Paracoccus saliphilus sp. nov., a halophilic bacterium isolated from a saline soil

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A Gram-negative, coccoid to short rod-shaped, non-spore-forming, moderately halophilic bacterium, designated strain YIM 90738^T, was isolated from a salt lake in Xinjiang province, north-west China, and was subjected to a polyphasic taxonomic study. The strain was non-motile and grew at pH 6.0–8.0 (optimal growth at pH 7.0), 10–55 °C (optimal growth at 37 °C) and salinities of 1–15 % NaCl (w/v, optimal growth at 8 % NaCl). Ubiquinone 10 was detected as the major respiratory quinone. The cellular fatty acid profile had C_{18:1ω7c} (80.4 % of the total) as the major component, similar to those of members of the genus *Paracoccus*. The nearest phylogenetic neighbour of strain YIM 90738^T was the type strain of *Paracoccus homiensis*, as determined by 16S rRNA gene sequence analysis (97.5 % similarity). The level of DNA–DNA relatedness between *P. homiensis* DSM 17862^T and strain YIM 90738^T was 51.5 %. The G + C content of the genomic DNA of strain YIM 90738^T was 60.3 mol%. On the basis of phenotypic and phylogenetic data and its genotypic distinctiveness, strain YIM 90738^T (=CCTCC AB 206074^T =KCTC 22163^T) is proposed as the type strain of a novel species of the genus *Paracoccus*, *Paracoccus saliphilus* sp. nov.

The genus *Paracoccus* Davis 1969 was proposed by Davis *et al.* (1969). At the time of writing, the genus comprised 26 recognized species. Owing to the physiological versatility of members of the genus, the description of the genus *Paracoccus* has been emended several times (Ludwig *et al.*, 1993; Katayama *et al.*, 1995; Liu *et al.*, 2008). The most recently described species at the time of writing are *Paracoccus halophilus* (Liu *et al.*, 2008) and *Paracoccus marinus* (Khan *et al.*, 2008). Species of the genus are characterized as Gram-negative and catalase- and oxidase-positive, with high metabolic versatility, being halophilic or halotolerant, having a large amount of C_{18:1ω7c} and having ubiquinone 10 as the major isoprenoid quinone. A moderately halophilic bacterium, designated strain YIM 90738^T, was isolated from a saline soil sample and is shown here to represent a novel species of the genus *Paracoccus*.

Strain YIM 90738^T was isolated from a saline soil sample taken from the edge of the Ebinur Lake wetland natural

reserve, which is located in the arid area of north-west Xinjiang province, north-west China; the wetland forms the lowest part of the west Junggar Basin. The sampling site is at 44° 34' 07" N 83° 44' 25" E. The major ion components of the soil (g l⁻¹) were Na⁺ (35.00), K⁺ (0.48 g), Ca²⁺ (0.38), Mg²⁺ (4.73), Cl⁻ (38.01), SO₄²⁻ (40.34) and HCO₃⁻ (0.32). Strain YIM 90738^T was isolated on modified ISP 5 medium by using the routine dilution plating method; the medium contained (per litre distilled water) 1 g L-asparagine, 10 g glycerol, 5 g yeast extract, 1 g K₂HPO₄, 5 g KNO₃, 100 g NaCl and 15 g agar. The medium was adjusted to pH 7.5. Colonies were picked and repeatedly restreaked onto modified ISP 5 medium until purity was confirmed.

Strain YIM 90738^T was cultivated on modified ISP 5 medium at 37 °C for investigation of morphological and physiological characteristics. The strain was maintained on modified ISP 5 agar slants containing 8 % NaCl (w/v) at 4 °C and as 20 % (w/v) glycerol suspensions at -20 °C. Cell biomass of strain YIM 90738^T for respiratory isoprenoid quinone analysis and for extraction of DNA was obtained by cultivation in shaken flasks (about

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150 r.p.m.) by using modified ISP 5 broth at 37 °C for about 1 week. For fatty acid methyl ester analysis, cell mass of strain YIM 90738^T was obtained from agar plates after incubation for 2 days on trypticase soy agar (TSA; Difco) containing 8 % NaCl at 37 °C.

