Pseudorhodobacter antarcticus sp. nov., isolated from Antarctic intertidal sandy sediment, and emended description of the genus *Pseudorhodobacter* Uchino *et al.* 2002 emend. Jung *et al.* 2012

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A Gram-negative, aerobic, non-motile, pink-pigmented and rod-shaped strain, designated ZS3-33^T, was isolated from Antarctic intertidal sandy sediment. The strain grew optimally at 15 °C and with 1.0 % (w/v) NaCl. It reduced nitrate to nitrite and hydrolysed Tween 20. It could not produce bacteriochlorophyll *a*. The predominant cellular fatty acid was $C_{18:1}\omega7c$ and the predominant respiratory quinone was Q-10. The major polar lipids were phosphatidylglycerol, phosphatidylcholine, two unidentified aminophospholipids and an unidentified aminolipid. Analyses of 16S rRNA gene sequences revealed that strain ZS3-33^T belonged to the genus *Pseudorhodobacter*, showing 97.4 % similarity to the type strain of *Pseudorhodobacter ferrugineus* and 95.3 % similarity to the type strain of *Pseudorhodobacter aquimaris*. Levels of *gyrB* gene sequence similarity between strain ZS3-33^T and the type strains of *P. ferrugineus* and *P. aquimaris* were 87.6 and 81.7 %, respectively. DNA–DNA relatedness between strain ZS3-33^T and *P. ferrugineus* DSM 5888^T was 56.6 %. The genomic DNA G+C content of strain ZS3-33^T was 57.1 mol%. Based on data from this polyphasic study, strain ZS3-33^T represents a novel species of the genus *Pseudorhodobacter*, for which the name *Pseudorhodobacter antarcticus* sp. nov. is proposed. The type strain is ZS3-33^T (=CGMCC 1.10836^T=KCTC 23700^T). An emended description of the genus *Pseudorhodobacter* Uchino *et al.* 2002 emend. Jung *et al.* 2012 is also proposed.

The genus *Pseudorhodobacter* within the class *Alphaproteobacteria* was originally created by Uchino *et al.* (2002) to reclassify *Agrobacterium ferrugineum* (Rüger & Höfle, 1992). At the time of writing, it contains two species: *Pseudorhodobacter ferrugineus* (type species) and *Pseudorhodobacter aquimaris*, both of which are aerobic, non-photosynthetic bacterial species of marine origin (Uchino *et al.*, 2002; Jung *et al.*, 2012). During study on the diversity of culturable bacteria from Antarctic intertidal sandy sediments, a pink-pigmented bacterial strain, designated ZS3-33^T, was isolated (Yu *et al.*, 2010). 16S rRNA gene sequence analysis indicated that the new isolate was affiliated with the genus *Pseudorhodobacter*. In this paper, the strain is further taxonomically characterized using a polyphasic approach and is proposed to represent a novel species of the genus *Pseudorhodobacter*.

Antarctic intertidal sediment samples were collected from the coastal area off the Chinese Antarctic Zhongshan Station on the Larsemann Hills during the 23rd Chinese National Antarctic Research Expedition in March 2007. Strain ZS3-33^T was isolated from the samples through the dilution-plating technique as described previously (Yu *et al.*, 2010). It was routinely cultivated in TYS broth [0.5 % tryptone (Oxoid), 0.1 % yeast extract (Oxoid) and artificial seawater] or on TYS agar (0.5 % tryptone, 0.1 % yeast extract, 1.5 % agar and artificial seawater) at 15 °C and

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequence and *gyrB* gene sequence of strain ZS3-33^T are FJ196030 and JN255731, respectively.

Five supplementary figures are available with the online version of this paper.

