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

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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 chemo-organotrophs, 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 (L-Lys–L-Ala), which was different from that of other *pediococci* (L-Lys–L-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 14800^T was obtained using primers Lac16S-f (5'-AAT GAG AGT TTG ATC CTG GCT-3') and Lac16S-r (5'-GAG GTG ATC CAG CCG CAG 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 (Polymed), 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* LMG 13989^T was obtained. It was almost identical to that deposited in GenBank/EMBL/DBJ 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. urinaeequi* IFO 12173 and *A. viridans* ATCC 11563^T 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. urinaeequi* LMG 13989^T and *A. viridans* DSM 20340^T was found, indicating that the two strains belong to separate species.

On the basis of these results it is proposed that the species *P. urinaeequi* be reclassified as a novel species within the genus *Aerococcus*, with the name *Aerococcus urinaeequi* 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. urinaeequi*, the description of the reclassified species *P. urinaeequi* from Garvie (1986) is reported.

Description of *Aerococcus urinaeequi* comb. nov.

Basonym: *Pediococcus urinaeequi* (ex Mees 1934) Garvie 1988.

Aerococcus urinaeequi (u.rin'ae.e.qui. n. L. *urina* -ae urine; L. n. gen. *equus* -i horse; gen. *equi* of a horse; *urinaeequi* 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 29723^T [=CCUG 28094^T=CIP 103442^T=LMG 13989^T=DSM 20341^T=NCIMB 701636^T (formerly NCDO 1636^T)].

References

- Collins, M. D., Williams, A. M. & Wallbanks, S. (1990). The phylogeny of *Aerococcus* and *Pediococcus* as determined by 16S rRNA sequence analysis: description of *Tetragenococcus* gen. nov. *FEMS Microbiol Lett* **58**, 255–262.
- Dellaglio, F., Bottazzi, V. & Battistotti, B. (1974). Caratteri e distribuzione della microflora pediococcica in alcuni formaggi italiani. *Ann Microbiol Enzimol* **24**, 325–334.
- Dellaglio, F., Trovatielli, L. D. & Sarra, P. G. (1981). DNA-DNA homology among representative strains of the genus *Pediococcus*. *Zentralbl Bakteriol Mikrobiol Hyg 1 Abt Orig C* **2**, 140–150.
- Fox, G. E., Wisotzkey, J. D. & Jurtshuk, P., Jr (1992). How close is close: 16S rRNA sequence identity may not be sufficient to guarantee species identity. *Int J Syst Bacteriol* **42**, 166–170.
- Garvie, E. I. (1986). Genus *Pediococcus* Claussen 1903, 68^{AL}. In *Bergey's Manual of Systematic Bacteriology*, vol. 2, pp. 1075–1079. Edited by P. H. A. Sneath, N. S. Mair, M. E. Sharpe & J. G. Holt. Baltimore: Williams & Wilkins.
- Garvie, E. I. (1988). *Pediococcus urinaeequi* nom. rev. In *Validation of the Publication of New Names and Combinations Previously Effectively Published Outside the IJSB*, List no. 25. *Int J Syst Bacteriol* **38**, 220–222.
- Kumar, S., Tamura, K., Jakobsen, I. B. & Nei, M. (2001). MEGA2: molecular evolutionary genetics analysis software. *Bioinformatics* **17**, 1244–1245.
- Lapage, S. P., Sneath, P. H. A., Lessel, E. F., Skerman, V. B. D., Seeliger, H. P. R. & Clark, W. A. (editors) (1992). *International Code of Nomenclature of Bacteria (1990 Revision)*. Bacteriological Code. Washington, DC: American Society for Microbiology.
- Simpson, W. J. & Taguchi, H. (1995). The genus *Pediococcus*, with notes on the genera *Tetragenococcus* and *Aerococcus*. In *The Lactic Acid Bacteria*, vol. 2, *The Genera of Lactic Acid Bacteria*, pp. 125–172. Edited by B. J. B. Wood & W. H. Holzapfel. London: Blackie Academic & Professional.
- Stiles, M. E. & Holzapfel, W. H. (1997). Lactic acid bacteria of food and their current taxonomy. *Int J Food Microbiol* **36**, 1–29.
- Strimmer, K. & von Haeseler, A. (1996). Quartet puzzling: a quartet maximum likelihood method for reconstructing tree topologies. *Mol Biol Evol* **13**, 964–969.
- Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. (1997). The CLUSTAL_X Windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* **24**, 4876–4882.
- Van de Peer, Y. & De Wachter, R. (1994). TREECON for Windows: a software package for the construction and drawing of evolutionary trees for the Microsoft Windows environment. *Comput Appl Biosci* **10**, 569–570.