Gram staining was carried out by the standard Gram reaction and was confirmed by using the KOH lysis test method (Cerny, 1978). Cell motility was confirmed by the presence of turbidity throughout a tube containing semi-solid medium (Leifson, 1960). Cell morphology was observed by light microscopy (model BH2; Olympus) and transmission electron microscopy (model H-800; Hitachi) by using cells from exponentially growing cultures. Colony morphology was observed on modified ISP 5 medium after incubation at 37 °C for 5 days. The methyl red and Voges–Proskauer tests were investigated as described by Lányi (1987). The temperature range for growth was determined by incubating cells for 1 week on modified ISP 5 medium at 4, 10, 15, 20, 28, 37, 45, 55 and 65 °C. The pH growth range was investigated between pH 4.0 and 10.0 at intervals of 1 pH unit, by using the buffer system described by Xu *et al.* (2005). Liquid cultures were incubated on a rotary shaker at 37 °C for 2–3 weeks using modified ISP 5 as the basal medium. Tolerance to chlorides of sodium, potassium and magnesium was tested by using the same basal medium, with concentrations of the above at 0, 1, 3, 5, 8, 10, 12, 15, 18, 20, 25 and 30 % (w/v). Catalase activity was determined by production of bubbles after the addition of a drop of 3 % H₂O₂. Oxidase activity was observed by oxidation of tetramethyl-*p*-phenylenediamine. Hydrolysis of casein, starch, CM-cellulose, gelatin, pectin, tyrosine and Tweens 20, 40 and 80 was determined as described by Cowan & Steel (1965). Metabolic properties and enzyme activities were determined by means of the API 20NE and API ZYM systems (bioMérieux) according to the manufacturer's instructions. Utilization of organic substrates was tested by using Biolog GN2 microplates. Cell suspensions were prepared by using pre-warmed sterile saline solution (8 % NaCl, w/v), within the density range specified by the manufacturer. The detailed physiological and biochemical properties of strain YIM 90738^T are given in Table 1 and in the species description.

Fatty acids were analysed as described by Sasser (1990) by using the Microbial Identification System (MIDI). Isoprenoid quinones were extracted and purified as described by Komagata & Suzuki (1987). The purified ubiquinones were dissolved in acetone and separated by reversed-phase HPLC.

Extraction of genomic DNA and PCR amplification of the 16S rRNA gene were done as described by Li *et al.* (2007). The genomic DNA G+C content was determined by reversed-phase HPLC (Mesbah *et al.*, 1989). The sequence obtained for strain YIM 90738^T was compared with other publicly available 16S rRNA gene sequences deposited in the GenBank/DDBJ/EMBL databases. The multiple

sequence alignment program CLUSTAL X (Thompson *et al.*, 1997) was used to align the 16S rRNA gene sequences of the new isolate and representative members of selected genera. Phylogenetic analyses were performed by using the neighbour-joining (Saitou & Nei, 1987), maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Fitch, 1971) methods. A phylogenetic tree was constructed according to the neighbour-joining method of Saitou & Nei (1987) from *K*_{nuc} values (Kimura, 1980) by using MEGA version 3.0 (Kumar *et al.*, 2004). The topology of the phylogenetic tree was evaluated by bootstrap resampling based on 1000 replicates (Felsenstein, 1985). DNA–DNA hybridization studies were performed according to the method of Ezaki *et al.* (1989), by using photobiotin-labelled DNA probes and microdilution wells. *Paracoccus homiensis* DSM 17862^T was used as a reference strain for DNA–DNA hybridizations. DNA–DNA hybridization experiments were performed in triplicate. The hybridization temperature was 42 °C, which is within the limit of validity for the filter method (De Ley & Tijtgat, 1970).

Cells of strain YIM 90738^T were Gram-negative, non-motile, cocci to short rods, 1.6–2.0 × 2.5–5.5 µm; no spores were observed. Optimum growth occurred at pH 7.0 and 37 °C on modified ISP 5 medium. At 37 °C, strain YIM 90738^T grew at total salt concentrations of 1–15 % (w/v). Strain YIM 90738^T did not grow in the absence of NaCl; optimal growth was at 8 % NaCl, which indicated that the isolate could be assigned to the group of moderately halophilic bacteria (Kushner & Kamekura, 1988). Strain YIM 90738^T was unable to grow in the presence of only KCl or MgCl₂ · 6H₂O on modified ISP 5 medium. Table 1 provides a comparison of the phenotypic characteristics of strain YIM 90738^T and closely related species of the genus *Paracoccus*.

