

Paracoccus niistensis sp nov., isolated from forest soil, India

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Abstract

A Gram-negative, non-motile, catalase-positive and oxidase-positive, aerobic bacterium designated as NII-0918^T was isolated from soil sample in Western *ghat* forest, India. 16S rRNA gene sequence analysis showed that strain NII-0918^T belongs to the subclass α -*Proteobacteria*, being related to genus *Paracoccus*, and sharing highest sequence similarity with *Paracoccus chinensis* NBRC 104937^T(99.4%), *Paracoccus marinus* NBRC 100640^T (97.3%), *Paracoccus koreensis* Ch05^T (97.1%) and *Paracoccus kondratievae* GB^T(97.0%). Other members of *Paracoccus* showing below 97.0% similarity. The DNA–DNA hybridization values between these four strains and NII-0918^T were 44.7, 28, 32 and 41% respectively. The major fatty acids of strain NII-0918^T were summed feature 7 (C_{18:1} ω 7c/ ω 9t/ ω 12t) (83.0 %) and C_{18:0} (12.5 %). Ubiquinone Q-10 was detected as the major respiratory quinone. The G+C content of genomic DNA of NII-0918^T was 66.6 mol%. On the basis of physiological, morphological, chemotaxonomical and DNA–DNA hybridization data, it is proposed that strain NII-0918^T should be placed as a novel species, for which we propose *Paracoccus niistensis* sp. nov. The type strain is NII-0918^T (CCTCC AA 209055^T = NCIM 5340^T=KCTC 22789^T)

Key words: *Paracoccus niistensis* sp. nov, Polyphasic taxonomy, 16S rRNA

Introduction

The genus *Paracoccus* was first described by Davis et al. (1969) for Gram-negative bacteria, spherical in shape or in the form of short rods, catalase and oxidase positive, nitrate reducing, aerobic and nonmotile. These species have attracted the attention of microbiologists because of their exclusive aerobic respiratory system, which has several components in common with those of the mitochondria (John & Whatley, 1975). Members of the genus exhibit a wide range of metabolic flexibility, particularly with respect to processes involving respiration and energy transduction and are generally found in the soil as well as natural and artificial brines (Daneshvar et al. 2003). The genus currently comprises 30 species with valid publication, with recent introduction of *Paracoccus fistulariae* (Kim et al. 2010), *Paracoccus sphaerophysae* (Deng et al. 2010), *Paracoccus isopora* (Chen et al. 2010), *Paracoccus marinus* (Khan et al. 2008), *Paracoccus halophilus* (Liu et al. 2008), *Paracoccus aestuarii* (Roh et al. 2009), *Paracoccus saliphilus* (Wang et al. 2009) and *Paracoccus chinensis* (Li et al. 2009).

Materials and Methods

Strain NII-0918^T was isolated from a soil sample collected from Western *ghat* forest soil in West coast of India [GPS coordinates for the sample site are 74° 52'E, 8° 18'N] incubated on R2A agar (Hi-Media, Mumbai) at 28°C for one week. The isolate was preserved on slants at 4°C and in 20 % (v/v) glycerol at -80 °C. Morphology was observed by light microscopy (BH 2; Olympus) and scanning electron microscopy (Model JSM5600LV; JEOL) after 5 days incubation on R2A agar at 28°C. Gram-staining was determined using 24 h cultures on R2A agar plates. Motility was determined using the hanging drop technique and tested by using stab cultures in semisolid R2A medium. Hydrolysis of carboxymethylcellulose, casein, starch, gelatin, lecithinase activity, lipase activity (Tween 80), production of indole and hydrogen sulfide, activities of arginine dihydrolase and urease, reduction of nitrate and nitrite were investigated using the methods of Smibert & Krieg (1994). Catalase activity was tested using the 3% (v/v) H₂O₂ drop method and oxidase activity was determined using a 1% solution of tetramethyl-p-phenylenediamine dihydrochloride. β -Galactosidase activity was tested by using the method of Gerhardt & Krieg (1981). Utilization of substrates for growth was examined using R2A basic medium supplemented with 0.01% (w/v) various carbohydrates and at a concentration of 0.1 % amino acids. All results were recorded after incubation for 7 days. Differential physiological characteristics of strain NII-0918^T and the most related type strains of species of the genus *Paracoccus* are given in Table

