Paracoccus chinensis sp. nov., isolated from sediment of a reservoir

Hai-Feng Li, Jian-Hang Qu, Jin-Shui Yang, Zhi-Jian Li and Hong-Li Yuan

Correspondence

Hong-Li Yuan
hlyuan@cau.edu.cn

State Key Lab for Agrobiotechnology, College of Biological Sciences, Key Laboratory of Agro-Microbial Resource and Utilization, Ministry of Agriculture, China Agricultural University, Beijing 100193, PR China

A Gram-negative, short ovoid- to coccus-shaped, aerobic, motile, non-spore-forming bacterium (designated strain KS-11^T) was isolated from sediment of the eutrophic Guanting reservoir in Beijing, China. Colonies grown on R2A agar plates were circular, convex and colourless to orange. The strain grew in the presence of up to 1 % NaCl (optimum, 0 % NaCl). Growth occurred at 25–40 °C (optimum, 28–37 °C) and at pH 6.0–9.5 (optimum, pH 7.5–9.0). On the basis of 16S rRNA gene sequence similarity, strain KS-11^T was shown to belong to the class *Alphaproteobacteria*, being closely related to *Paracoccus marinus* (96.9 % 16S rRNA gene sequence similarity), followed by *Paracoccus koreensis* (96.8 %), *Paracoccus solventivorans* (96.8 %), *Paracoccus alkenifer* (96.2 %) and *Paracoccus kocurii* (95.8 %). The major fatty acids of strain KS-11^T were summed feature 7 (C_{18:1ω7cl/ω9tl/ω12t}) (83.8 %) and C_{18:0} (6.5 %) and the G+C content of the genomic DNA was 69.0 mol%. Based on comparative analysis of physiological and chemotaxonomic data, it is proposed that strain KS-11^T represents a novel species of the genus *Paracoccus*, named *Paracoccus chinensis* sp. nov. The type strain is KS-11^T (=CGMCC 1.7655^T=NBRC 104937^T).

The Guanting reservoir lies in the north-west of Beijing, China and has been eutrophic for several decades. Several novel bacterial strains have been isolated from the reservoir sediment (Qu & Yuan, 2008a; Qu *et al.*, 2008b). One of the isolates, designated strain KS-11^T, was found to be a member of the genus *Paracoccus* in the *Alphaproteobacteria* according to the 16S rRNA gene sequence phylogenetic analysis.

The genus *Paracoccus* was first reported by Davis *et al.* (1969). To date, the genus is composed of more than 20 species with validly published names. The Gram-negative, catalase- and oxidase-positive, coccus or short-rod members of this genus show a variety of metabolic properties. One species is able to grow methylotrophically with methyl compounds such as methylamine or *N,N*-dimethylformamide (Urakami *et al.*, 1990). Another species can oxidize reduced sulfur compounds (Rainey *et al.*, 1999), whereas some strains are able to reduce nitrate to N₂ under anaerobic conditions (Siller *et al.*, 1996). *Paracoccus koreensis* (La *et al.*, 2005), *Paracoccus homiensis* (Kim *et al.*, 2006), *Paracoccus marinus* (Khan *et al.*, 2008) and *Paracoccus halophilus* (Liu *et al.*, 2008) were described recently.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain KS-11^T is EU660389.

Minimum-evolution and maximum-parsimony phylogenetic trees based on the 16S rRNA gene sequences are available as supplementary material with the online version of this paper.

Strain KS-11^T was isolated from surface sediment of the Guanting reservoir. The samples were suspended and serially diluted in 50 mM phosphate buffer (pH 7.0) and spread on R2A agar plates (containing, l⁻¹ distilled water: 0.5 g glucose, 0.5 g soluble starch, 0.5 g casein hydrolysate, 0.5 g yeast extract, 0.5 g peptone, 0.05 g MgSO₄·7H₂O, 0.3 g KH₂PO₄ and 16 g agar, with the pH adjusted to 7.2 prior to autoclaving). After incubation at 28 °C for 3 weeks, well-separated colonies were picked randomly and purified by subculture on new plates. This procedure was performed several times. Strain KS-11^T comprised one of these colonies and was cultured routinely on R2A agar plates and maintained as a glycerol suspension (20 %, v/v) at –80 °C. The temperature range and optimum for growth were determined at 4, 20, 28, 37 and 45 °C on R2A agar plates for 20 days. The pH range and optimum for growth were examined by incubating cells in R2A broth at pH 5.0–10.0 (at 0.5 pH unit intervals), with the pH adjusted using 1 M HCl or 1 M NaOH. Tolerance to salinity was investigated in R2A broth supplemented with 0, 0.5, 1, 2, 3, 4, 5, 10 and 15 % (w/v) NaCl.