Strain YIM 90738^T contained ubiquinone Q-10 as the predominant isoprenoid quinone. The major cellular fatty acid was C_{18:1}ω7c (80.4 % of the total); C_{18:0} (6.1 %), C_{10:0} 3-OH (3.3 %) and C_{19:0} cyclo ω8c (2.6 %) were detected as minor components. This fatty acid profile (Table 2) and ubiquinone Q-10 as the predominant isoprenoid quinone were consistent with data for recognized members of the genus *Paracoccus* (Kim *et al.*, 2006). The DNA G+C content of strain YIM 90738^T was 60.3 mol%, lower than that of some species of the genus *Paracoccus* (Table 1), but similar to that of *Paracoccus sulfuroxidans* (61.3 mol%; Liu *et al.*, 2006) and *Paracoccus kondratievae* (62.5 mol%; Doronina *et al.*, 2002).

An almost-complete 16S rRNA gene sequence (1389 bp) was determined for strain YIM 90738^T and was used for BLAST searches and phylogenetic analysis. Sequence alignment showed that strain YIM 90738^T had highest 16S rRNA sequence similarity to members of the genus *Paracoccus*. Phylogenetic analysis was based on sequences of representative strains of the genus *Paracoccus*, and the type strain of *Rhodobacter capsulatus* was used as the outgroup. In the phylogenetic tree based on the neighbour-joining algorithm, strain YIM 90738^T shared the same

Table 1. Differential characteristics between strain YIM 90738^T and the type strains of related species of the genus *Paracoccus*

Strains: 1, YIM 90738^T; 2, *P. homiensis* DSM 17862^T (data from Kim *et al.*, 2006); 3, *P. zeaxanthinifaciens* ATCC 21588^T (Berry *et al.*, 2003); 4, *P. carotinifaciens* IFO 16121^T (Tsubokura *et al.*, 1999); 5, *P. haeundaensis* KCCM 10460^T (Lee *et al.*, 2004); 6, *P. marcusii* DSM 11574^T (Harker *et al.*, 1998); 7, *P. seriniphilus* DSM 14827^T (Pukall *et al.*, 2003). ND, No data available.

Characteristic	1	2	3	4	5	6	7
Cell morphology*	C-SR	R-O	C-SR	R	R	C-SR	C-R
Pigmentation†	NP-YB	NP-CW	Y-OR	OR-RD	OR-RD	OR	NP-CW
Flagellation	—	+	—	+	—	—	—
Motility	—	+	—	+	—	—	—
Growth at 55 °C	+	—	—	—	—	—	—
Growth at 15 % (w/v) NaCl	+	+	—	—	—	—	—
pH range	6.0–8.0	5.0–9.0	6.1–9.1	ND	6.0–10.5	ND	5.0–10.0
Nitrate reduction	—	—	—	—	+	—	+
Growth on:							
Acetic acid	+	+	—	ND	ND	—	+
Adonitol	+	—	—	+	—	+	+
L-Aspartate	+	+	+	+	ND	—	+
L-Asparagine	+	—	+	+	—	—	+
i-Erythritol	—	+	—	+	ND	+	+
Gentiobiose	+	—	—	+	ND	+	—
L-Phenylalanine	—	+	—	ND	ND	—	—
Propionic acid	—	+	—	ND	ND	+	+
Quinic acid	+	—	—	+	ND	+	—
L-Rhamnose	—	+	—	ND	—	—	—
D-Sorbitol	+	+	—	+	—	+	+
Succinic acid	+	+	—	ND	ND	+	—
Xylitol	+	—	—	+	ND	+	+
DNA G + C content (mol%)	60.3	63.0	66.9–67.7‡	67	66.9	66	63.3

*C, Cocci; O, ovoid; R, rods; SR, short rods.

†NP, Colourless; CW, creamy white; OR, orange; RD, red; Y, yellow; YB, yellow-brown.

‡Range of values for five strains including the type strain.

branch as *P. homiensis* DD-R11^T and *Paracoccus zeaxanthinifaciens* R-1506 (Fig. 1) and showed the highest levels of 16S rRNA gene sequence similarity to these strains (97.5 and 96.3 %, respectively); levels of similarity to the type strains of other recognized species of the genus *Paracoccus* were less than 96.3 %. The topologies of phylogenetic trees built with the maximum-likelihood and maximum-parsimony algorithms were similar to that of the neighbour-joining tree (data not shown).

