Pseudoalteromonas lipolytica sp. nov., isolated from the Yangtze River estuary

Xue-Wei Xu,[1,2] Yue-Hong Wu,[1,2] Chun-Sheng Wang,[1,2] Xiao-Hui Gao,[1,2] Xiao-Gu Wang[1,2] and Min Wu[3]

Correspondence
Xue-Wei Xu
xuxw@sio.org.cn

[1]Laboratory of Marine Ecosystem and Biogeochemistry, State Oceanic Administration, Hangzhou 310012, PR China

[2]Second Institute of Oceanography, State Oceanic Administration, Hangzhou 310012, PR China

[3]College of Life Sciences, Zhejiang University, Hangzhou 310058, PR China

A strictly aerobic, Gram-negative, non-pigmented bacterial strain, designated LMEB 39^T, was isolated from a seawater sample collected from the Yangtze River estuary near the East China Sea and was examined physiologically, chemotaxonomically and phylogenetically. The novel isolate was motile by a single polar flagellum and positive for nitrate reduction and decomposition of casein, gelatin, Tween 20 and Tween 80, but negative for indole production. Chemotaxonomic analysis revealed ubiquinone-8 as the predominant respiratory quinone and phosphatidylethanolamine and phosphatidylglycerol as major polar lipids. The major fatty acids were $C_{16:1}\omega7c$/iso-$C_{15:0}$ 2-OH, $C_{16:0}$, $C_{18:1}\omega7c$, $C_{12:0}$ 3-OH, $C_{17:1}\omega8c$ and $C_{17:0}$. The genomic DNA G+C content was 42.3 mol%. Comparative 16S rRNA gene sequence analysis revealed that the isolate belongs to the genus *Pseudoalteromonas*. Strain LMEB 39^T exhibited the closest phylogenetic affinity to *Pseudoalteromonas byunsanensis* JCM 12483^T (97.4 % sequence similarity). The DNA–DNA reassociation values between strain LMEB 39^T and *P. byunsanensis* JCM 12483^T and *Pseudoalteromonas undina* DSM 6065^T (97.2 % sequence similarity) were 31.7 and 30.3 %, respectively. On the basis of phenotypic and genotypic data, strain LMEB 39^T represents a novel species of the genus *Pseudoalteromonas*, for which the name *Pseudoalteromonas lipolytica* sp. nov. is proposed; the type strain is LMEB 39^T (=CGMCC 1.8499^T=JCM 15903^T).

The genus *Pseudoalteromonas* was proposed by Gauthier *et al.* (1995) and represents a clade of marine bacteria grouped by their phylogenetic relationships based on 16S rRNA gene sequences. Members of the genus *Pseudoalteromonas* are Gram-negative, strictly aerobic, rod-shaped cells. They are motile by a single polar flagellum and have a chemoheterotrophic metabolism (Bowman & McMeekin, 2005). At the time of writing, the genus *Pseudoalteromonas* comprises 34 species with validly published names (Gauthier *et al.*, 1995; Ivanova *et al.*, 1996, 1998, 2000, 2001, 2002a, b, c, d, 2004; Bozal *et al.*, 1997; Euzéby, 1997; Bowman, 1998; Holmström *et al.*, 1998; Sawabe *et al.*, 2000; Venkateswaran & Dohmoto, 2000; Egan *et al.*, 2001; Isnansetyo & Kamei, 2003; Romanenko *et al.*, 2003a, b; Lau *et al.*, 2005; Park *et al.*, 2005; Nam *et al.*, 2007; Al Khudary *et al.*, 2008). Some strains produce yellow, red, violet or melanin-like pigments and have the ability to form antibiotic substances (Bowman, 2007). Here, a novel non-pigmented *Pseudoalteromonas* strain isolated from the Yangtze River estuary was studied using a polyphasic approach.

A seawater sample was collected in October 2007 from the Yangtze River estuary (31° 00′ 04″ N 122° 37′ 26″ E) near the East China Sea at a depth of 21 m (temperature 21 °C, salinity 30.5 ‰). The sample was stored in darkness until micro-organisms were isolated in July 2008. Approximately 80 µl seawater was plated on halophilic medium (HM) containing 10 % (w/v) NaCl by using a tenfold dilution-series method. After 3 days aerobic incubation at 28 °C, one non-pigmented colony, designated LMEB 39^T, was picked. The isolate was purified by repeated restreaking; purity was confirmed by the uniformity of cell morphology. Unless otherwise stated, strain LMEB 39^T was cultured routinely on HM medium containing 3 % (w/v) NaCl at 28 °C and maintained as a glycerol suspension (30 % v/v) at −80 °C. The HM medium (pH 7.5) contained

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain LMEB 39^T is FJ404721.