Cell morphology was observed under an Olympus light microscope at ×1000. Gram-staining was determined using 24 h cultures on R2A agar plates (Skerman, 1967). Motility was determined using the hanging drop technique and tested by using stab cultures in semisolid R2A medium. Hydrolysis of carboxymethylcellulose, starch and gelatin, lecithinase activity, lipase activity (Tween 80),

production of indole and hydrogen sulfide, activities of arginine dihydrolase and urease, and reduction of nitrate and nitrite were all investigated using the methods of Smibert & Krieg (1994). Denitrification was determined according to the method of Dong & Cai (2001).

Catalase activity was tested using the 3 % (v/v) H₂O₂ drop method with young cells grown on R2A agar. 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 basic medium [containing, l⁻¹ distilled water: 1 g (NH₄)₂HPO₄, 0.3 g K₂HPO₄ and 0.05 g MgSO₄·7H₂O; pH 7.2], supplemented with 0.02 % (w/v) yeast extract, various carbohydrates and alcohols at a concentration of 0.5 %, and organic acids and amino acids at 0.1 %. All results were recorded after incubation for 7 days. Differential physiological characteristics of strain KS-11^T and the most related type strains of species of the genus *Paracoccus* are given in Table 1.

For pigment analysis, cells were grown on King's B and R2A agar. Determination of pigment production and spectral characteristics were performed by using extraction with methanol, according to Hildebrand *et al.* (1994), with

a visible–UV LabTach UV-1000 spectrophotometer. Strain KS-11^T produced a water-insoluble yellow pigment with a major peak at 470 nm; a fluorescent pigment was also detected.

For the determination of the phylogenetic position of strain KS-11^T, genomic DNA was extracted using the method of Marmur (1961) from cells cultured for 2 days in R2A liquid medium. The 16S rRNA gene was amplified using the bacterial universal primers 27F (5'-GAGAG-TTGATCCTGGCTCAG-3', *Escherichia coli* position 27–46) and 1495R (5'-CTACGGCTACCTTGTTACGA-3', *E. coli* position 1476–1495). Based on the 16S rRNA sequence of strain KS-11^T, a BLAST search showed that the strain was closely related to the genus *Paracoccus*. The identification of phylogenetic neighbours and calculation of pairwise 16S rRNA gene sequence similarity were achieved using the EzTaxon server (<http://www.eztaxon.org/>; Chun *et al.*, 2007). Strain KS-11^T showed the highest similarity with *P. marinus* NBRC 100637^T (96.9 %), followed by *P. koreensis* KCTC 12238^T (96.8 %), *Paracoccus solventivorans* KCTC 12190^T (96.8 %), *Paracoccus alkenifer* KCTC 12188^T (96.2 %) and *Paracoccus kocurii* KCTC 12189^T (95.8 %). The 16S rRNA gene sequences of related taxa were obtained from GenBank/EMBL/DDBJ databases. Multiple alignments were performed using the CLUSTAL W program

Table 1. Differential phenotypic characteristics of strain KS-11^T and closely related species of the genus *Paracoccus*

Strains: 1, KS-11^T (*P. chinensis* sp. nov.; data from this study); 2, *P. kocurii* KCTC 12189^T (Van Spanning *et al.*, 2005); 3, *P. alkenifer* KCTC 12188^T (Van Spanning *et al.*, 2005); 4, *P. solventivorans* KCTC 12190^T (Van Spanning *et al.*, 2005); 5, *P. koreensis* KCTC 12238^T (La *et al.*, 2005); 6, *P. marinus* NBRC 100637^T (Khan *et al.*, 2008). +, Positive; –, negative; NT, not tested.