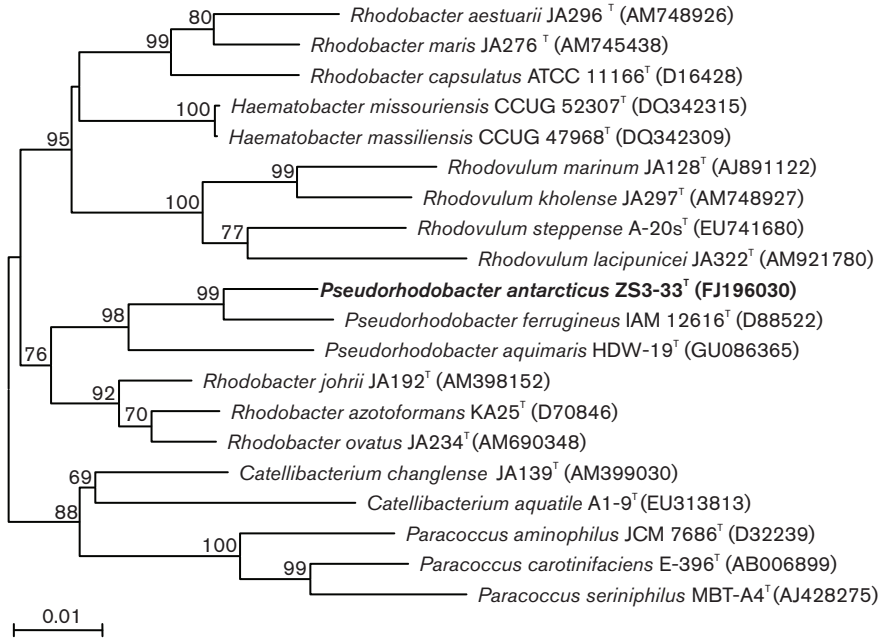

Fig. 1. Neighbour-joining tree based on 16S rRNA gene sequences showing the phylogenetic positions of strain ZS3-33^T (in bold) and the type strains of related species. Percentage bootstrap values above 50 are shown at nodes. Bar, 0.01 substitutions per nucleotide position.

stored at −80 °C in TYS broth supplemented with 30 % glycerol. Artificial seawater was prepared with Sigma sea salts (3 %) and distilled water. *P. ferrugineus* DSM 5888^T, which was obtained from DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany) and used as a reference strain in some tests, was routinely grown on TYS agar or in TYS broth at 15 °C. *P. aquimaris* KCTC 23043^T, which was used as a reference strain in polar lipid analysis, was routinely grown on TYS agar or in TYS broth at 30 °C.

Colonial morphology was observed after 3–7 days of incubation at 15 °C on TYS agar. Cell morphology was examined by transmission electron microscopy (JEM–100CX II). Cells for the test were grown at 15 °C in TYS broth for 5 days and negatively stained with a 1.0 % phosphotungstic acid solution. Growth at various temperatures (4, 10, 15, 20, 25, 30, 35, 37 and 40 °C) was determined in TYS broth. Growth at different pH (4.0–10.5, at 0.5 unit intervals) was measured at 15 °C in TYS broth. The medium used was amended with 50 mM MES, MOPS, Tris or CHES and adjusted with HCl or NaOH to the expected pH. The NaCl concentration range (0–25 %, at 0.5 % intervals) for growth was tested at 15 °C in TYS broth except that artificial seawater was replaced with distilled water supplemented with appropriate amounts of NaCl. Growth under anaerobic conditions was examined in Hungate tubes in TYS broth supplemented with potassium nitrate (0.1 %, w/v), cysteine hydrochloride (0.05 %, w/v) and sodium sulfide (0.05 %, w/v). Resazurin was used as a redox indicator.

The Gram reaction was determined using the KOH (3 %) lysis method (Buck, 1982) and the Hucker staining method (Murray *et al.*, 1994). Catalase activity was detected by observing bubble formation in 3 % hydrogen peroxide solution. Oxidase activity was determined using commercial oxidase strips (Merck). DNA hydrolysis was tested on DNase test agar (Oxoid) prepared with artificial seawater.