Supplementary tables showing detailed fatty acid profiles of strain LMEB 39^T, *P. byunsanensis* JCM 12483^T and *P. undina* DSM 6065^T and differential characteristics of strain LMEB 39^T and related strains, and a transmission electron micrograph of strain LMEB 39^T, are available with the online version of this paper.

[(l distilled water)$^{-1}$]: NaCl as indicated, 2.0 g KCl, 1.0 g $MgSO_4 . 7H_2O$, 0.36 g $CaCl_2 . 2H_2O$, 0.23 g NaBr, 0.06 g $NaHCO_3$, trace $FeCl_3$, 10.0 g yeast extract (BD), 5.0 g peptone (BD) and 1.0 g glucose (Ventosa *et al.*, 1982).

Optimal conditions for growth were determined using marine broth 2216 (MB) with different NaCl concentrations (0, 0.5, 1.0, 3.0, 5.0, 7.5, 10.0, 12.0, 15.0, 20.0 and 25.0 %, w/v). The pH range for growth was determined by adding MES (pH 5.0–6.0), PIPES (pH 6.5–7.0), Tricine (pH 7.5–8.5) and CAPSO (pH 9.0–10.5) to MB at a concentration of 50 mM. The temperature range for growth was determined by incubating the isolate at 4, 15, 20, 25, 30, 35, 30, 37, 45, 50 and 55 °C. Cell motility and morphology were examined using optical microscopy (BX40; Olympus) and transmission electron microscopy (JEM-1230; JEOL).

Growth under anaerobic conditions was tested in anaerobically prepared MB under a gas phase containing 10 % (v/v) CO_2, 10 % (v/v) H_2 and 80 % (v/v) N_2. Pigments were extracted with acetone/methanol (7 : 2), the extract was cleared by centrifugation and the absorption spectrum (315–800 nm) was determined in a Beckman DU 800 spectrophotometer. Oxidase and catalase activity, H_2S production, nitrate reduction and the ability to hydrolyse aesculin, casein, DNA, Tweens 20 and 80 and tyrosine were determined according to Dong & Cai (2001). Acid production was tested using marine oxidation–fermentation medium supplemented with 0.5 % sugars (Leifson, 1963). Sensitivity to antimicrobial agents was determined on marine agar 2216 (MA; BD) for at least 3 days. Additional enzyme activities and biochemical characteristics were determined using API 20E, API 20NE and API ZYM kits (bioMérieux) at 28 °C. Enzyme activities were tested using the API ZYM kit as recommended by the manufacturer. Strips were inoculated with a heavy bacterial suspension (MacFarland 5 standard) in AUX medium supplemented with 2 % (w/v) sea salts (Sigma) according to Park *et al.* (2005). *Pseudoalteromonas byunsanensis* JCM 12483^T and *Pseudoalteromonas undina* DSM 6065^T were used as controls in the tests.

Genomic DNA was obtained by using the method of Marmur (1961). The DNA G+C content was determined by thermal denaturation (T_m) (Marmur & Doty, 1962) using *Escherichia coli* K-12 DNA as calibration standard. Cellular fatty acid methyl esters obtained from cells grown in MA for 48 h at 30 °C were analysed by using GC-MS (Kuykendall *et al.*, 1988) according to the instructions of the Microbial Identification System (MIDI). Isoprenoid quinones were extracted from freeze-dried cells (200 mg) with chloroform/methanol (2 : 1) and analysed by reversed-phase HPLC. Total lipids were extracted by the modified method of Kamekura & Kates (1988). Phospholipids and glycolipids were separated on silica gel plates (10 × 10 cm) by TLC and analysed according to Xu *et al.* (2007a).