Characteristic	1	2	3	4	5	6
Motility	+	–	–	–	–	–
Temperature for growth (°C)	20–37	25–30	30	30–37	15–37	10–35
pH for growth	6–9.5	6.6–8.2	6–9	7–8	6–8	6–9
Size of single cell (µm)	1.0–1.3 × 1.5–2.0	0.5–0.8 × 0.7–1.1	0.4–0.6 × 0.9–1.7	0.4–0.5 × 0.9–1.5	0.5–1.0 × 1.0–1.5	0.5–0.8 × 0.8–1.2
Denitrification	–	+	+	+	–	NT
Carbon utilization						
D-Glucose	+	–	–	NT	–	+
L-Alanine	+	–	–	–	+	NT
Gluconate	+	–	–	–	–	NT
Histidine	+	–	+	+	+	NT
Malonate	–	–	+	+	–	NT
L-Proline	+	–	+	+	+	NT
D-Ribose	+	+	–	–	+	NT
D-Sorbitol	+	–	–	–	+	–
Fructose	+	–	–	–	NT	+
Galactose	+	–	–	–	NT	+
Maltose	+	–	–	NT	–	–
Sucrose	+	–	–	–	–	–
Xylose	+	–	–	NT	NT	NT
Inositol	+	–	–	NT	–	–
Mannose	+	NT	NT	NT	–	–
Trehalose	+	–	–	NT	–	–
Citrate	+	–	–	+	–	NT
DNA G + C content (mol%)	69.0	71.0	70.2	68.5	69.0	69.0

(Thompson *et al.*, 1994). A phylogenetic tree was generated with the neighbour-joining method (Saitou & Nei, 1987) using the MEGA 3.1 program (Kumar *et al.*, 2004); the bootstrap values were evaluated based on 1000 replications (Felsenstein, 1985). Two other algorithms (minimum-evolution and maximum-parsimony) were also used to construct phylogenetic trees. These showed similar topologies to that of the neighbour-joining tree (see Supplementary Fig. S1, available in IJSEM Online). The phylogenetic results indicated that strain KS-11^T belonged to the genus *Paracoccus*. The positions of strain KS-11^T and related species of the genus *Paracoccus* are shown in Fig. 1.

Analysis of cellular fatty acids was performed according to the protocol of the Sherlock Microbial Identification System (MIDI) with cells grown in R2A broth for 2 days. The fatty acids were analysed by gas chromatography (AGILENT 6890; Hewlett Packard) and identified using the Microbial Identification software package (Sasser, 1990). The fatty acid profile of strain KS-11^T comprised mainly C_{18:1}ω7c/ω9t/ω12t (summed feature 7; 83.8%), C_{18:0} (6.5%), C_{16:0} (2.9%) and C_{10:0} 3-OH (2.1%). Fatty acid profiles of strain KS-11^T and the type strains of related recognized species are shown in Table 2.

The G+C content of the chromosomal DNA was determined by using the *T_m* method as described by De Ley (1970). The G+C content of the genomic DNA of strain KS-11^T was 69.0 mol%, within the range 63–71 mol% for members of the genus *Paracoccus* (Harker *et al.*, 1998).

Based on the data and observations given above, strain KS-11^T should be assigned to the genus *Paracoccus* as representing a novel species, for which the name *Paracoccus chinensis* sp. nov. is proposed.

Description of *Paracoccus chinensis* sp. nov.

Paracoccus chinensis (chi.nen'sis. N.L. masc. adj. *chinensis* pertaining to China, where the type strain was isolated and studied).

Cells are Gram-negative, aerobic, non-spore-forming, motile, ovoid- to coccus-shaped, 1.0 × 1.3–1.5–2.0 μm in size, and occur singly, in pairs, small clusters or short chains on R2A agar incubated for 3 days. Colonies on R2A agar are circular, smooth, moist, non-glossy, low-convex and colourless to orange. Growth occurs at 25–40 °C (optimum, 28–37 °C), pH 6.0–9.5 (optimum, pH 7.5–9.0) and with less than 1% NaCl. Does not grow anaerobically using nitrate as electron acceptor. A water-insoluble yellow pigment is produced on R2A agar, with a major absorbance peak at 470 nm. A fluorescent pigment is also produced at a low rate on King's B medium. Positive for catalase, oxidase, urease, lecithinase and hydrolysis of casein. Negative for arginine dihydrolase, β-galactosidase, lipase activity (Tween 80), production of indole and hydrogen sulfide, and hydrolysis of starch, gelatin and carboxymethylcellulose. Nitrate is reduced but not nitrite. Utilizes D-fructose, D-galactose, D-xylose, melibiose, melezitose, lactose, soluble starch, maltose, D-mannose, D-ribose, L-fucose, D-glucose, sucrose, D-sorbitol, inositol, salicin, inulin, gluconate, L-alanine, L-histidine, L-proline, L-tyrosine, L-asparagine, pyruvate, citrate and acetate, but not raffinose, D-arabinose, L-rhamnose, D-sorbose, L-arabinose, malonate, mannitol, succinic acid, glycerol, lactate, succinate, L-glycine, L-cysteine, L-glutamic acid, methanol, ethanol or acetone. Major cellular fatty acids are C_{18:1}ω7c/ω9t/ω12t (summed feature 7) and C_{18:0}. The G+C content of the genomic DNA of the type strain is 69.0 mol%.