Table 1. Fatty acid composition (%) of strain ZS3-33^T and the type strains of recognized *Pseudorhodobacter* species

Strains: 1, ZS3-33^T (data from this study); 2, *P. ferrugineus* DSM 5888^T (this study); 3, *P. aquimaris* HDW-19^T (Jung *et al.*, 2012). −, Not detected.

Fatty acid	1	2	3
$C_{16:0}$	2.9	1.6	1.1
$C_{17:0}$	0.7	0.4	−
$C_{18:0}$	3.8	3.6	3.2
$C_{10:0}$ 3-OH	3.5	4.0	2.8
$C_{18:1}\omega 9c$	3.5	1.8	−
$C_{18:1}\omega 7c$	84.4	82.1	86.4
Summed feature 3*	0.6	1.2	2.8
Summed feature 7†	1.5	0.5	0.2
ECL 11.799‡	−	3.0	2.7

*Summed feature 3 contained $C_{16:1}\omega 7c$ and/or iso-$C_{15:0}$ 2-OH.
†Summed feature 7 contained $C_{19:1}\omega 6c$, $C_{19:0}\omega 10c$ cyclo and/or unknown fatty acid ECL 18.846.
‡ECL, Equivalent chain-length.

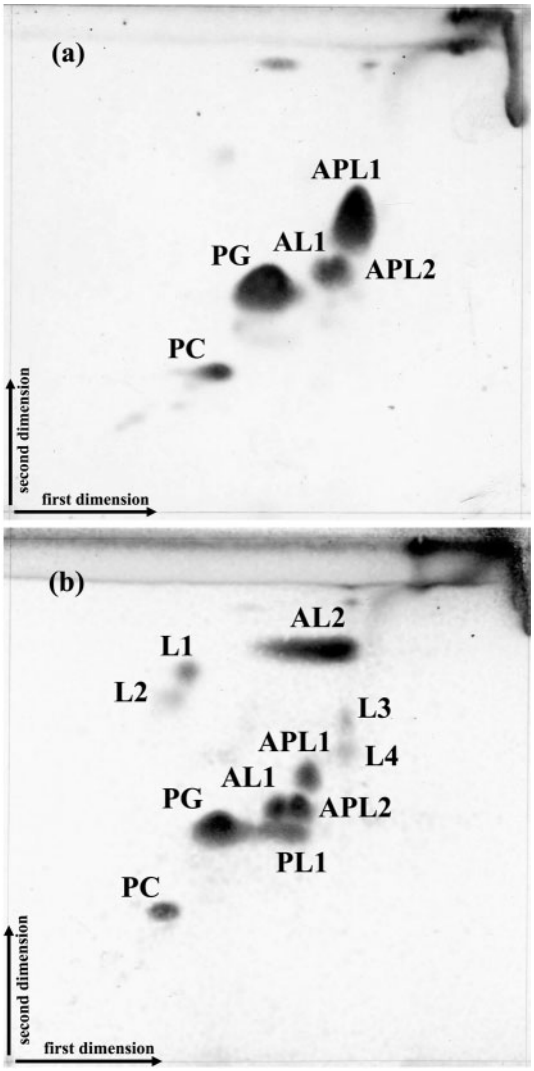

Fig. 2. Two-dimensional thin-layer chromatographs of polar lipids of strain ZS3-33^T (a) and *Pseudorhodobacter aquimaris* KCTC 23043^T (b), which were stained with ethanolic molybdatophosphoric acid. PC, Phosphatidylcholine; PG, phosphatidylglycerol; APL1–2, unidentified aminophospholipids; AL1–2, unidentified aminolipids; PL1, an unidentified phospholipid; L1–4, unidentified lipids.

Hydrolysis of starch, casein and Tweens 20, 40, 60 and 80 was tested on TYS agar supplemented with 1 % (w/v) substrate at 15 °C based on the methods described previously (Smibert & Krieg, 1994). Hydrolysis of tyrosine, xanthine and hypoxanthine (0.5 %, w/v) was tested on TYS agar according to Cowan & Steel (1965). *In vivo* pigment absorption spectrum analysis was conducted as described by Jung *et al.* (2012) using a UV/VIS-550 spectrophotometer (Jasco). Cells for the analysis were cultivated in the dark at 15 °C in TYS broth for 5 days.

