Reclassification of *Pediococcus urinaeequi* (ex Mees 1934) Garvie 1988 as *Aerococcus urinaeequi* comb. nov.

Giovanna E. Felis, Sandra Torriani and Franco Dellaglio

Dipartimento Scientifico e Tecnologico, Università degli Studi di Verona, Strada le Grazie 15, 37134 Verona, Italy

The taxonomic status of *Pediococcus urinaeequi* is described, and the transfer of the species to the genus *Aerococcus* with the name *Aerococcus urinaeequi* comb. nov. is proposed, on the basis of the analysis of 16S rRNA gene sequence and DNA–DNA hybridization data.

The formal description of the species *Pediococcus urinaeequi* was formulated by E. I. Garvie in 1986, and the name was validated in the Approved List of Bacterial Names (Garvie, 1988). The species consists of alkaline-tolerant, tetrad-forming cocci. Pediococci are lactic acid bacteria, characterized by spherical cells that divide alternately in two planes at right angles to form tetrads. They are chemoheterotrophs, requiring a rich medium for growth. Gas is not formed from fermentation of carbohydrates, which usually leads to the formation of DL- or L-lactate. Garvie (1986) suggested that the separation of the genus *Pediococcus* from the genus *Aerococcus* required further clarification, with particular reference to the taxonomic position of *P. urinaeequi*, on the basis of the following observations. High DNA–DNA hybridization values were shared by strains of *P. urinaeequi* and *Aerococcus viridans* (Dellaglio et al., 1974), no DNA–DNA relatedness between some *P. urinaeequi* strains and either *Pediococcus halophilus* (now *Tetragenococcus halophilus*) or other *A. viridans* strains was found by Dellaglio et al. (1981) and, finally, strains of *P. urinaeequi* and *A. viridans* showed the same cross-linkage in their cell wall peptidoglycan (1-Lys–1-Ala), which was different from that of other pediococci (1-Lys–1-Ala–D-Asp).

A subsequent phylogenetic analysis performed on 16S rRNA gene sequences by Collins et al. (1990) confirmed that the type strain of *P. urinaeequi* was more similar to strains of the genus *Aerococcus*, and to the species *A. viridans* in particular, than to other *Pediococcus* species. Based on this result, Simpson & Taguchi (1995) and Stiles & Holzapfel (1997) reported that the species *P. urinaeequi* did not belong to the genus *Pediococcus*.

No formal reclassification has ever been proposed; therefore *P. urinaeequi* is still a species with a validly published name belonging to the genus *Pediococcus*. The aim of the present study was the reinvestigation and clarification of its taxonomic position.

Small subunit (16S) rRNA gene sequences of *P. urinaeequi* and other bacterial species were obtained from GenBank; sequence accession numbers and strain numbers are given in Fig. 1. The 16S rRNA gene sequence for *Pediococcus clausenii* DSM 14800T was obtained using primers Lac16S-f (5′-TGA GAG GTT ATC CTC GCT-3′) and Lac16S-r (5′-GAG GTG ATC CAG CCG GAG GTT-3′). The reaction mixture (20 μl) contained 30 ng template DNA, 1·5 mM MgCl₂, 0·2 mM dNTPs, 1 μM of each primer and 1 U *Taq* DNA polymerase (Promega), in a standard reaction buffer. After an initial denaturation of 4 min at 94 °C, 25 cycles of 1 min at 94 °C, 1·5 min at 50 °C, 2 min at 72 °C and final extension at 72 °C for 7 min were performed. The 1·6 kb amplification products were extracted from an agarose gel (Promega elution kit) and sequenced at the Biomolecular Research (BMR) Center at Padua University (Italy).

Using the same procedure, the 16S rRNA gene sequence of *P. urinaeequi* DSM 13989T was obtained. It was almost identical to that deposited in GenBank/EMBL/DDBJ for *P. urinaeequi* IFO 12173 (accession no. D87677).

Sequences were aligned with the CLUSTAL_X program (Thompson et al., 1997). Positions ambiguously aligned, not available or not identified (N in the sequence) were removed from all the sequences. Several phylogenetic trees were obtained with various distance formulas (Kimura, Tajima–Nei, Tamura three-parameter) and neighbour-joining reconstruction, as implemented in MEGA version 2.1 (Kumar et al., 2001), and with the Galtier–Gouy model as implemented in TREECON (Van de Peer & De Wachter, 1994). Parsimony analysis was performed as implemented in MEGA version 2.1 (Kumar et al., 2001), and a maximum-likelihood phylogenetic reconstruction was also obtained.
**Fig. 1.** Phylogenetic tree based on 16S rRNA gene sequences, inferred using the Tamura three-parameter distance and neighbour-joining tree reconstruction. Bar, number of nucleotide substitutions per site.
with models HKY and SH as implemented in TREE-PUZZLE (Strimmer & von Haeseler, 1996). The results obtained were very similar in all cases. An example is given in Fig. 1.

The close relationship of *P. urinaeaequi* IFO 12173 and *A. viridans* ATCC 11563T is evident, and is due to a similarity value of 99.9% (1407 identical nucleotides out of 1409 unambiguously aligned and not degenerated available positions) between the two sequences. This low divergence may indicate that the two species form the same taxon. Since 16S rRNA gene identity may not be sufficient to guarantee species identity (Fox et al., 1992), a DNA–DNA hybridization test was performed at the DSMZ: a 50-9% (replicate 51-3%) total DNA–DNA similarity between *P. urinaeaequi* LMG 13989T and *A. viridans* DSM 20340T was found, indicating that the two strains belong to separate species.

On the basis of these results it is proposed that the species *P. urinaeaequi* be reclassified as a novel species within the genus *Aerococcus*, with the name *Aerococcus urinaeaequi* comb. nov. (Rule 41a of the Bacteriological Code; Lapage et al., 1992).

The genus *Aerococcus* is therefore constituted of six species instead of five. For an easier consultation of data concerning the species *A. urinaeaequi*, the description of the reclassified species *P. urinaeaequi* from Garvie (1986) is reported.

**Description of Aerococcus urinaeaequi**

**comb. nov.**


*Aerococcus urinaeaequi* (*u.rin’ae.equi.* n. L. *urina* -ae urine; L. n. *genus* *equus* -i horse; *genus* *equi* of a horse; *urinaeaequi* from the urine of a horse, source of isolation of the strain).

Cells are spherical, never elongated. Division occurs alternately in two planes at right angles to form tetrads. Gram-positive, non-motile, non-spore-forming, facultative anaerobes. Cells might form irregular clusters when grown in liquid medium. Catalase-negative. According to previous descriptions, the optimum pH value for growth is between 8.5 and 9.0, with growth commencing in medium at an initial pH of 6.5 to 7.0 (Simpson & Taguchi, 1995). Grows well in Trypticase Soy broth containing yeast extract (0.3%, w/v), at a final pH of 7.2 ± 0.2. Optimum temperature is 25–30°C. (+)-L-Lactate is formed from glucose. Acid is produced from maltose, sucrose, trehalose and dextrin, but not from melezitose, starch, glycerol or sorbitol. Strain-dependent reactions are obtained with arabinose, xylose, lactose and mannitol. G+C content of the DNA is 39.5 mol%.

The type strain is ATCC 29723T (=CCUG 28094T =CIP 103442T =LMG 13989T =DSM 20341T =NCIMB 701636T (formerly NCDO 1636T)).

**References**


