Antonie van Leeuwenhoek (2011) 99:501–506
DOI 10.1007/s10482-010-9515-4

ORIGINAL PAPER

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

Syed G. Dastager · C. K. Deepa · Wen-Jun Li · Shu-Kun Tang · Ashok Pandey

Received: 20 July 2010 / Accepted: 17 September 2010 / Published online: 9 October 2010

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 the 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* showed 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 (C18:1 ω7c/ω 9t/ω 12t) (83.0%) and C18:0 (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).

Keywords *Paracoccus niistensis* sp. nov · Polyphasic taxonomy · 16S rRNA

S. G. Dastager (✉)
Biological Oceanography Division, National Institute of Oceanography, Dona Paula, Panaji 403004, Goa, India
e-mail: sdastager@nio.org; syedmicro@gmail.com

C. K. Deepa · A. Pandey
National Institute for Interdisciplinary Science and Technology (CSIR), Trivandrum 695019, India

W.-J. Li · S.-K. Tang
Laboratory for Conservation and Utilization of Bio-Resources, Yunnan Institute of Microbiology, Yunnan University, Kunming, Yunnan 650091, People's Republic of China

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 non-motile. 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 and 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 isoporae* (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 and Krieg (1994). Catalase activity was tested using the 3% (v/v) H_2O_2 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 and 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

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

Characteristics	NII-0918^T	*P.chinensis* NBRC 104937^T	*P.marinus* 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

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

+ Positive or present, − negative, *w* weakly positive

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 and 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 KH_2PO4/0.1 M NaOH; pH 9.0–10.0: 0.1 M $NaHCO_3$/0.1 M Na_2CO_3.

Chemotaxonomy

Biomass for chemical and molecular studies was obtained by cultivation in the shaking flasks (150 rpm) 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 and 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*nuc 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 1,000 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 72 h 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 (C18:1 ω7c/ω 9t/ω 12t) (83.0%) and C18:0 (12.5%) as the major hydroxy fatty acid. Other fatty acids detected were C13:0 (3.0%), ai-C13:0 (2.0%) and C10:0 3-OH (2.0%). The following fatty acids were detected in trace amounts: i-C13:0, C17:0, and C19:0. 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 1397 bp 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