The 16S rRNA gene was amplified and analysed as described previously (Xu *et al.*, 2007b). PCR products were cloned into vector pMD 19-T (TaKaRa) and then sequenced to determine the almost-complete 16S rRNA gene sequence. Sequence data were aligned with CLUSTAL W 1.8 (Thompson *et al.*, 1994). The sequence was compared with closely related sequences of reference organisms from the EzTaxon service (Chun *et al.*, 2007). Phylogenetic trees were constructed by the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Fitch, 1971) methods with the MEGA4 program package (Tamura *et al.*, 2007) and by the maximum-likelihood method (Felsenstein, 1981) with the program TreePuzzle 5.2. Evolutionary distances were calculated

Table 1. Phenotypic characteristics differentiating strain LMEB 39^T from related *Pseudoalteromonas* species

Strains: 1, LMEB 39^T; 2, *P. byunsanensis* JCM 12483^T; 3, *P. undina* DSM 6065^T. +, Positive; −, negative; W, weakly positive; ND, no data available. Data were obtained from this study under identical growth conditions. All strains are positive for catalase and oxidase. All strains are negative for indole production and hydrolysis of aesculin, DNA, gelatin, Tweens 20 and 80 and tyrosine.

Characteristic	1	2	3
Pigmentation	−	+	−
Growth at 10 % NaCl	+	−	ND
Growth on MacConkey agar	+	−	−
Nitrate reduced to nitrite	+	−	+
Voges–Proskauer reaction	+	−	+
Utilization of:			
L-Arabinose	+	−	+
D-Mannose	−	−	+
Maltose	+	−	+
N-Acetylglucosamine	+	−	+
Adipate	W	−	W
Caprate	+	−	+
Malate	+	−	+
Phenylacetate	−	−	W
Acid production from:			
L-Arabinose	+	−	+
D-Fructose	+	−	−
Glucose	−	W	+
D-Mannose	+	−	−
Sucrose	+	−	−
Trehalose	−	+	+
Sensitive to:			
Ampicillin (10 μg)	+	−	−
Carbenicillin (100 μg)	+	−	−
Kanamycin (30 μg)	−	+	−
Nitrofurantoin (300 μg)	−	+	−
Novobiocin (30 μg)	−	+	+
Tetracycline (30 μg)	+	−	−
API ZYM:			
N-Acetyl-β-glucosaminidase	+	−	−
α-Glucosidase	+	−	−
Lipase (C14)	W	+	W
α-Mannosidase	+	−	−

according to the algorithm of the Kimura two-parameter model (Kimura, 1980) for the neighbour-joining method.

Cells of strain LMEB 39^T were Gram-negative, rod-shaped and approximately 0.5–0.8 μm wide and 1.0–2.0 μm long. Cells were motile by means of a polar flagellum (Supplementary Fig. S1, available in IJSEM Online). Detailed phenotypic characteristics of strain LMEB 39^T are given in the species description. A comparison of the phenotypic properties of strain LMEB 39^T, *P. byunsanensis* JCM 12483^T and *P. undina* DSM 6065^T is shown in Table 1.

The almost-complete 16S rRNA gene sequence (1503 nt) of strain LMEB 39^T was obtained. Sequence comparisons to representative bacteria with validly published names indicated that strain LMEB 39^T belongs to the genus *Pseudoalteromonas*, being related most closely to the type strains of *P. byunsanensis* (97.4 % similarity) and *P. undina* (97.2 %); sequence similarities with respect to the type strains of other recognized *Pseudoalteromonas* species were 92.7–97.1 %. Phylogenetic analysis based on the neighbour-joining method showed that strain LMEB 39^T formed a monophyletic clade adjacent to the type strain of *P. byunsanensis* (Fig. 1). Similar topology was found in phylogenetic trees constructed by the maximum-likelihood and maximum-parsimony algorithms using 16S rRNA gene sequences of all currently recognized species of the genus *Pseudoalteromonas*.

