter) (Felsenstein, 1989). 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) with the programs DNABOOT, DNADIST, NEIGHBOR and CONSENSE (Felsenstein, 1989).

Morphologically, the two isolates consisted of Gram-positive cocci that occurred in pairs, tetrads or small groups. They produced α -haemolytic reactions on blood agar and were non-motile. Utilizing the commercial API Rapid ID32S system, both strains were biochemically unreactive, failing to produce acid from D-arabitol, L-arabinose, cyclodextrin, glycogen, lactose, melibiose, mannitol, maltose, melezitose, methyl β -D-glucopyranoside, pullulan, raffinose, ribose, sorbitol, sucrose, tagatose or trehalose. Both strains tested negative for alkaline phosphatase, alanine-phenylalanine-proline arylamidase, arginine dihydrolase, *N*-acetyl β -glucosaminidase, β -galactosidase, α -galacto-

sidase, β -galacturonidase, β -glucosidase, β -glucuronidase, glycyl-tryptophan arylamidase, pyroglutamic acid arylamidase, β -mannosidase and urease. They were also Voges-Proskauer-negative. Indeed, the hydrolysis of hippurate was the only positive test observed with the API Rapid ID32S system. An examination of the cell wall of strain CCUG 28831^T revealed a directly cross-linked murein based on L-lysine (type A1 α). This murein type is found in several Gram-positive, catalase-negative taxa such as the aerococci, *Alloiococcus otitis*, *Abiotrophia defectiva* and *Globicatella sanguinis*.

PAGE analysis of whole-cell proteins further demonstrated the close phenotypic relatedness of the two isolates. A dendrogram depicting the relationships of the two isolates is shown in Fig. 1 and illustrates clearly that they represent a taxon distinct from all other reference Gram-positive, catalase-negative cocci examined to date. To determine the phylogenetic relatedness of the two isolates, their 16S rRNA genes were amplified by PCR and sequenced. Pairwise analysis showed that the isolates were genealogically identical

Fig. 2. Unrooted tree showing the phylogenetic relationships of *A. christensenii* sp. nov. and some other low-G+C content, Gram-positive bacteria. With the exception of *A. christensenii*, all taxa are represented by their type strains. The tree, constructed by 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 represents 1% sequence divergence.

Table 1. Tests useful for differentiating *A. christensenii* sp. nov. from *A. urinae* and *A. viridans*

Test	<i>A. christensenii</i>	<i>A. urinae</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	—	—	+

v, Variable.

(100% sequence identity). Sequence database searches showed that the unknown bacterium was phylogenetically most closely related to the aerococci (approx. 94–96% identity). Considerably lower levels of relatedness (<92% identity) were shown to other Gram-positive taxa (data not shown). A tree constructed by using the neighbour-joining method and showing the phylogenetic position of the unknown bacterium within the 'lactic acid' group of bacteria is illustrated in Fig. 2. Treeing analysis showed that the unknown bacterium was a member of the *Aerococcus* clade and was most closely related to *A. urinae* (approx. 4% 16S rRNA sequence divergence). This association with *A. urinae* was supported in 100% of bootstrapped trees. The next nearest relative corresponded to *A. viridans*, which displayed about 6% 16S rRNA sequence divergence from the unknown coccus.

It is clear from the comparative 16S rRNA gene sequence analysis that the unidentified coccus is a

member of the genus *Aerococcus*. Sequence divergence values of 4–6% from *A. urinae* and *A. viridans*, however, demonstrate unequivocally that the unknown bacterium represents a hitherto undescribed species of the genus. It is evident from both sequence divergence values and tree topology considerations that the bacterium is more closely related phylogenetically to *A. urinae* than to *A. viridans*. Christensen *et al.* (1997) have shown that *A. urinae* as presently defined embraces two biotypes, which share high DNA relatedness. The two isolates reported here can be readily distinguished from both *A. urinae* biotypes by their failure to produce acid from mannitol and sucrose. Using conventional testing, Christensen *et al.* (1997) reported that all 22 strains of *A. urinae* examined by them produced acid from these substrates. Using the commercial API Rapid ID32S system, we examined 38 strains of *A. urinae* and found that all fermented mannitol and 36 of 38 also fermented sucrose. Similarly, by using the API Rapid ID32S

system, neither of the two human isolates produced β -glucuronidase. However, 37 of 38 strains of *A. urinae* produced this enzyme. The unknown bacterium could also be distinguished readily from *A. viridans* by using the API Rapid ID32S system. *A. viridans* tests positive for pyroglutamic acid arylamidase and produces acid from lactose, maltose, sucrose and trehalose. By contrast, strains CCUG 28826 and CCUG 28831^T were negative for these tests (Table 1). Thus, on the basis of phylogenetic evidence and their distinctive biochemical characteristics, we consider that the two human vaginal isolates merit classification as a new species of the genus *Aerococcus*, for which the name *Aerococcus christensenii* sp. nov. is proposed.

Description of *Aerococcus christensenii* sp. nov.

Aerococcus christensenii (christ.en.sen'i.i. M.L. gen. n. *christensenii* named after the Danish microbiologist, Jens J. Christensen).

Cells are Gram-positive, non-spore-forming cocci that occur in pairs, tetrads or small groups. Non-pigmented and non-motile. Grows in 6.5% NaCl and on blood agar producing an α -haemolytic reaction. Facultatively anaerobic. Catalase- and oxidase-negative. Using the commercial API Rapid ID32S system, acid is not produced from D-arabitol, L-arabinose, cyclo-dextrin, glycogen, lactose, melibiose, mannitol, maltose, melezitose, methyl β -D-glucopyranoside, pullulan, raffinose, ribose, sorbitol, sucrose, tagatose or trehalose. Alkaline phosphatase, alanine-phenylalanine-proline arylamidase, arginine dihydrolase, N-acetyl β -glucosaminidase, glycyl-tryptophan arylamidase, α -galactosidase, β -galactosidase, β -galacturonidase, β -glucosidase, β -glucuronidase, pyroglutamic acid arylamidase, β -mannosidase and urease activity are not detected. Acetoin is not produced. Hippurate is hydrolysed. The cell wall murein is of the L-lysine directly cross-linked type. The G+C content of the DNA is 38.5 mol% (T_m). Isolated from the human vagina. The type strain is CCUG 28831^T.

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