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-182T, was isolated from sediments of the South China Sea. Cells of strain HN-182T are coccolid to short rods, Gram-negative, non-spore-forming and non-motile. Strain HN-182T 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-182T contains ubiquinone-10 as sole respiratory quinone. The major polar lipids are phosphatidylglycerol, dipropionatidylglycerol, phosphatidylcholine, an unidentified phospholipid and an unidentified glycolipid. Major cellular fatty acids are C18:1ω7c (60.7 %), C16:0 (12.5 %) and C18:0 (8.1 %). DNA G+C content is 67.2 mol % (by Tm). The analysis of 16S rRNA gene sequences indicated that strain HN-182T 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-182T is a member of Paracoccus. Based on these results, it is concluded that strain HN-182T represents a novel species of the genus Paracoccus, for which the name Paracoccus halophilus sp. nov. is proposed. The type strain is HN-182T (=CGMCC 1.6117T=JCM 14014T).

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

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

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-182T 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-182T 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-182T 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
HN-182T was cultivated for 2 weeks in Allen’s medium (Allen, 1959), which was modified by addition of elemental sulfur (1 %), sulfide (1 %), thiosulfate (1 %) or sulfite as energy source and NaCl (4.5 %), and the pH was adjusted to 6.5. Cell growth was estimated by monitoring the increase in turbidity at 600 nm. Cellular fatty acids were extracted from cells grown in MA at 30 °C for 2 days and subsequently analysed as described previously (Hu et al., 2004). Polar lipids were examined by two-dimensional TLC and visualized by spraying reagents specific for z-glycols (periodate-Schiff), sugars (z-naphthol/H2SO4, anisaldehyde/H2SO4), free amino groups (ninhydrin) and phosphate (Zindzadze) (Ventosa et al., 1993). A 50 % sulphuric acid solution was used to detect spots of all kinds of lipids (Fujii et al., 2003). Quinones were determined according to Collins (1985) and Wu et al. (1989). DNA base composition was determined by thermal denaturation (Marmur & Doty, 1962). The 16S rRNA gene was amplified as described previously (Zhang et al., 2006), and 16S rRNA gene sequence alignment was performed using the CLUSTAL_X program (version 1.64b; Thompson et al., 1997). A phylogenetic tree (Fig. 1) was constructed by the neighbour-joining method with Kimura’s two-parameter calculation model in MEGA version 3.1 (Kumar et al., 2004).

Compared with other Paracoccus species, strain HN-182T is characterized by its growth at lower temperature (7 °C) and higher NaCl concentration (8 %, w/v), as well as its ability to utilize D-xylose and some other carbon sources. Strain HN-182T was not capable of growing autotrophically on reduced sulfur. More physiological and biochemical properties of strain HN-182T are provided in the species description (see below) and comparisons of strain HN-182T with other Paracoccus species are listed in Table 1.

strain HN-182T contains ubiquinone-10 (Q-10) as the sole respiratory quinone. Strain HN-182T contains five polar lipids (Supplementary Fig. S2, in IJSEM Online): diphosthatidylglycerol (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-182T. Within the Rhodobacteraceae, the absence of PE was also reported for Rubellimicrobium thermophilum (Denner et al., 2006), Antarctobacter heliotermus, 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 C18:1ω7c (60.7 %), C16:0 (12.5 %), and C18:0 (8.1 %). A list of all detected fatty acids is provided in the species description. The molar G+C content of strain HN-182T was determined to be 67.2 mol% (by Tm).

The almost complete 16S rRNA gene (1428 nt) of strain HN-182T was amplified and sequenced. Database search by using BLAST on NCBI (Altschul et al., 1990) showed that strain HN-182T was related to P. versutus ATCC 25364T with the highest similarity of 96.7 %. Other closely related genera were Paracoccus denitrificans, Paracoccus freudenreichii, and Thalassobacter stenotrophicus. Phylogenetic tree constructed with the neighbour-joining method based on 16S rRNA gene evolutionary distances among strain HN-182T and other Paracoccus species. Bootstrap values (expressed as percentages of 1000 replications) >50 % are shown at the branching points. Roseobacter denitrificans OCH 114T was used as outgroup. Bar, evolutionary distance (Km) of 0.01.

![Fig. 1. Phylogenetic tree constructed with the neighbour-joining method based on 16S rRNA gene evolutionary distances among strain HN-182T and other Paracoccus species. Bootstrap values (expressed as percentages of 1000 replications) >50 % are shown at the branching points. Roseobacter denitrificans OCH 114T was used as outgroup. Bar, evolutionary distance (Km) of 0.01.](image-url)
Table 1. Differential properties of strain HN-182T and closely related Paracoccus species

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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-182T 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-182T 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-182T and P. sulfuroxidans showed a discontinuity in the sequence alignment corresponding to nucleotides 935–979 of the HN-182T sequence.

Combining the above phenotypic and genotypic characteristics, it is concluded that strain HN-182T 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. halos, halos salt; Gr. adj. philos loving; N.L. masc. adj. halophilus salt-loving).

Cells are Gram-negative, aerobic, non-motile, non-spore-forming, coccoïd to short rods, 0.4–0.5 x 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-xylene. Does not utilize adonitol, ethanol, inositol, lactose, melibiose, melizitose, 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-182T 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 C₁₈:₁₀₀₇c (60.7%), C₁₆:₀ (12.5%), C₁₈:₀ (8.1%), 11-methyl C₁₈:₁₀₀₇c (3.5%), C₁₇:₀ (3.0%), C₁₀:₀ 3-OH (1.8%), C₁₄:₀ (2.2%), C₁₈:₀₉c (1.3%), iso-C₁₅:₁ (1.1%), C₁₇:₁₀₀₈c (1.0%) and C₁₄:₀ 3-OH (0.8%). The following fatty acids were not detected: C₁₂:₀₉c, C₁₆:₁₀₀₇c and C₁₉:₀ cyclo. The G+C content is 67.2 mol% (T₀).

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

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References


Berry, A., Janssens, D., Hümblin, M., Jore, J. P. M., Hoste, B., Cleenwerck, I., Vancanneyt, M., Bretzel, W., Mayer, A. F. & other