Utilization of different substrates (1 %, w/v) as sole carbon and energy sources was determined as described by Shieh *et al.* (2004). Acid production was determined as described by Leifson (1963). API ZYM and API 20NE strips (bioMérieux) were used for further examination of enzyme activities and other biochemical properties following the manufacturer's instructions with the modification that cells for inoculation were suspended in artificial seawater.

For cellular fatty acid analyses, strain ZS3-33^T and the reference strain *P. ferrugineus* DSM 5888^T were cultivated in TYS broth at 15 °C for 5 days. Cellular fatty acid methyl esters were extracted following the instructions of the Sherlock Microbial Identification System. Fatty acid methyl esters were further analysed by GC (Hewlett Packard 6890) and the Sherlock Microbial Identification System software (version 4.5). Quinones were extracted as described previously (Komagata & Suzuki, 1987) and analysed using reversed-phase HPLC. Polar lipids were extracted according to the methods of Komagata & Suzuki (1987) and analysed using two-dimensional TLC followed by spraying with appropriate reagents, including ethanolic molybdophosphoric acid (total lipids), ninhydrin (aminolipids) and Zinzadze reagent (phospholipids).

Genomic DNAs used for PCR amplification were extracted using a bacterial genomic DNA isolation kit (Bioteke). The 16S rRNA genes were amplified by PCR with the primers 27F and 1492R (Lane, 1991) as in Hu & Li (2007). The DNA gyrase B subunit gene (*gyrB*) was amplified with primers UP-1 and UP-2r as in Yamamoto & Harayama (1995). PCR products were purified with an ENZA gel extraction kit (Omega Bio-Tek) and ligated into pMD 18-T vectors (TakaRa) and sequenced at Biosune Inc. (Shanghai, China) using an automated DNA sequencer (Applied Biosystems, model 3730). The obtained sequences were aligned with reference sequences retrieved from the GenBank database using MEGA version 5 (Tamura *et al.*, 2011). Phylogenetic trees were constructed using the neighbour-joining (Saitou & Nei, 1987) and maximum-likelihood (Felsenstein, 1981) methods with the same software. Tree topologies were evaluated by bootstrap analyses (1000 replications) (Felsenstein, 1985). Pairwise sequence similarities were calculated through the EzTaxon server 2.1 (www.eztaxon.org, Chun *et al.*, 2007).

Genomic DNAs were extracted following the method of Marmur (1961). Genomic DNA G+C content was determined by the thermal denaturation temperature (T_m) method (Marmur & Doty, 1962). DNA–DNA hybridization experiments between strain ZS3-33^T and *P. ferrugineus* DSM 5888^T were conducted using the thermal denaturation and renaturation method (De Ley *et al.*, 1970; Huß *et al.*, 1983) with a Beckman DU800 spectrophotometer.

The nearly complete 16S rRNA gene sequence of strain ZS3-33^T (1446 bp) was determined. Strain ZS3-33^T was found to share highest 16S rRNA gene sequence similarity with the type strain of *P. ferrugineus* (97.4 %), followed by the type strain of *P. aquimaris* (95.3 %). Sequence similarities with known species in other genera were all

Table 2. Differential characteristics of strain ZS3-33^T and the type strains of recognized *Pseudorhodobacter* species

Strains: 1, ZS3-33^T (data from this study); 2, *P. ferrugineus* DSM 5888^T (this study); 3, *P. aquimaris* HDW-19^T (Jung *et al.*, 2012). +, Positive; −, negative; w, weakly positive.