1. All physiological data were obtained during this study under identical growth conditions which were mentioned for *Paracoccus chinensis* NBRC 104937^T and *Paracoccus marinus* NBRC 100640^T. Growth at different temperatures (4, 10, 15, 20, 28, 37, 40, 45, 55, 65 °C) was tested with R2A medium (pH 7.0). Growth at different NaCl concentrations was tested using R2A medium (pH 7.0) as the basal medium with different NaCl concentrations ranged from 0 to 15% (V/V), at interval of 1% unit. The pH growth range was investigated between 4.0-10.0 at interval of 1 pH unit, using the buffer system: pH 4.0–5.0: 0.1 M citric acid/0.1 M sodium citrate; pH 6.0–8.0: 0.1 M KH₂PO₄/0.1 M NaOH; pH 9.0–10.0: 0.1 M NaHCO₃/0.1 M Na₂CO₃.

Chemotaxonomy

Biomass for chemical and molecular studies was obtained by cultivation in the shaking flasks (150 r.p.m) with tryptone soy broth (pH 7.0) at 28 °C for five days. Isoprenoid quinones were analysed by HPLC as described by Minnikin et al. (1984) and Kroppenstedt, (1982). To determine the cellular fatty acid composition, cells were cultivated on tryptone soy broth agar at 28°C for 5 days. Fatty acid methyl esters were prepared and analyzed by methods described by Sasser (1990) using the protocol of MIDI Sherlock Microbial Identification System.

Molecular systematic

Methods used for extraction of genomic DNA and PCR amplification of 16S rRNA gene were done as described by Li et al. (2007). Multiple alignments with sequences of most closely related *Paracoccus* and calculations of levels of sequence similarity were carried out by using EzTaxon server 2.0 (Chun et al. 2007). Phylogenetic analysis was performed using three tree-making algorithms that were the neighbor-joining (Saitou & Nei, 1987), maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Fitch, 1971) methods. A phylogenetic tree was constructed using the neighbor-joining method of Saitou and Nei (1987) from *K*_{nu} values (Kimura, 1980) using MEGA version 4.0 (Tamura et al. 2007). The topology of the phylogenetic tree was evaluated by the bootstrap resampling method of Felsenstein (1985) with 1000 replicates. The genomic DNA of the isolate for the determination of G+C content was prepared according to the method of Marmur (1961). The G+C content of the DNA was determined by reverse-phase HPLC of nucleosides according to Mesbah et al. (1989)

Results and discussion

Gram-negative, coccid shaped bacterium was isolated from soil samples. Colonies on nutrient agar were circular, convex, smooth and vivid orange in colour. Growth occurs between 10 and 40°C (optimum 28–30°C) and at pH 6–12 (optimum pH 7.0–8.0). Strain grows in 0–7% NaCl (w/v) concentration. All results were recorded after incubation for 72h of incubation. Differential morphological, physiological characteristics of strain NII-0918^T and the most related type strains of species of the genus *Paracoccus* are given in Table 1.

Chemotaxonomic data for the new isolate was consistent with their assignment to the genus *Paracoccus*. The predominant quinone was Q-10. The major fatty acids of strain NII-0918^T were summed feature 7 (C_{18:1} ω7c/ ω 9t/ ω 12t) (83.0 %) and C_{18:0} (12.5 %) as the major hydroxy fatty acid. Other fatty acids detected were C_{13:0} (3.0%), ai-C_{13:0} (2.0%) and C_{10:0} 3-OH (2.0 %). The following fatty acids were detected in trace amounts: i-C_{13:0}, C_{17:0}, and C_{19:0}. This fatty acid profile is characteristic of the *Alphaproteobacteria*, including members of the genus *Paracoccus* (Kelly et al. 2006).

Full length for 16S rRNA gene sequence of strain NII-0918^T was 1397bp and its 16S rRNA gene sequence was analyzed by preliminary comparison of the sequences from the GenBank database (<http://www.ncbi.nlm.nih.gov>). The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain NII-0918^T is FJ842690.