The major fatty acids of strain LMEB 39^T were $C_{16:1}\omega7c$/iso-$C_{15:0}$ 2-OH, $C_{16:0}$, $C_{18:1}\omega7c$, $C_{12:0}$ 3-OH, $C_{17:1}\omega8c$ and $C_{17:0}$. This profile differed from those of *P. byunsanensis* JCM 12483^T and *P. undina* DSM 6065^T (Supplementary Table S1, available in IJSEM Online). The presence of iso-branched fatty acids is a distinct characteristic enabling strain LMEB 39^T to be differentiated from *P. byunsanensis* JCM 12483^T. *P. byunsanensis* JCM 12483^T synthesized a violet pigment when grown on MA and showed maximal absorption at 573 nm, whereas strain LMEB 39^T did not (data not shown). Strain LMEB 39^T could also be distinguished from *P. byunsanensis* JCM 12483^T by a number of phenotypic characteristics, such as nitrate reduction, utilization of sugars and organic acids, acid production from sugars, susceptibility to antibiotics and

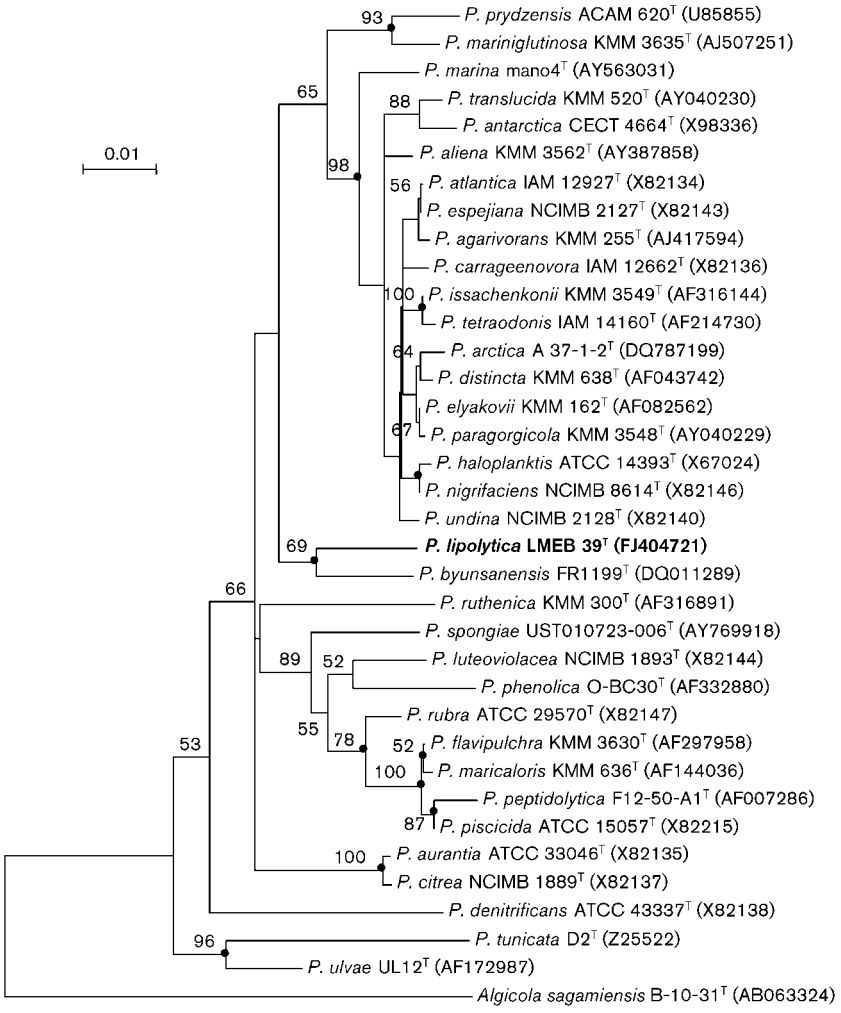

Fig. 1. Neighbour-joining tree based on 16S rRNA gene sequences showing phylogenetic relationships of the novel isolate and related taxa in the genus *Pseudoalteromonas*. Bootstrap values are based on 1000 replicates; only values >50 % are shown. Bar, 0.01 substitutions per nucleotide position. Filled circles indicate branches of the tree that were also formed using the maximum-parsimony and maximum-likelihood methods.

enzyme activities (Table 1; Supplementary Table S2, available in IJSEM Online).

DNA–DNA hybridizations were performed by the thermal-denaturation and renaturation method of De Ley *et al.* (1970), as modified by Huß *et al.* (1983), using a Beckman DU 800 spectrophotometer. The DNA–DNA relatedness values of strain LMEB 39^T with *P. byunsanensis* JCM 12483^T and *P. undina* DSM 6065^T (31.7 and 30.3 %, respectively) were significantly below the value of 70 % that is considered to be the threshold for the delineation of species (Wayne *et al.*, 1987).