Characteristic	1	2	3
Colony colour	Pink	Creamy	Creamy
Cell size (μm)	0.5−0.8 × 1.0−2.5	0.6−1.6 × 1.0−4.0	0.5−1.0 × 1.0−2.0
Motility	−	−	+
Optimum growth at/in:			
Temperature (°C)	15	25	30
NaCl (%, w/v)	1.0	1.0	2.0−3.0
pH	7.5	7.5	7.0−8.0
Growth in the absence of NaCl	+	+	−
Hydrolysis of:			
Tween 20	+	−	−
Casein	−	−	w
Aesculin	w	−	−
Hypoxanthine	−	−	+
Utilization of:			
Acetate	+	−	+
Citrate	−	−	+
Succinate	−	−	+
Acid production from:			
D-Sorbitol	−	−	w
Inositol	−	−	+
D-Xylose	−	+	−
D-Mannitol	−	−	+
Melibiose	−	+	−
D-Mannose	−	−	−
Enzyme activity (API ZYM)			
Alkaline phosphatase	+	+	−
Esterase (C4)	w	w	+
Esterase lipase (C8)	w	−	w
Acid phosphatase	+	w	−
Valine arylamidase	w	−	−
Cysteine arylamidase	w	−	−
Nitrate reduction to nitrite	+	−	−
DNA G+C content (mol%)	57.1	58.0*	60.9

*Data from Uchino *et al.* (2002).

below 95.2 %. In the neighbour-joining phylogenetic tree based on 16S rRNA gene sequences (Fig. 1), strain ZS3-33^T fell within the cluster comprising the genus *Pseudorhodobacter* and formed a sub-branch (99 % bootstrap support) with *P. ferrugineus*. The maximum-likelihood phylogenetic tree based on 16S rRNA gene sequences (Fig. S1) exhibited a similar topology. The phylogenetic trees based on *gyrB* gene sequences also showed that strain ZS3-33^T clustered with known species in the genus *Pseudorhodobacter* (Figs S2 and S3). Levels of *gyrB* gene sequence similarity between strain ZS3-33^T and the type strains of *P. ferrugineus* and *P. aquimaris* were 87.6 and 81.7 %, respectively. DNA–DNA relatedness between strain ZS3-33^T and the type strain of *P. ferrugineus* was 56.6 %, less than the threshold value of 70 % recommended to discriminate species and suggesting that they represent two separate species in the genus *Pseudorhodobacter* (Wayne *et al.*, 1987; Stackebrandt & Goebel, 1994).

The DNA G+C content of strain ZS3-33^T was 57.1 mol%, close to the values reported for the type strains of the two recognized *Pseudorhodobacter* species (58 mol% for *P. ferrugineus* and 60.9 mol% for *P. aquimaris*). The fatty acid profile of strain ZS3-33^T showed a predominance of $C_{18:1}\omega7c$ (84.4 %) with $C_{16:0}$ (2.9 %), $C_{18:0}$ (3.8 %), $C_{10:0}$ 3-OH (3.5 %) and $C_{18:1}\omega9c$ (3.5 %) as minor components (>2 %), which resembled those of the type strains of the two recognized *Pseudorhodobacter* species (Table 1). Strain ZS3-33^T contained Q-10 as the predominant respiratory quinone, in accordance with known *Pseudorhodobacter* species. The major polar lipids of strain ZS3-33^T were phosphatidylcholine (PC), phosphatidylglycerol (PG), two

unidentified aminophospholipids (APL1 and APL2) and an unidentified aminolipid (AL1) (Fig. 2), basically similar to those of *P. aquimaris* KCTC 23043^T. However, *P. aquimaris* KCTC 23043^T contained an unidentified aminolipid (AL2) and an unidentified phospholipid (PL1) which were not detected in strain ZS3-33^T (Fig. 2).

Strain ZS3-33^T was a Gram-negative, non-flagellated and rod-shaped bacterium (Fig. S4). It formed pink-coloured colonies on TYS agar, whereas the type strains of the two recognized *Pseudorhodobacter* species formed cream to brown-coloured colonies on the same agar. The sonicated cell extracts showed no absorption maxima above 600 nm (Fig. S5), indicating that the strain was not able to produce bacteriochlorophyll *a*. Other physiological and biochemical characteristics of strain ZS3-33^T are given in the species description.