The results indicated the new isolate had higher sequence similarity with members of the genus *Paracoccus*. Phylogenetic analysis showed NII-0918^T fell into one separate subclade with *Paracoccus chinensis* NBRC 104937^T (99.4%) and *Paracoccus marinus* (Fig. 1). However, the new strain NII-0918^T showed highest gene sequence similarity with *Paracoccus chinensis* NBRC 104937^T (99.4%), *Paracoccus marinus* NBRC 100640^T (97.3%), *Paracoccus koreensis* Ch05^T (97.1%) and *Paracoccus kondratievae* GB^T (97%). DNA–DNA relatedness tests were performed between strain NII-0918^T with its closest neighbors using the optical renaturation method (De Ley et al. 1970; Huß et al. 1983; Jahnke, 1992). The experiments were performed with three replications, the level of DNA-DNA relatedness of them was determined and values were 44.7±1.2, 28±5.0, 32±2.0, and 41.0±2.0% (SD1.5-3.0%). The determined DNA–DNA hybridization values were less than 70 %, the recommended threshold value for the delineation of genomic species (Stackebrandt & Goebel, 1994) for assigning strains to the same species, and confirm the separation of strain NII-0918^T from its nearest phylogenetic neighbor. The

G+C content of the genomic DNA from strain NII-0918^T was 66.6 mol%. By using a battery of cultural characteristics strain NII-0918^T differs from other members of the *Paracoccus* genus and represents a novel species for which we propose the name *Paracoccus niistensis* sp. nov.

Description of *Paracoccus niistensis* sp. nov

Paracoccus niistensis (ni.is.ten'sis. N.L. masc. adj. niistensis pertaining to NIIST, the acronym of the National Institute for Interdisciplinary Science and Technology, NIIST, where the taxonomic studies on this novel species were performed).

Gram-negative, aerobic, non-motile, short rod-shaped cells, 0.5–0.7 μm wide and 0.7–1.0 μm long. Colonies on nutrient agar are circular, convex, smooth and vivid orange in colour. Catalase- and oxidase-positive, but negative for β-glucosidase and β-galactosidase. Growth occurs between 10 and 40°C (optimum 28–30°C) and at pH 6–12 (optimum pH 7.0–8.0). Strain grows in 0–7% NaCl (w/v) concentration. Utilizes peptone, but not ammonium sulfate, sodium glutamate, sodium nitrate or casamino acids as nitrogen sources. Positive for utilization of glycogen and gluconate, weakly positive for D,L-arabinose, ribose, D,L-xylose, adonitol and tween 80. But D,L-arabitol, arbutin, cellobiose, esculin, D-galactose, D-glucose, D-fructose, inositol, 2-ketogluconate, 5-ketogluconate, lactose, melibiose, mannitol, mannose, salicin, sorbitol, sucrose and raffinose are not utilized. Negative for casein hydrolysis, but positive urease activity. Indole is not produced from tryptophan and acid is not produced from glucose. Nitrate and nitrite are not reduced. The major fatty acid were summed feature 7 (C_{18:1} ω7c/ ω9t/ ω12t) (83.0 %) and C_{18:0} (12.5 %). Ubiquinone-10 is the major respiratory quinone.

The type strain is NII-0918^T (CCTCC AA 209055^T = NCIM 5340^T = KCTC 22789^T), isolated from the forest soil of Western *ghat*, in India. The DNA G+C content of the type strain is 66.6 mol%.

