Paracoccus halophilus sp. nov., isolated from marine sediment of the South China Sea, China, and emended description of genus *Paracoccus* Davis 1969

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An aerobic bacterial isolate, strain HN-182^T, was isolated from sediments of the South China Sea. Cells of strain HN-182^T are coccoid to short rods, Gram-negative, non-spore-forming and non-motile. Strain HN-182^T is heterotrophic and grows well on marine broth (Difco 2216), and is not capable of growing autotrophically on reduced sulfur. Grows at temperatures ranging from 7 to 42 °C (optimum at 25 °C), but not at 4 or 45 °C, and at pH 5.0–9.0 (optimum at pH 7.0), but not at pH 4.5 or 9.5. NaCl is required for growth [0.5–8.5 % (w/v)] with an optimum of 4.5 %. Cells are positive for catalase, oxidase and urease activities. Nitrate is not reduced. Strain HN-182^T contains ubiquinone-10 as sole respiratory quinone. The major polar lipids are phosphatidylglycerol, diphosphatidylglycerol, phosphatidylcholine, an unidentified phospholipid and an unidentified glycolipid. Major cellular fatty acids are C18:1 ω 7c (60.7 %), C16:0 (12.5 %) and C18:0 (8.1 %). DNA G+C content is 67.2 mol% (by *T_m*). The analysis of 16S rRNA gene sequences indicated that strain HN-182^T was related to members of the genus *Paracoccus*, with similarities ranging from 91.2 to 96.7 % (highest to *Paracoccus versutus*) and a close relationship with *Paracoccus sulfuroxidans*, indicating that strain HN-182^T is a member of *Paracoccus*. Based on these results, it is concluded that strain HN-182^T represents a novel species of the genus *Paracoccus*, for which the name *Paracoccus halophilus* sp. nov. is proposed. The type strain is HN-182^T (=CGMCC 1.6117^T=JCM 14014^T).

The genus *Paracoccus* was first described by Davis *et al.* (1969) and the description was emended by Katayama *et al.* (1995). At the time of writing, the genus comprises 22 recognized species names (see Fig. 1, below). Representatives of *Paracoccus* are able to grow heterotrophically on a wide range of organic compounds. A number of *Paracoccus* species can also grow chemoautotrophically and use nitrate as electron acceptor (such as *Paracoccus denitrificans*, Ludwig *et al.*, 1993), or use hydrogen as electron donor (such as *Paracoccus versutus*, Katayama *et al.*, 1995). Members of the genus *Paracoccus* are metabolically versatile and are widely distributed in soil (Siller *et al.*, 1996; Tsubokura *et al.*, 1999), bioreactors (La *et al.*, 2005; Lipski *et al.*, 1998), activated sludge (Katayama *et al.*, 1995; Liu *et al.*, 2006) and in the marine environment (Pukall *et al.*, 2003; Berry *et al.*, 2003; Lee *et al.*, 2004; Kim

et al., 2006). In this study, a marine bacterial isolate, the strain HN-182^T that is able to grow at 7 °C and high salt concentrations (8 % NaCl), was investigated for its taxonomic status. Based on phenotypic and genotypic studies it is concluded that this marine isolate represents a novel species within the genus *Paracoccus*.

Strain HN-182^T was isolated from marine sediment (GPS coordinates for the sampling site are 21° 18.65' N 115° 15.90' E) with marine agar 2216 (MA; Difco), during an ecological survey on the bacterial diversity of the South China Sea. Tenfold dilutions of the sample were prepared with sterile saline solution, and 0.1 ml of each dilution was spread out on MA plates (pH 7.0). The plates were incubated at 30 °C for 2 days. Strain HN-182^T was obtained after several streakings and transfers on MA plates.

Routine cultivation was conducted at 30 °C with MA media. Gram reactions were determined by staining cells grown on MA at 30 °C for 24 h, according to the method described by Gerhardt *et al.* (1994). Endospore formation was detected by malachite green staining. Flagellation was

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain HN-182^T is DQ423482.

An electron micrograph of strain HN-182^T and a figure showing its polar lipids profile are available as supplementary material with the online version of this paper.

by the neighbour-joining method with Kimura's two-parameter calculation model in MEGA version 3.1 (Kumar *et al.*, 2004).

Strain HN-182^T contains ubiquinone-10 (Q-10) as the sole respiratory quinone. Strain HN-182^T contains five polar lipids (Supplementary Fig. S2, in IJSEM Online): diphosphatidylglycerol (DPG), phosphatidylcholine (PC), phosphatidylglycerol (PG), an unidentified glycolipid (GL1) and an unidentified phospholipid (PL1). Among these, PG, PL1 and GL1 are the major polar lipids. Phosphatidylethanolamine (PE), which is commonly present in Gram-negative bacteria, was not detected in the lipid extracts of strain HN-182^T. Within the *Rhodobacteraceae*, the absence of PE was also reported for *Rubellimicrobium thermophilum* (Denner *et al.*, 2006), *Antarctobacter heliothermus*, *Roseobacter denitrificans*, *Roseobacter litoralis*, *Roseisalinus antarcticus* and *Thalassobacter stenotrophicus* (Labrenz *et al.*, 1998, 1999, 2005; Macián *et al.*, 2005). The major cellular fatty acids (>5 %) were C_{18:1}ω7c (60.7 %), C_{16:0} (12.5 %), and C_{18:0} (8.1 %). A list of all detected fatty acids is provided in the species description. The molar G + C content of strain HN-182^T was determined to be 67.2 mol% (by T_m).