Strain ZS3-33^T could be differentiated from other *Pseudorhodobacter* species by combinations of various phenotypic characteristics, such as colony colour, optimum growth temperature, nitrate reduction, hydrolysis of substrates, utilization of carbon sources, acid production from carbohydrates and API ZYM enzyme activities, as shown in Table 2. Based on phylogenetic analyses of 16S rRNA gene and *gyrB* gene sequences, chemotaxonomic characteristics and DNA–DNA hybridization as well as phenotypic distinctiveness, strain ZS3-33^T should be placed within the genus *Pseudorhodobacter* and represents a novel species in the genus, for which the name *Pseudorhodobacter antarcticus* sp. nov. is proposed.

Emended description of the genus *Pseudorhodobacter* Uchino *et al.* 2002 emend. Jung *et al.* 2012

The description is as given by Uchino *et al.* (2002) and Jung *et al.* (2012) with the following amendments. Optimal growth temperature is between 15 and 30 °C. Major polar lipids are phosphatidylglycerol, phosphatidylcholine, two unidentified aminophospholipids and an unidentified aminolipid.

Description of *Pseudorhodobacter antarcticus* sp. nov.

Pseudorhodobacter antarcticus (ant.arc′ti.cus. L. masc. adj. *antarcticus* southern, belonging to Antarctica).

Cells are Gram-negative, aerobic, catalase- and oxidase-positive, non-motile rods (0.5–0.8 × 1.0–2.5 μm). Colonies on TYS agar are pink, circular (1.0–1.5 mm in diameter) and convex with a smooth surface. Grows at 4–30 °C (optimum, 15 °C), at pH 5.0–10.0 (optimum, pH 7.5) and with 0–10 % (w/v) NaCl (optimum, 1.0 % NaCl). Nitrate is reduced to nitrite. H_2S is not produced from cysteine. Bacteriochlorophyll *a* is not produced. Hydrolyses Tween 20, but not DNA, Tweens 40, 60 or 80, starch, tyrosine, xanthine or hypoxanthine. Utilizes acetate but not citrate, succinate, L-arabinose, D-xylose, D-fructose, cellobiose, D-galactose, D-glucose, glycerol, D-mannose, L-glutamine, sucrose, maltose, pyruvate, trehalose, L-lactic acid or L-serine as sole carbon and energy sources. In API 20NE tests, cells are positive for reduction of nitrate to nitrite, β-galactosidase and assimilation of D-glucose and weakly positive for aesculin hydrolysis, but negative for indole production, arginine dihydrolase, urease, gelatinase, and assimilation of arabinose, mannose, mannitol, *N*-acetyl-glucosamine, maltose, gluconate, caprate, adipate, malate, citrate and phenylacetate. With the API ZYM strips, activities of alkaline phosphatase, acid phosphatase, leucine arylamidase, esterase (C4) (weakly), esterase lipase (C8) (weakly), valine arylamidase (weakly) and cystine arylamidase (weakly) are detected, but those of lipase (C14), trypsin, α-chymotrypsin, α-galactosidase, naphthol-AS-BI-phosphohydrolase, β-galactosidase, α-glucosidase, β-glucosidase, β-glucuronidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase are not detected. The major polar lipids are phosphatidylcholine, phosphatidylglycerol, two unidentified aminophospholipids and an unidentified aminolipid. The predominant respiratory quinone is Q-10. The predominant fatty acid is $C_{18:1}\omega7c$.

The type strain, ZS3-33^T (=CGMCC 1.10836^T=KCTC 23700^T), was isolated from intertidal sandy sediment collected from the Antarctic. The genomic DNA G+C content of the type strain is 57.1 mol%.