Based on DNA–DNA hybridizations, strain YIM 90738^T showed a level of DNA–DNA relatedness of $51.5 \pm 4\%$ (mean $\pm$ SD of three determinations) to *P. homiensis* DSM 17862^T. This, combined with levels of 16S rRNA gene sequence similarity, supports the genomic distinction of strain YIM 90738^T from recognized species of the genus *Paracoccus* (Stackebrandt & Goebel, 1994; Wayne *et al.*, 1987). Strain YIM 90738^T could also be differentiated from recognized *Paracoccus* species based on differences in several phenotypic characteristics. Therefore, on the basis of the data presented, strain YIM 90738^T is considered to represent a novel species of the genus *Paracoccus*, for which we propose the name *Paracoccus saliphilus* sp. nov.

Description of *Paracoccus saliphilus* sp. nov.

Paracoccus saliphilus (sa.li.phi'lus. L. n. *sal*, *salis* salt; Gr. adj. *philos* loving; N.L. masc. adj. *saliphilus* salt-loving).

Cells are aerobic, Gram-negative, non-spore-forming, non-motile, coccoid to rod-shaped, $1.6\text{--}2.0 \times 2.5\text{--}5.5$ μm . Colonies are circular, smooth, flat and colourless to yellow-brown. Moderately halophilic; grows over a wide range (1–15 %, w/v) of salt concentrations, with optimal growth at 8 % (w/v) NaCl. No growth in the absence of NaCl. Growth occurs at 10–55 °C and at pH 6.0–8.0; optimal growth is at 37 °C and pH 7.0. Positive for oxidase, catalase and the Voges–Proskauer test. Indole and H₂S are not produced. Nitrate is not reduced. Negative for arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase and methyl red. Gelatin, urea and aesculin are hydrolysed, but casein, starch, CM-cellulose, pectin, tyrosine and Tweens 20, 40 and 80 are not. Enzyme activities (tested in the API ZYM system) are observed for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, acid phosphatase, naphthol-AS-BI-phospho-

Table 2. Comparison of the fatty acid profiles of strain YIM 90738^T and its nearest phylogenetic neighbours within the genus *Paracoccus*

Strains: 1, YIM 90738^T (grown on TSA at 37 °C, pH 7.3 and 8 % NaCl; data from the present study); 2, *P. homiensis* DSM 17862^T (marine agar, 28 °C, pH 7.0; Kim *et al.*, 2006); 3, *P. zeaxanthinifaciens* ATCC 21588^T (TSA, 28 °C, pH 7.3; Kim *et al.*, 2006); 4, *P. carotinifaciens* IFO 16121^T (TSA, 28 °C, pH 7.3; Kim *et al.*, 2006); 5, *P. haeundaensis* KCCM 10460^T (TSA, 25 °C, pH 8.0; Lee *et al.*, 2004); 6, *P. marcusii* DSM 11574^T (TSA, 28 °C, pH 7.3; Kim *et al.*, 2006); 7, *P. seriniphilus* DSM 14827^T (TSA, 30 °C, pH 7.3; Pukall *et al.*, 2003). Values are percentages of total fatty acids. —, Not detected or <1 %; NA, not applicable (listed as a component of a summed feature).

Fatty acid	1	2	3	4	5	6	7
C _{10:0} 3-OH	3.3	2.6	4.9	3.4	2.1	6.2	7.9
Unidentified ECL 11.799	—	2.4	3.6	2.8	—	4.9	—
Unidentified ECL 15.275	—	—	1.6	1.1	—	2.9	—
C _{12:1} ω7c	—	—	—	—	2.0	—	—
C _{12:0} ALDE	—	1.8	—	—	—	—	—
C _{14:0} 3-OH	—	—	NA	NA	1.5	NA	—
C _{18:1} ω7c	80.4	78.9	80.2	84.0	84.3	80.3	83.5
C _{18:0}	6.1	4.5	3.6	5.2	7.8	2.6	2.5
C _{18:0} 3-OH	—	1.1	—	—	—	—	—
C _{19:0} cyclo ω8c	2.6	—	—	—	—	—	—
11-Methyl C _{18:1} ω7c	—	5.8	—	—	—	—	1.5
Summed feature 2*	—	—	2.9	2.1	—	3.0	—

*Summed feature 2 comprised C_{14:0} 3-OH and/or C_{16:1} iso I.

