Reevaluating the Classification of *Paracoccus halodenitrificans* with Sequence Comparisons of 16S Ribosomal DNA

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The results of phylogenetic analysis in which 16S ribosomal DNA sequences were compared confirmed previous chemotaxonomic data which suggested that *Paracoccus halodenitrificans* is inappropriately placed in the genus *Paracoccus*, which belongs in the α subclass of the *Proteobacteria*. *P. halodenitrificans* should be placed in the family *Halomonadaceae*, which belongs in the γ subclass of the *Proteobacteria*.

A phylogenetic relationship between *Paracoccus denitrificans* and *Paracoccus halodenitrificans* has never been established. The G+C contents of these two species are comparable (64 to 67 mol%) and are consistent with the physiological similarities that prompted the initial congeneric classification of the organisms (11). rRNA catalog and sequence data for the type species of the genus *Paracoccus*, *P. denitrificans*, place this organism in the α subclass of the *Proteobacteria* (4, 8–10), but no phylogenetic data for *P. halodenitrificans* are available.

The results of a quinone and fatty acid analysis of members of the genus *Paracoccus* strongly suggested that the species *P. halodenitrificans* should be excluded from the genus (14). The major respiratory quinone of *P. denitrificans* is ubiquinone 10, and its principal cellular fatty acids are 18:0, 10:0, and 14:0. *P. halodenitrificans*, however, has the same lipid profile as members of the genus *Halomonas* in the γ subclass of the *Proteobacteria* (5), which have ubiquinone 9 as their major respiratory quinone and contain 18:1, 16:0, 16:1, cy19:0, and cy17:0 as their principal fatty acids. Furthermore, the range of G+C contents of the halomonads (52 to 68 mol% [6]) accommodates the G+C content of *P. halodenitrificans* (64 to 66 mol%).

To clarify the taxonomic status of *P. halodenitrificans*, we compared the sequence of its 16S rRNA gene with the sequence of the *P. denitrificans* (8) 16S rRNA gene and with the 16S rRNA gene sequences of members of the family *Halomonadaceae*.

The type strain of *P. halodenitrificans*, strain ATCC 13511 (= DSM 735), was cultured in nutrient broth (Difco) supplemented with 6% NaCl. DNA was extracted from the cells, and the 16S rRNA gene was amplified by PCR and sequenced as previously described (2).

The 16S rRNA sequence of *P. halodenitrificans* was aligned with previously described 16S rRNA sequences of species belonging to the *Halomonadaceae*. The PHYLIP package (version 3.4) was used to establish phylogenetic relationships between species, and Jukes-Cantor distances were calculated with the DNADIST program. A phylogenetic tree was derived with the FITCH program, with a randomized order of input sequences and global rearrangement (3). The species *Escherichia coli*, *Pseudomonas aeruginosa*, and *Oceanospirillum linum*, which are also members of the γ subgroup of the *Proteobacteria*, were used as reference organisms in the analysis. Our results placed *P. halodenitrificans* in the family

Halomonadaceae (Fig. 1). The percentages of similarity between *P. halodenitrificans* and the other species examined are shown in Table 1.

The 16S rRNA sequence of *P. halodenitrificans* was examined for the 17 signatures which define the *Halomonadaceae* (2). All of these signatures occur in the *P. halodenitrificans* sequence, including the cytosine at position 486 that distinguishes members of the family *Halomonadaceae* (2).

We therefore propose that *P. halodenitrificans* should be transferred to the family *Halomonadaceae* in the γ subclass of the *Proteobacteria*. It is not possible at this time to place *P.*

FIG. 1. Phylogenetic tree derived by performing a distance analysis of 16S rRNA sequences, showing the relationships among *P. denitrificans*, *P. halodenitrificans*, members of the family *Halomonadaceae*, and other members of the γ subclass of the *Proteobacteria*. (All sequences were obtained from the Ribosomal Database unless indicated otherwise in the text.) Bar = 0.03 evolutionary distance unit as calculated by the Jukes-Cantor method. Abbreviations: *H.*, *Halomonas*; *D.*, *Deleya*; *Hv.*, *Halovibrio*; *P.*, *Paracoccus*; *E.*, *Escherichia*; *Ps.*, *Pseudomonas*; *O.*, *Oceanospirillum*.

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TABLE 1. Levels of 16S rRNA similarity between *P. halodenitrificans* and some other members of the *Proteobacteria*^a

Species	% Similarity
<i>Deleya halophila</i>	94.3
<i>Halomonas elongata</i>	93.8
<i>Deleya aquamarina</i>	93.8
<i>Halomonas subglaciescola</i>	93.7
<i>Deleya marina</i>	93.5
<i>Halomonas halmophila</i>	92.3
<i>Halovibrio variabilis</i>	91.7
<i>Oceanospirillum linum</i>	88.3
<i>Pseudomonas aeruginosa</i>	86.3
<i>Escherichia coli</i>	84.6
<i>Paracoccus denitrificans</i>	79.9

^a Values were calculated from pairwise mean distance values derived PAUP, version 3.0 (13). For more extensive distance data for members of the *Halomonadaceae* see reference 2.

halodenitrificans in one of the three genera of this family. The genera *Deleya*, *Halomonas*, and *Halovibrio* have been shown to be indistinguishable by phenotypic, chemotaxonomic, and molecular criteria (1, 2, 5, 7, 12). Until the internal structure of the family *Halomonadaceae* is clarified at the genus level, assigning *P. halodenitrificans* to a genus would be arbitrary and artificial.

Nucleotide sequence accession numbers. The sequence of the 16S rRNA gene of *P. halodenitrificans* ATCC 13511^T has been deposited in the GenBank data base under accession number L04942.

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