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References

Buck, J. D. (1982). Nonstaining (KOH) method for determination of gram reactions of marine bacteria. *Appl Environ Microbiol* **44**, 992–993.

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.

Cowan, S. T. & Steel, K. J. (1965). *Manual for the Identification of Medical Bacteria*. London: Cambridge University Press.

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

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.

Hu, Z. Y. & Li, Y. (2007). *Pseudidiomarina sediminum* sp. nov., a marine bacterium isolated from coastal sediments of Luoyuan Bay in China. *Int J Syst Evol Microbiol* **57**, 2572–2577.

Huß, V. A. R., Festl, H. & Schleifer, K. H. (1983). Studies on the spectrophotometric determination of DNA hybridization from renaturation rates. *Syst Appl Microbiol* **4**, 184–192.

Jung, Y. T., Oh, K. H., Oh, T. K. & Yoon, J. H. (2012). *Pseudorhodobacter aquimaris* sp. nov., isolated from seawater, and emended description of the genus *Pseudorhodobacter* Uchino *et al.* 2002. *Int J Syst Evol Microbiol* **62**, 100–105.

Komagata, K. & Suzuki, K. (1987). Lipid and cell-wall analysis in bacterial systematics. *Methods Microbiol* **19**, 161–207.

Lane, D. J. (1991). 16S/23S rRNA sequencing. In *Nucleic Acid Techniques in Bacterial Systematics*, pp. 115–175. Edited by E. Stackebrandt & M. Goodfellow. Chichester: Wiley.

Leifson, E. (1963). Determination of carbohydrate metabolism of marine bacteria. *J Bacteriol* **85**, 1183–1184.

Marmur, J. (1961). A procedure for the isolation of deoxyribonucleic acid from micro-organisms. *J Mol Biol* **3**, 208–218.

Marmur, J. & Doty, P. (1962). Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature. *J Mol Biol* **5**, 109–118.

Murray, R. G. E., Doetsch, R. N. & Robinow, C. F. (1994). Determinative and cytological light microscopy. In *Methods for General and Molecular Bacteriology*, pp. 21–41. Edited by P. Gerhardt, R. G. E. Murray, W. A. Wood & N. R. Krieg. Washington, DC: American Society for Microbiology.

Rüger, H. J. & Höfle, M. G. (1992). Marine star-shaped-aggregate-forming bacteria: *Agrobacterium atlanticum* sp. nov.; *Agrobacterium meteori* sp. nov.; *Agrobacterium ferrugineum* sp. nov., nom. rev.; *Agrobacterium gelatinovorum* sp. nov., nom. rev.; and *Agrobacterium stellulatum* sp. nov., nom. rev. *Int J Syst Bacteriol* **42**, 133–143.

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

Shieh, W. Y., Lin, Y. T. & Jean, W. D. (2004). *Pseudovibrio denitrificans* gen. nov., sp. nov., a marine, facultatively anaerobic, fermentative bacterium capable of denitrification. *Int J Syst Evol Microbiol* **54**, 2307–2312.

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.

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.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. & Kumar, S. (2011). MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol Biol Evol* **28**, 2731–2739.

Uchino, Y., Hamada, T. & Yokota, A. (2002). Proposal of *Pseudorhodobacter ferrugineus* gen. nov., comb. nov., for a non-photosynthetic marine bacterium, *Agrobacterium ferrugineum*, related to the genus *Rhodobacter*. *J Gen Appl Microbiol* **48**, 309–319.

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.

Yamamoto, S. & Harayama, S. (1995). PCR amplification and direct sequencing of *gyrB* genes with universal primers and their application to the detection and taxonomic analysis of *Pseudomonas putida* strains. *Appl Environ Microbiol* **61**, 1104–1109.

Yu, Y., Li, H.-R., Zeng, Y.-X. & Chen, B. (2010). Phylogenetic diversity of culturable bacteria from Antarctic sandy intertidal sediments. *Polar Biol* **33**, 869–875.