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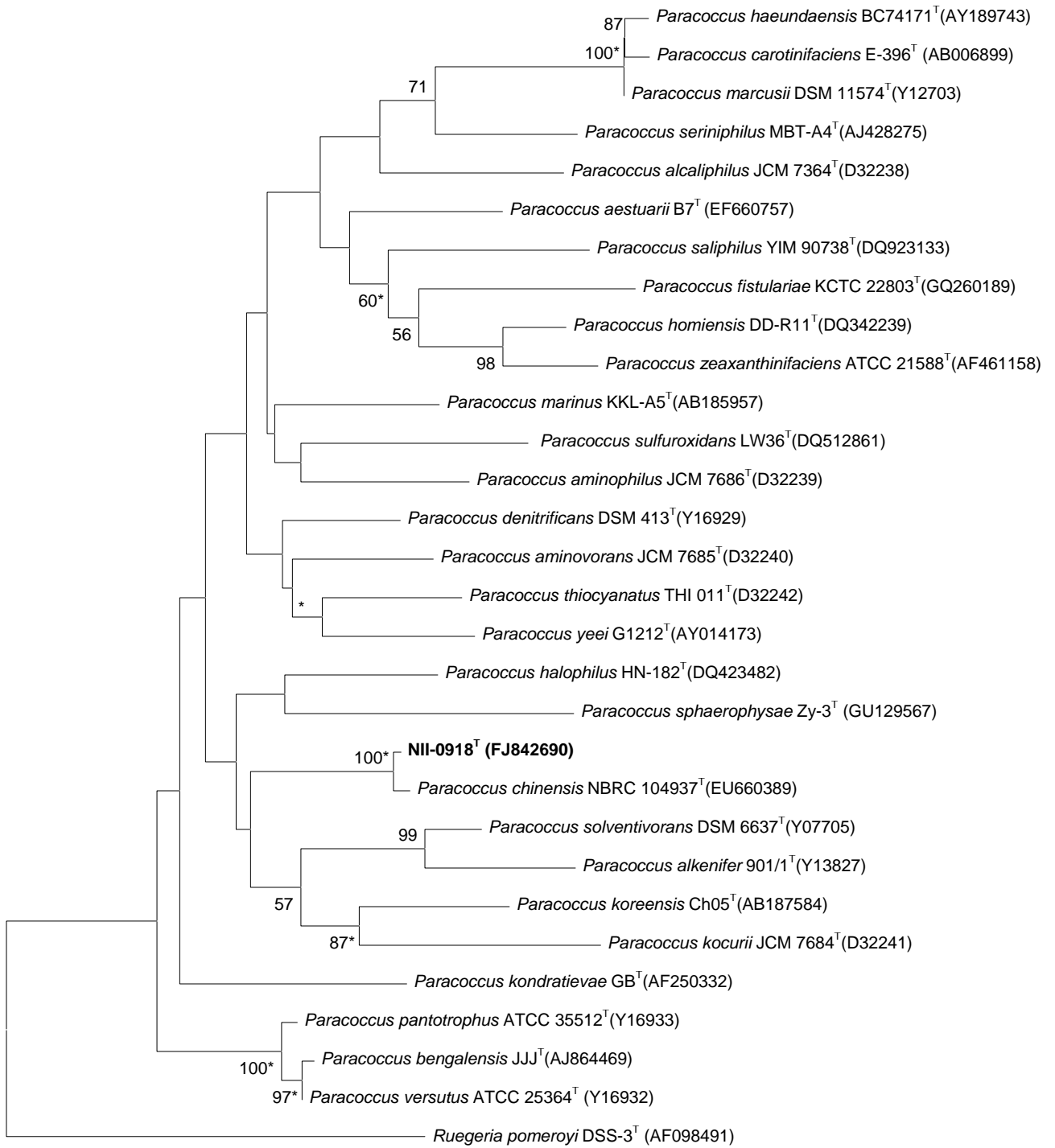
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Table 1. Differences in phenotypic characteristics of strain *Paracoccus* NII-0918^T and its related species.

Characteristics	NII-0918^T	<i>P.chinensis</i> NBRC 104937^T	<i>P.marinus</i> NBRC 100637^T
Colony colour	Vivid orange	Colour less-orange	Dull orange
Cell size	0.5-0.7×0.7-1.0	1.0-1.3×1.5-2.0	0.5-0.8×0.8-1.2
Cell shape	Short rods	Coccus shape	Short rods
Growth at (°C)			
37	+	+	W
40	+	+	-
NaCl range (%)	0-7	<1.0	1-4
Utilization of			
Mannitol	-	-	-
Inositol	-	+	-
Fructose	-	+	+
Galactose	-	+	+
Glucose	-	+	+
Lactose	-	+	+
Sorbitol	-	+	-
Casein	-	+	-
Urease	+	+	-
Tween 80	+	-	-
DNA G+C (mol %)	66.6	69	69
Source of Isolation	Forest soil	Sea water	Sea water

+, Positive or present; -, negative, w, weakly positive. All strains are negative for growth at 4°C, starch and gelatin hydrolysis, β -galactosidase, β -glucosidase and indole. All data were obtained during this study under identical growth conditions, except where indicated otherwise.

Fig. 1. Neighbour-joining phylogenetic dendrogram based on 16S rRNA sequences showing relationships between strain NII-0918^T and related taxa. Asterisks indicate branches that were recovered using least-squares (Fitch & Margoliash, 1967), maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Kluge & Farris, 1969) algorithms. *Ruegeria pomeroyi* DSS-3^T (AF098491) was used as an outgroup. The numbers represent the percentage of bootstrap support from 1000 replicate bootstrap sampling. Only the bootstrap percentages higher than 50% are shown at branching points. Bar, 0.01 substitutions per nucleotide position.



0.01