Fig. 1. Unrooted phylogenetic tree, showing the relationships of *P. chinensis* KS-11^T and other related species. Bootstrap values (expressed as percentages of 1000 replications) greater than 50% are shown at branch points. Bar, 0.5 substitutions per 100 nt positions.

Table 2. Cellular fatty acid profiles (%) of strain KS-11^T and related type strains of *Paracoccus* species

Strains: 1, KS-11^T (*P. chinensis* sp. nov.; data from this study); 2, *P. kocurii* KCTC 12189^T (La *et al.*, 2005); 3, *P. alkenifer* KCTC 12188^T (La *et al.*, 2005); 4, *P. solventivorans* KCTC 12190^T (La *et al.*, 2005); 5, *P. koreensis* KCTC 12238^T (La *et al.*, 2005); 6, *P. marinus* NBRC 100637^T and NBRC 100640 (Khan *et al.*, 2008). tr, Trace amount (<1%); –, not detected.

Fatty acid	1	2	3	4	5	6
Saturated						
C _{12:0}	–	1.1	–	–	–	–
C _{13:0} iso	tr	–	–	–	–	–
C _{13:0} anteiso	1.4	–	–	–	–	–
C _{14:0}	–	tr	–	–	–	–
C _{16:0}	2.9	4.0	1.6	–	2.3	tr
C _{17:0}	tr	3.2	2.3	2.2	–	1–2
C _{18:0}	6.5	14.8	4.5	7.1	13.4	4–5
C _{19:0}	–	1.0	0.9	–	–	tr
Unsaturated						
C _{20:1ω9t}	–	2.6	1.7	1.4	–	–
Hydroxy						
C _{16:0} 2-OH	–	1.1	–	–	–	–
C _{10:0} 3-OH	2.1	–	–	–	2.4	2
C _{19:0} cyclo ω8c	–	34.5	–	–	–	–
Summed features*						
3	–	tr	2.3	3.2	–	–
7	83.8	36.5	86.7	86.1	79.6	85–87
Unknown†						
ECL 11.799	2.0	–	–	–	–	2.0

*Summed features are groups of two or three fatty acids that cannot be separated by GLC with the MIDI system. Summed feature 3 contains C_{14:0} 3-OH and/or C_{16:1} iso I; summed feature 7 contains C_{18:1ω7c/ω9t/ω12t}.

†Unknown fatty acids have no name listed in the peak library file of the MIDI system and therefore cannot be identified. Equivalent chain-length (ECL) is given.

The type strain, KS-11^T (=CGMCC 1.7655^T=NBRC 104937^T), was isolated from sediment of the Guanting reservoir in China.

Acknowledgements

This work was supported by project no. 30670071 of the National Natural Science Foundation of China. It was also supported by the Special Program for water pollution control of Taihu Lake in Jiangsu Province (no. BK2007741).

References

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

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

De Ley, J. (1970). Reexamination of the association between melting point, buoyant density, and chemical base composition of deoxyribonucleic acid. *J Bacteriol* **101**, 738–754.

Dong, X. Z. & Cai, M. Y. (2001). *Determinative Manual for Routine Bacteriology*. Beijing: Scientific Press.

Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.

Gerhardt, P. & Krieg, N. R. (1981). General characterization. In *Manual of Methods for General Bacteriology*, pp. 409–443. Edited by N. R. Krieg. Washington, DC: American Society for Microbiology.

Harker, M., Hirschberg, J. & Oren, A. (1998). *Paracoccus marcusii* sp. nov., an orange Gram-negative coccus. *Int J Syst Bacteriol* **48**, 543–548.