On the basis of the phenotypic and phylogenetic data presented in this study, strain LMEB 39^T represents a novel species within the genus *Pseudoalteromonas*, for which the name *Pseudoalteromonas lipolytica* sp. nov. is proposed.

Description of *Pseudoalteromonas lipolytica* sp. nov.

Pseudoalteromonas lipolytica (li.po.ly′ti.ca. Gr. n. *lipos* fat; N.L. fem. adj. *lytica* from Gr. fem. adj. *lutikê* able to dissolve; N.L. fem. adj. *lipolytica* fat-dissolving, referring to the property of being able to hydrolyse lipids).

Gram-negative and motile by means of a polar flagellum. Cells are straight to slightly curved and rod-shaped (0.5–0.8 μm in width and 1.0–2.0 μm in length) with rounded ends. Chemo-organotrophic. Colonies on MA are 1–2 mm in diameter, smooth, circular and non-pigmented after 48 h. Requires Na^+ or seawater for growth. Growth is observed in the presence of 0.5–15.0 % NaCl. Optimal NaCl concentration for growth is 3.0 %. Grows at pH 5.5–9.5 and 15–37 °C (optimum growth at pH 7.0–8.0 and 25 °C). No growth is detected at 4 °C or above 45 °C. Growth occurs on MacConkey agar (red colonies), but not on cetrimide agar. Positive for oxidase and catalase. Nitrate is reduced to nitrite, but not further to N_2O or N_2. Aesculin, casein, DNA, gelatin, Tweens 20 and 80 and tyrosine are hydrolysed. Agar is not hydrolysed. H_2S is produced from L-cysteine or thiosulfate. Glucose fermentation, indole production and ONPG hydrolysis are negative. Voges–Proskauer reaction is positive. Negative for arginine dihydrolase, β-galactosidase, lysine and ornithine decarboxylases, tryptophan deaminase and urease. The following constitutive enzyme activities are detected in API ZYM tests: *N*-acetyl-β-glucosaminidase, acid and alkaline phosphatases, α-chymotrypsin, esterase (C4), esterase lipase (C8), lipase (C14), α-glucosidase, leucine arylamidase, α-mannosidase, naphthol-AS-BI-phosphohydrolase, valine arylamidase, cystine arylamidase and trypsin. The following activities are not observed: α- and β-galactosidases, β-glucuronidase, β-glucosidase and β-fucosidase. The following compounds are utilized as sole carbon and energy sources: adipate, L-arabinose, caprate, glucosamine, D-glucose, malate and maltose. The following compounds are not utilized as sole carbon and energy sources: citrate, gluconate, D-mannitol, D-mannose and phenylacetate. Acid is produced from L-arabinose, D-fructose, maltose, D-mannose, salicin and sucrose, but not from D-galactose, glucose, lactose, mannitol, *myo*-inositol, rhamnose, raffinose, D-ribose, L-sorbitol, sorbose, trehalose or D-xylose. Susceptible to (μg unless otherwise stated) ampicillin (10), carbenicillin (100), chloramphenicol (30), erythromycin (15), gentamicin (10), neomycin (30), polymyxin B (300 IU), rifamycin (5), tetracycline (30) and tobramycin (10), but not to bacitracin (0.04 IU), cefotaxime (30), kanamycin (30), nitrofurantoin (300), novobiocin (30) or nystatin (100). Principal fatty acids (>5 %) are $C_{16:1}\omega7c$/iso-$C_{15:0}$ 2-OH, $C_{16:0}$, $C_{18:1}\omega7c$, $C_{12:0}$ 3-OH, $C_{17:1}\omega8c$ and $C_{17:0}$. Ubiquinone-8 is the major respiratory quinone. The polar lipid profiles include phosphatidylethanolamine and phosphatidylglycerol as the major compounds and minor to trace amounts of diphosphatidylglycerol, two unidentified phospholipids and two unidentified glycolipids.

The type strain is LMEB 39^T (=CGMCC 1.8499^T=JCM 15903^T), isolated from the Yangtze River estuary near the East China Sea. The DNA G+C content of the type strain is 42.3 mol% (T_m).