hydrolase, β-galactosidase, α-glucosidase, β-glucosidase and N-acetyl-β-glucosaminidase; no activity is observed for lipase (C14), α-chymotrypsin, α-galactosidase, β-glucuronidase, α-mannosidase or α-fucosidase. The following substrates are utilized as sole carbon or nitrogen and energy sources with the Biolog GN2 system: α-cyclodextrin, dextrin, glycogen, N-acetyl-D-galactosamine, adonitol, L-arabinose, D-arabitol, L-fucose, D-fructose, D-galactose, gentiobiose, α-D-glucose, α-D-lactose, D-mannose, melibiose, D-psicose, D-sorbitol, sucrose, turanose, xylitol, methyl pyruvate, monomethyl succinate, acetic acid, cis-aconitic acid, citric acid, formic acid, D-galacturonic acid, D-gluconic acid, D-glucosaminic acid, D-glucuronic acid, α-hydroxybutyric acid, β-hydroxyphenylacetic acid, γ-hydroxybutyric acid, α-ketobutyric acid, α-ketoglutaric acid, DL-lactic acid, quinic acid, D-saccharic acid, succinic acid, bromosuccinic acid, succinamic acid, glucuronamide, L-alaninamide, DL-alanine, L-alanyl glycine, L-asparagine, L-aspartic acid, L-glutamic acid, glycyl L-aspartic acid, glycyl L-glutamic acid, L-histidine, hydroxy-L-proline, L-leucine, L-ornithine, L-proline, DL-serine, L-threonine, DL-carnitine, γ-aminobutyric acid, urocanic acid, inosine, uridine, thymidine, phenylethylamine, putrescine, 2-aminoethanol, 2,3-butanediol, glycerol, DL-α-glycerol phosphate, glucose 1-phosphate and glucose 6-phosphate. The following substrates are not utilized: Tweens 40 and 80, N-acetyl-D-glucosamine, cellobiose, i-erythritol, myo-inositol, lactose, lactulose, maltose, D-mannitol, methyl β-D-glucoside, raffinose, L-rhamnose, trehalose, D-galactonic acid lactone, p-hydroxybutyric acid, α-ketovaleric acid, malonic acid, propionic acid, sebacic acid, L-phenylalanine, L-pyroglyutamic acid and phenylethylamine. Ubiquinone Q-10 is the predominant isoprenoid quinone. The major fatty acid is C_{18:1} ω7c. The G+C content of the type strain is 60.3 mol%.

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the position of strain YIM 90738^T among species of the genus *Paracoccus*. Numbers at branch points are bootstrap values (percentages based on 1000 resamplings; only values above 50 % are shown). The sequence of *Rhodobacter capsulatus* ATCC 11166^T was used as the outgroup. Bar, 0.5 % sequence divergence.

The type strain, YIM 90738^T (=CCTCC AB 206074^T =KCTC 22163^T), was isolated from a salt lake in Xinjiang Province, north-west China.