Hildebrand, D. C., Palleroni, N. J., Hendson, M., Toth, J. & Johnson, J. L. (1994). *Pseudomonas flavescens* sp. nov., isolated from walnut blight cankers. *Int J Syst Bacteriol* **44**, 410–415.

Khan, S. T., 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, B. Y., Weon, H. Y. & Yoo, S. H. (2006). *Paracoccus homiensis* sp. nov., isolated from a sea-sand sample. *Int J Syst Evol Microbiol* **56**, 2387–2390.

Kumar, S., Tamura, K. & Nei, M. (2004). MEGA3: integrated software for Molecular Evolutionary Genetics Analysis and sequence alignment. *Brief Bioinform* **5**, 150–163.

La, H. J., Im, W. T., Ten, L. N., Kang, M. S., Shin, D. Y. & Lee, S. T. (2005). *Paracoccus koreensis* sp. nov., isolated from anaerobic granules in an upflow anaerobic sludgeblanket (UASB) reactor. *Int J Syst Evol Microbiol* **55**, 1657–1660.

Liu, Z. P., Wang, B. J., Liu, X. Y., Dai, X., Liu, Y. H. & Liu, S. J. (2008). *Paracoccus halophilus* sp. nov., isolated from marine sediment of the South China Sea, China, and emended description of 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.

Qu, J. H. & Yuan, H. L. (2008a). *Sediminibacterium salmoneum* gen. nov., sp. nov., a novel member of the phylum *Bacteroidetes* isolated from sediment of a eutrophic reservoir. *Int J Syst Evol Microbiol* **58**, 2191–2194.

Qu, J. H., Li, H. F., Yang, J. S. & Yuan, H. L. (2008b). *Flavobacterium cheniae* sp. nov., isolated from sediment of a eutrophic reservoir. *Int J Syst Evol Microbiol* **58**, 2186–2190.

Rainey, F. A., Kelly, D. P., Stackbrandt, E., Burghardt, J., Hiraishi, A., Katayama, Y. & Wood, A. (1999). A re-evaluation of the taxonomy of *Paracoccus denitrificans* and a proposal for the combination *Paracoccus pantotrophus* comb. nov. *Int J Syst Bacteriol* **49**, 645–651.

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

Sasser, M. (1990). *Identification of bacteria by gas chromatography of cellular fatty acids*, MIDI Technical Note 101. Newark, DE: MIDI Inc.

Siller, H., Fred, A. R., Stackbrandt, E. & Winter, J. (1996). Isolation and characterization of a new Gram-negative, acetone-degrading, nitrate-reducing bacterium from soil, *Paracoccus solventivorans* sp. nov. *Int J Syst Bacteriol* **46**, 1125–1130.

Skerman, V. B. D. (1967). *A Guide to the Identification of the Genera of Bacteria*, 2nd edn. Baltimore: Williams & Wilkins.

Smibert, R. M. & Krieg, N. R. (1994). Phenotypic characterization. In *Methods for General and Molecular Bacteriology*, pp. 607–654. Edited

by P. Gerhardt, R. G. E. Murray, W. A. Wood & N. R. Krieg. Washington, DC: American Society for Microbiology.

Thompson, J. D., Higgins, D. G. & Gibson, T. J. (1994). CLUSTAL w: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res* **22**, 4673–4680.

Urakami, T., Araki, H., Oyanagi, H., Suzuki, K. & Komagata, K. (1990). *Paracoccus aminophilus* sp. nov. and *Paracoccus aminovorans*

sp. nov., which utilize *N,N*-dimethylformamide. *Int J Syst Bacteriol* **40**, 287–291.

Van Spanning, R. J. M., Stouthamer, A. H., Baker, S. C. & Van Verseveld, H. W. (2005). Genus XII. *Paracoccus* Davis 1969, 384^{AL} emend. Ludwig, Mittenhuber and Friedrich 1993, 366. In *Bergey's Manual of Systematic Bacteriology*, 2nd edn, vol. 2 (*The Proteobacteria*), part C (*The Alpha-, Beta-, Delta-, and Epsilonproteobacteria*), pp. 197–204. Edited by D. J. Brenner, N. R. Krieg, J. T. Staley & G. M. Garrity. New York: Springer.