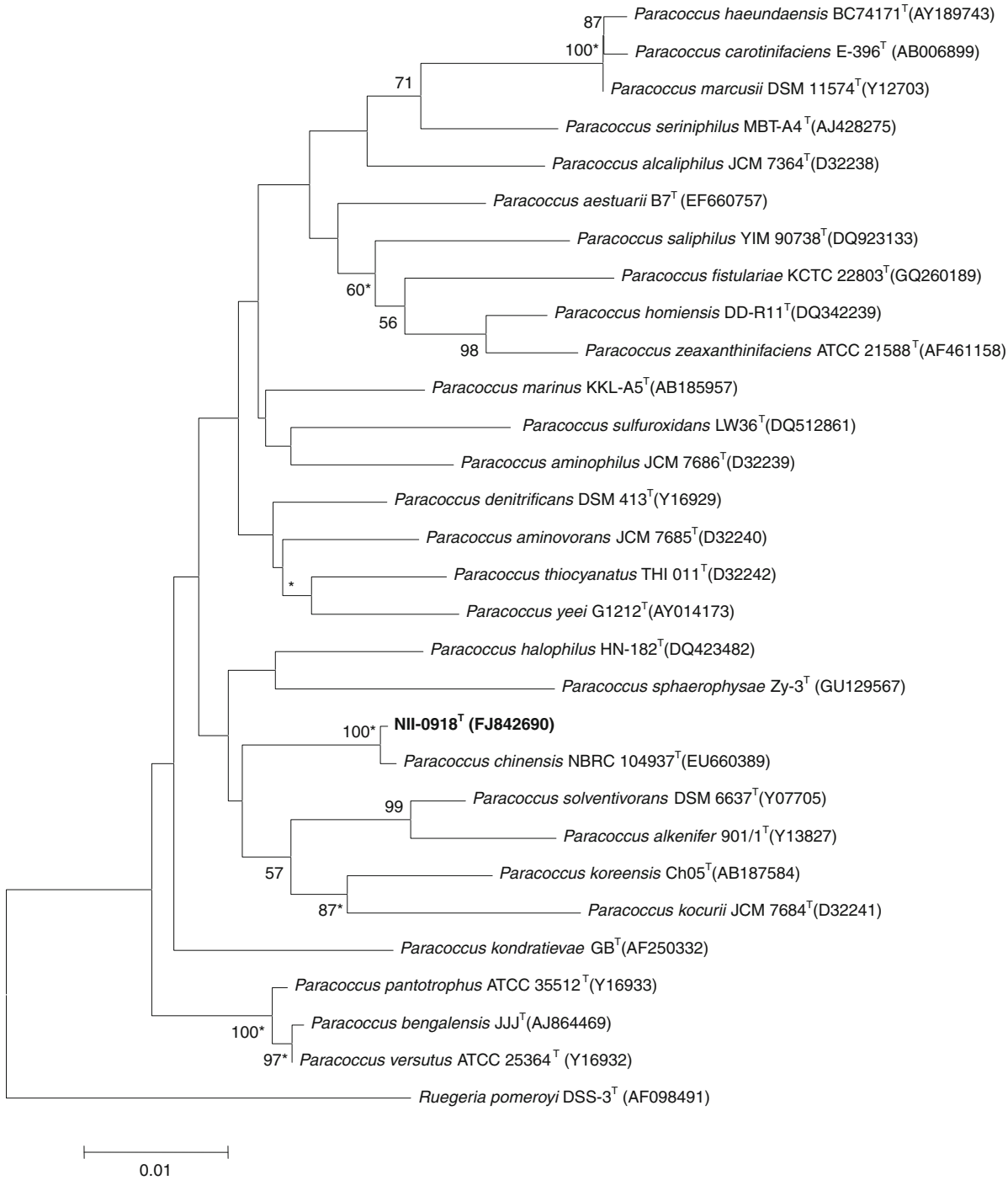

Fig. 1 Neighbour-joining phylogenetic dendrogram based on 16S rRNA sequences showing relationships between strain NII-0918^T and related taxa. Asterisks indicate branches that were recovered using least-squares (Fitch & Margoliash 1967), maximum-likelihood (Felsenstein 1981) and maximum-parsimony (Kluge and Farris 1969) algorithms. *Ruegeria pomeroyi* DSS-3^T (AF098491) was used as an outgroup. The numbers represent the percentage of bootstrap support from 1,000 replicate bootstrap sampling. Only the bootstrap percentages higher than 50% are shown at branching points. *Bar* 0.01 substitutions per nucleotide position

41.0 ± 2.0% (SD 1.5–3.0%). The determined DNA–DNA hybridization values were less than 70%, the recommended threshold value for the delineation of genomic species (Stackebrandt and 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 mm wide and 0.7–1.0 mm 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 (C18:1 ω7c/ω 9t/ω 12t) (83.0%) and C18:0 (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%.

Acknowledgment The authors would like to thank CSIR Task force network programme on Exploration of India's Rich Microbial Diversity (NWP 0006) for providing the financial support.

References

Chen MH, Sheu SY, Chen CA, Wang JT, Chen WM (2010) *Paracoccus isoporae* sp. nov., isolated from the reef-building coral *Isopora palifera*. Int J Syst Evol Microbiol. doi:ijs.0.023333-0v1-ijs.0.023333-0

Chun J, Lee J-H, Jung Y, Kim M, Kim S, Kim BK, Lim YW (2007) EzTaxon: a web-based tool for the identification of prokaryotes based on 16S ribosomal RNA gene sequence. Int J Syst Evol Microbiol 57:2259–2261

Daneshvar MI, Hollis DG, Weyant RS, Steigerwalt AG, Whitney AM (2003) *Paracoccus yeeii* sp. nov. (formerly CDC Group EO-2), a novel bacterial species associated with human infection. J Clin Microbiol 41:1289–1294

Davis DH, Doudoroff M, Stanier RY, Mandel M (1969) Proposal to reject the genus *Hydrogenomonas*: taxonomic implications. Int J Syst Bacteriol 19:375–390

De Ley J, Cattoir H, Reynaerts A (1970) The quantitative measurement of DNA hybridization from renaturation rates. Eur J Biochem 12:133–142

Deng ZS, Zhao LF, Xu L, Kong ZY, Zhao P, Qin W, Chang JL, Wei GH (2010) *Paracoccus sphaerophysae* sp. nov., a siderophore-producing, endophytic bacterium isolated from root nodules of a *Sphaerophysa salsula* growing in northwestern China. Int J Syst Evol Microbiol. doi: 10.1099/ijs.0.021071-0

Felsenstein J (1981) Evolutionary trees from DNA sequences: a maximum likelihood approach. J Mol Evol 17:368–376

Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39:783–789

Fitch WM (1971) Toward defining the course of evolution: minimum change for a specified tree topology. Syst Zool 20:406–416

Fitch WM, Margoliash E (1967) Construction of a phylogenetic tree. Science 155:279–284

Gerhardt P, Krieg NR (1981) General characterization. In: Krieg NR (ed) Manual of methods for general bacteriology. American Society for Microbiology, Washington, DC, pp 409–443