The almost complete 16S rRNA gene (1428 nt) of strain HN-182^T was amplified and sequenced. Database search by using BLAST on NCBI (Altschul *et al.*, 1990) showed that strain HN-182^T was related to *P. versutus* ATCC 25364^T with the highest similarity of 96.7 %. Other closely related

Table 1. Differential properties of strain HN-182^T and closely related *Paracoccus* species

Taxa: 1, HN-182^T; 2, *P. sulfuroxidans* (data from Liu *et al.*, 2006); 3, *P. versutus* (Katayama *et al.*, 1995); 4, *P. denitrificans* (Doronina *et al.*, 1998; Rainey *et al.*, 1999; Kelly *et al.*, 2006a, b); 5, *P. pantotrophus* (Robertson & Kuenen, 1983; Rainey *et al.*, 1999); 6, *P. kondratievae* (Doronina *et al.*, 2002); 7, *P. bengalensis* (Ghosh *et al.*, 2006). +, Positive or present; –, negative or absent; NA, no data available.

Characteristic	1	2	3	4	5	6	7
Utilization of:							
L-Arabinose	+	+	+	+	–	+	+
D-Galactose	+	–	+	+	–	+	+
Maltose	+	+	+	+	NA	–	+
D-Mannose	+	–	+	+	+	–	–
D-Fructose	+	–	+	+	–	+	+
Lactose	–	–	–	–	–	–	+
Inositol	–	–	NA	+	NA	+	–
Sucrose	–	–	+	+	+	–	+
D-Xylose	+	–	+	+	NA	–	–
Sulfur compounds used for energy							
Sulfide	–	+	+	+	+	–	+
Thiosulfate	–	+	+	+	+	+	+
Nitrate to N ₂	–	–	+	+	+	+	+
Growth at/in:							
7 °C	+	–	–	–	–	–	–
45 °C	–	–	–	–	–	+	–
8 % NaCl	+	–	–	–	–	–	–
DNA G+C content (mol%)	67.2	61.3	66.8	66.5	64–68	62.5	65.3

species are *Paracoccus bengalensis* (96.5%, Ghosh *et al.*, 2006), *Paracoccus pantotrophus* (96.2%) and *Paracoccus sulfuroxidans* (95.8%). Phylogenetic trees were constructed with maximum-parsimony, maximum-likelihood and neighbour-joining methods. These methods generated a slightly different topology of the tree, but strain HN-182^T and seven other species, including *P. sulfuroxidans*, generated a coherent cluster for all three trees. A neighbour-joining tree is shown in Fig. 1. It is interesting that strain HN-182^T had the highest 16S rRNA gene identity to that of *P. versutus*, but showed a closer relationship with *P. sulfuroxidans* in the phylogenetic trees. A possible explanation to this might be that the two sequences of strain HN-182^T and *P. sulfuroxidans* showed a discontinuity in the sequence alignment corresponding to nucleotides 935–979 of the HN-182^T sequence.

Combining the above phenotypic and genotypic characteristics, it is concluded that strain HN-182^T represents a novel species of the genus *Paracoccus*, for which the name *Paracoccus halophilus* sp. nov. is proposed.

Emended description of genus *Paracoccus* Davis 1969

The formal descriptions and emendations given by Davis *et al.* (1969), Katayama *et al.* (1995) and Ludwig *et al.*

(1993) remain correct. Some species of the genus *Paracoccus* are halophilic and halotolerant.

Description of *Paracoccus halophilus* sp. nov.

Paracoccus halophilus (ha.lo.phi'lus. Gr. n. *hals*, *halos* salt; Gr. adj. *philos* loving; N.L. masc. adj. *halophilus* salt-loving).

Cells are Gram-negative, aerobic, non-motile, non-spore-forming, coccoid to short rods, 0.4–0.5 × 0.7–1.1 µm in size. Colonies grown on MA for 2 days are 2 mm in diameter, smooth, circular, non-glossy, creamy white and convex. Growth occurs at temperatures of 7–42 °C and optimally at 25 °C, but not at 4 or 45 °C. Cells grow at pH 5.0–9.0 and optimally at 7.0, but not at pH 4.5 or 9.5. NaCl is required for growth [0.5–8.5% (w/v)], with an optimum of 4.5%. Positive for catalase, oxidase and urease activities. Negative for amylase and lipase activities. Agar, casein, starch, gelatin and Tweens are not hydrolysed. Utilizes glycerol, L-arabinose, cellobiose, D-fructose, D-galactose, glucose, maltose, D-mannose, L-rhamnose, D-ribose, mannitol, D-sorbitol and D-xylose. Does not utilize adonitol, ethanol, inositol, lactose, melibiose, melezitose, raffinose, sucrose or trehalose. Acid is produced from glucose. Nitrate is not reduced to nitrogen. Reduced sulfur does not support autotrophic growth. The sole respiratory ubiquinone of strain HN-182^T is Q-10. The polar lipids are phosphatidylglycerol (PG), diphosphatidylglycerol (DPG), phosphatidylcholine (PC), an unidentified glycolipid (GL1) and an unidentified phospholipid (PL1). The cellular fatty acids (listed according to percentage) are C_{18:1ω7c} (60.7%), C_{16:0} (12.5%), C_{18:0} (8.1%), 11-methyl C_{18:1ω7c} (3.5%), C_{17:0} (3.0%), C_{10:0} 3-OH (1.8%), C_{14:0} (2.2%), C_{18:1ω9c} (1.3%), iso-C_{15:1} I (1.1%), C_{17:1ω8c} (1.0%) and C_{14:0} 3-OH (0.8%). The following fatty acids were not detected: C_{12:1ω7c}, C_{16:1ω7c} and C_{19:0} cyclo. The G+C content is 67.2 mol% (*T_m*).

The type strain, HN-182^T (=CGMCC 1.6117^T=JCM 14014^T), was isolated from a marine sediment from the South China Sea, China.

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