Acknowledgements

We thank Jean Euzéby for his help with the specific etymology and nomenclature and Aharon Oren for critical reading of the manuscript. This work was supported by grants from the Ministry of Science and Technology of China (973 Program, 2004CB719604-3; 863 Program, 2007AA021305), the National Natural Science Foundation of China (40806066) and the Chinese Offshore Investigation and Assessment (908-ZC-I-02).

References

Al Khudary, R., Stößer, N. I., Qoura, F. & Antranikian, G. (2008). *Pseudoalteromonas arctica* sp. nov., an aerobic, psychrotolerant, marine bacterium isolated from Spitzbergen. *Int J Syst Evol Microbiol* **58**, 2018–2024.

Bowman, J. P. (1998). *Pseudoalteromonas prydzensis* sp. nov., a psychrotrophic, halotolerant bacterium from Antarctic sea ice. *Int J Syst Bacteriol* **48**, 1037–1041.

Bowman, J. P. (2007). Bioactive compound synthetic capacity and ecological significance of marine bacterial genus *Pseudoalteromonas*. *Mar Drugs* **5**, 220–241.

Bowman, J. P. & McMeekin, T. A. (2005). Genus XI. *Pseudoalteromonas* Gauthier, Gauthier and Christen 1995a, 759^{VP}. In *Bergey's Manual of Systematic Bacteriology*, 2nd edn, vol. 2B, pp. 467–478. Edited by D. J. Brenner, N. R. Krieg, J. T. Staley & G. M. Garrity. New York: Springer.

Bozal, N., Tudela, E., Rosselló-Mora, R., Lalucat, J. & Guinea, J. (1997). *Pseudoalteromonas antarctica* sp. nov., isolated from an Antarctic coastal environment. *Int J Syst Bacteriol* **47**, 345–351.

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.

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

Dong, X.-Z. & Cai, M.-Y. (2001). Determination of biochemical properties. In *Manual for the Systematic Identification of General Bacteria*, pp. 370–398. Edited by X.-Z. Dong & M.-Y. Cai. Beijing: Science Press (in Chinese).

Egan, S., Holmström, C. & Kjelleberg, S. (2001). *Pseudoalteromonas ulvae* sp. nov., a bacterium with antifouling activities isolated from the surface of a marine alga. *Int J Syst Evol Microbiol* **51**, 1499–1504.

Euzéby, J. P. (1997). List of Bacterial Names with Standing in Nomenclature: a folder available on the Internet. *Int J Syst Bacteriol* **47**, 590–592. http://www.bacterio.cict.fr

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

Fitch, W. M. (1971). Toward defining the course of evolution: minimum change for a specific tree topology. *Syst Zool* **20**, 406–416.

Gauthier, G., Gauthier, M. & Christen, R. (1995). Phylogenetic analysis of the genera *Alteromonas*, *Shewanella*, and *Moritella* using genes coding for small-subunit rRNA sequences and division of the genus *Alteromonas* into two genera, *Alteromonas* (emended) and *Pseudoalteromonas* gen. nov., and proposal of twelve new species combinations. *Int J Syst Bacteriol* **45**, 755–761.

Holmström, C., James, S., Neilan, B. A., White, D. C. & Kjelleberg, S. (1998). *Pseudoalteromonas tunicata* sp. nov., a bacterium that produces antifouling agents. *Int J Syst Bacteriol* **48**, 1205–1212.

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.

Isnansetyo, A. & Kamei, Y. (2003). *Pseudoalteromonas phenolica* sp. nov., a novel marine bacterium that produces phenolic anti-methicillin-resistant *Staphylococcus aureus* substances. *Int J Syst Evol Microbiol* **53**, 583–588.

Ivanova, E. P., Kiprianova, E. A., Mikhailov, V. V., Levanova, G. F., Garagulya, A. D., Gorshkova, N. M., Yumoto, N. & Yoshikawa, S. (1996). Characterization and identification of marine *Alteromonas nigrifaciens* strains and emendation of the description. *Int J Syst Bacteriol* **46**, 223–228.

Ivanova, E. P., Kiprianova, E. A., Mikhailov, V. V., Levanova, G. F., Garagulya, A. D., Gorshkova, N. M., Vysotskii, M. V., Nicolau, D. V., Yumoto, N. & other authors (1998). Phenotypic diversity of *Pseudoalteromonas citrea* from different marine habitats and emendation of the description. *Int J Syst Bacteriol* **48**, 247–256.