Huß VAR, Festl H, Schleifer KH (1983) Studies on the spectrophotometric determination of DNA hybridization from renaturation rates. Syst Appl Microbiol 4:184–192

Jahnke KD (1992) BASIC computer program for evaluation of spectroscopic DNA renaturation data from GILFORD SYSTEM 2600 spectrophotometer on a PC/XT/AT type personal computer. J Microbiol Methods 15:61–73

John P, Whatley FR (1975) *Paracoccus denitrificans* and the evolutionary origin of the mitochondrion. Nature 254: 495–498

Kelly DP, Rainey FA, Wood AP (2006) The genus *Paracoccus*. In: Dworkin M, Falkow S, Rosenberg E, Schleifer KH, Stackebrandt E (eds) The prokaryotes. A handbook on the biology of bacteria, vol. 5, 3rd edn. Springer, New York, pp 232–249

Khan ST, Takaichi S, Harayama S (2008) *Paracoccus marinus* sp. nov., an adonixanthin diglucoside-producing bacterium isolated from coastal seawater in Tokyo Bay. Int J Syst Evol Microbiol 58:383–386

Kim YO, Kong HJ, Park S, Kang SJ, Kim KK, Moon DY, Oh TK, Yoon JH (2010) *Paracoccus fistulariae* sp. nov., a lipolytic bacterium isolated from bluespotted cornetfish, *Fistularia commersonii*. Int J Syst Evol Microbiol. doi: 10.1099/ijs.0.021808-0

Kimura M (1980) A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J Mol Evol 16:111–120

Kluge AG, Farris FS (1969) Quantitative phyletics and the evolution of anurans. Syst Zool 18:1–32

Kroppenstedt RM (1982) Separation of bacterial menaquinones by HPLC using reverse phase (RP 18) and a silver loaded ion exchange as stationary phases. J Liq Chromatogr 5: 2359–2387

Li WJ, Xu P, Schumann P, Zhang YQ, Pukall R, Xu LH, Stackebrandt E, Jiang CL (2007) *Georgenia ruanii* sp. nov., a novel actinobacterium isolated from forest soil in Yunnan (China) and emended description of the genus *Georgenia*. Int J Syst Evol Microbiol 57:1424–1428

Li H-F, Qu J-H, Yang J-S, Li J-Z, Yuan H-L (2009) Paracoccus chinensis sp. nov., isolated from sediment of a reservoir. Int J Syst Evol Microbiol 59:2670–2674

Liu ZP, Wang BJ, Liu XY, Dai X, Liu YH, Liu SJ (2008) *Paracoccus halophilus* sp. nov., isolated from marine sediment of the South China Sea, China, and emended description of the genus *Paracoccus* Davis 1969. Int J Syst Evol Microbiol 58:257–261

Marmur J (1961) A procedure for the isolation of deoxyribonucleic acid from microorganisms. J Mol Biol 3:208–218

Mesbah M, Premachandran U, Whitman WB (1989) Precise measurement of the G+C content of deoxyribonucleic acid by high-performance liquid chromatography. Int J Syst Bacteriol 39:159–167

Minnikin DE, O'Donnell AG, Goodfellow M, Alderson G, Athalye M, Schaal A, Parlett JH (1984) An integrated procedure for the extraction of isoprenoid quinines and polar lipids. J Microbiol Methods 2:233–241

Roh SW, Nam YD, Chang HW, Kim KH, Kim MS, Shin KS, Yoon JH, Oh HM, Bae JW (2009) *Paracoccus aestuarii* sp. nov., isolated from tidal flat sediment. Int J Syst Evol Microbiol 59:790–794

Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic tree. Mol Biol Evol 4:406–425

Sasser M (1990) Identification of bacteria by gas chromatography of cellular fatty acids. USFCC Newsl 20:1–6

Smibert RM, Krieg NR (1994) Phenotypic characterization. In: Gerhardt P, Murray RGE, Wood WA, Krieg NR (eds) Methods for general, molecular bacteriology. American Society for Microbiology, Washington, DC, pp 607–654

Stackebrandt E, Goebel BM (1994) Taxonomic Note: a place for DNA–DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. Int J Syst Bacteriol 44:846–849

Tamura K, Dudley J, Nei M, Kumar S (2007) MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. Mol Biol Evolu 24:1596–1599

Wang Y, Tang SK, Lou K, Mao PH, Jin X, Jiang CL, Xu LH, Li WJ (2009) *Paracoccus saliphilus* sp. nov., a halophilic bacterium isolated from a saline soil. Int J Syst Evol Microbiol 59:1924–1928