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References

- Berry, A., Janssens, D., Hübelin, M., Jore, J. P. M., Hoste, B., Cleenwerck, I., Vancanneyt, M., Bretzek, W., Mayer, A. F. & other authors (2003). *Paracoccus zeaxanthinifaciens* sp. nov., a zeaxanthin-producing bacterium. *Int J Syst Evol Microbiol* **53**, 231–238.
- Cerny, G. (1978). Studies on aminopeptidase for the distinction of Gram-negative from Gram-positive bacteria. *Eur J Appl Microbiol Biotechnol* **5**, 113–122.
- Cowan, S. T. & Steel, K. J. (1965). *Manual for the Identification of Medical Bacteria*. London: Cambridge University Press.
- 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. & Tijtgaat, R. (1970). Evaluation of membrane filter methods for DNA-DNA hybridization. *Antonie van Leeuwenhoek* **36**, 461–474.
- Doronina, N. V., Trotsenko, Y. A., Kuznetsov, B. B. & Tourova, T. P. (2002). Emended description of *Paracoccus kondratievae*. *Int J Syst Evol Microbiol* **52**, 679–682.
- Ezaki, T., Hashimoto, Y. & Yabuuchi, E. (1989). Fluorometric deoxyribonucleic acid-deoxyribonucleic acid hybridization in micro-dilution wells as an alternative to membrane filter hybridization in which radioisotopes are used to determine genetic relatedness among bacterial strains. *Int J Syst Bacteriol* **39**, 224–229.
- 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**, 783–791.
- Fitch, W. M. (1971). Toward defining the course of evolution: minimum change for a specific tree topology. *Syst Zool* **20**, 406–416.
- Harker, M., Hirschberg, J. & Oren, A. (1998). *Paracoccus marcusii* sp. nov., an orange Gram-negative coccus. *Int J Syst Bacteriol* **48**, 543–548.
- Katayama, Y., Hiraishi, A. & Kuraishi, H. (1995). *Paracoccus thiocyanatus* sp. nov., a new species of thiocyanate-utilizing facultative chemolithotroph, and transfer of *Thiobacillus versutus* to the genus *Paracoccus* as *Paracoccus versutus* comb. nov. with emendation of the genus. *Microbiology* **141**, 1469–1477.
- 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., Kwon, S. W., Cho, Y. H., Stackebrandt, E. & Go, S. J. (2006). *Paracoccus homiensis* sp. nov., isolated from a sea-sand sample. *Int J Syst Evol Microbiol* **56**, 2387–2390.
- Kimura, M. (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequence. *J Mol Evol* **16**, 111–120.
- Komagata, K. & Suzuki, K. (1987). Lipid and cell-wall analysis in bacterial systematics. *Methods Microbiol* **19**, 161–207.
- Kumar, S., Tamura, K. & Nei, M. (2004). MEGA3: integrated software for molecular evolutionary genetics analysis and sequence alignment. *Brief Bioinform* **5**, 150–163.
- Kushner, D. J. & Kamekura, M. (1988). Physiology of halophilic eubacteria. In *Halophilic Bacteria*, vol. I, pp. 109–140. Edited by F. Rodríguez-Valera. Boca Raton, FL: CRC Press.
- Lányi, B. (1987). Classical and rapid identification methods for medically important bacteria. *Methods Microbiol* **19**, 1–67.
- Lee, J. H., Kim, Y. S., Choi, T.-J., Lee, W. J. & Kim, Y. T. (2004). *Paracoccus haeundaensis* sp. nov., a Gram-negative, halophilic, astaxanthin-producing bacterium. *Int J Syst Evol Microbiol* **54**, 1699–1702.
- Leifson, E. (1960). *Atlas of Bacterial Flagellation*. London: Academic Press.
- Li, W. J., Xu, P., Schumann, P., Zhang, Y. Q., Pukall, R., Xu, L. H., Stackebrandt, E. & Jiang, C. L. (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.
- Liu, X.-Y., Wang, B.-J., Jiang, C.-Y. & Liu, S.-J. (2006). *Paracoccus sulfuroxidans* sp. nov., a sulfur oxidizer from activated sludge. *Int J Syst Evol Microbiol* **56**, 2693–2695.
- 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.
- Ludwig, W., Mittenhuber, G. & Friedrich, C. G. (1993). Transfer of *Thiosphaera pantotropha* to *Paracoccus denitrificans*. *Int J Syst Bacteriol* **43**, 363–367.
- Mesbah, M., Premachandran, U. & Whitman, W. B. (1989). Precise measurement of the G + C content of deoxyribonucleic acid by high-performance liquid chromatography. *Int J Syst Bacteriol* **39**, 159–167.
- Pukall, R., Laroche, M., Kroppenstedt, R. M., Schumann, P., Stackebrandt, E. & Ulber, R. (2003). *Paracoccus seriniphilus* sp. nov., an L-serine-dehydratase-producing coccus isolated from the marine bryozoan *Bugula plumosa*. *Int J Syst Evol Microbiol* **53**, 443–447.
- 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. *USFCC Newsl* **20**, 16.
- Stackebrandt, E. & Goebel, B. M. (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.
- Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. (1997). The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* **25**, 4876–4882.
- Tsubokura, A., Yoneda, H. & Mizuta, H. (1999). *Paracoccus carotinifaciens* sp. nov., a new aerobic Gram-negative astaxanthin-producing bacterium. *Int J Syst Bacteriol* **49**, 277–282.
- Wayne, L. G., Brenner, D. J., Colwell, R. R., Grimont, P. A. D., Kandler, O., Krichevsky, M. I., Moore, L. H., Moore, W. E. C., Murray, R. G. E. & other authors (1987). International Committee on Systematic Bacteriology. Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int J Syst Bacteriol* **37**, 463–464.
- Xu, P., Li, W.-J., Tang, S.-K., Zhang, Y.-Q., Chen, G.-Z., Chen, H.-H., Xu, L.-H. & Jiang, C.-L. (2005). *Naxibacter alkalitolerans* gen. nov., sp. nov., a novel member of the family ‘Oxalobacteraceae’ isolated from China. *Int J Syst Evol Microbiol* **55**, 1149–1153.