Ivanova, E. P., Chun, J., Romanenko, L. A., Matte, M. E., Mikhailov, V. V., Frolova, G. M., Huq, A. & Colwell, R. R. (2000). Reclassification of *Alteromonas distincta* Romanenko *et al.* 1995 as *Pseudoalteromonas distincta* comb. nov. *Int J Syst Evol Microbiol* **50**, 141–144.

Ivanova, E. P., Romanenko, L. A., Matté, M. H., Matté, G. R., Lysenko, A. M., Simidu, U., Kita-Tsukamoto, K., Sawabe, T., Vysotskii, M. V. & other authors (2001). Retrieval of the species *Alteromonas tetraodonis* Simidu et al. 1990 as *Pseudoalteromonas tetraodonis* comb. nov. and emendation of description. *Int J Syst Evol Microbiol* **51**, 1071–1078.

Ivanova, E. P., Sawabe, T., Alexeeva, Y. V., Lysenko, A. M., Gorshkova, N. M., Hayashi, K., Zukova, N. V., Christen, R. & Mikhailov, V. V. (2002a). *Pseudoalteromonas issachenkonii* sp. nov., a bacterium that degrades the thallus of the brown alga *Fucus evanescens*. *Int J Syst Evol Microbiol* **52**, 229–234.

Ivanova, E. P., Sawabe, T., Lysenko, A. M., Gorshkova, N. M., Svetashev, V. I., Nicolau, D. V., Yumoto, N., Taguchi, T., Yoshikawa, S. & other authors (2002b). *Pseudoalteromonas ruthenica* sp. nov., isolated from marine invertebrates. *Int J Syst Evol Microbiol* **52**, 235–240.

Ivanova, E. P., Sawabe, T., Lysenko, A. M., Gorshkova, N. M., Hayashi, K., Zhukova, N. V., Nicolau, D. V., Christen, R. & Mikhailov, V. V. (2002c). *Pseudoalteromonas translucida* sp. nov. and *Pseudoalteromonas paragorgicola* sp. nov., and emended description of the genus. *Int J Syst Evol Microbiol* **52**, 1759–1766.

Ivanova, E. P., Shevchenko, L. S., Sawabe, T., Lysenko, A. M., Svetashev, V. I., Gorshkova, N. M., Satomi, M., Christen, R. & Mikhailov, V. V. (2002d). *Pseudoalteromonas maricaloris* sp. nov., isolated from an Australian sponge, and reclassification of [*Pseudoalteromonas aurantia*] NCIMB 2033 as *Pseudoalteromonas flavipulchra* sp. nov. *Int J Syst Evol Microbiol* **52**, 263–271.

Ivanova, E. P., Gorshkova, N. M., Zhukova, N. V., Lysenko, A. M., Zelepuga, E. A., Prokof'eva, N. G., Mikhailov, V. V., Nicolau, D. V. & Christen, R. (2004). Characterization of *Pseudoalteromonas distincta*-like sea-water isolates and description of *Pseudoalteromonas aliena* sp. nov. *Int J Syst Evol Microbiol* **54**, 1431–1437.

Kamekura, M. & Kates, M. (1988). Lipids of halophilic archaebacteria. In *Halophilic Bacteria II*, pp. 25–54. Edited by F. Rodriguez-Valera. Boca Raton, FL: CRC Press.

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.

Kuykendall, L. D., Roy, M. A., O'Neill, J. J. & Devine, T. E. (1988). Fatty acids, antibiotic resistance, and deoxyribonucleic acid homology groups of *Bradyrhizobium japonicum*. *Int J Syst Bacteriol* **38**, 358–361.

Lau, S. C., Tsoi, M. M., Li, X., Dobretsov, S., Plakhotnikova, Y., Wong, P. K. & Qian, P. Y. (2005). *Pseudoalteromonas spongiae* sp. nov., a novel member of the *γ-Proteobacteria* isolated from the sponge *Mycale adhaerens* in Hong Kong waters. *Int J Syst Evol Microbiol* **55**, 1593–1596.

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 microorganisms. *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.

Nam, Y.-D., Chang, H.-W., Park, J. R., Kwon, H.-Y., Quan, Z.-X., Park, Y.-H., Lee, J.-S., Yoon, J.-H. & Bae, J.-W. (2007). *Pseudoalteromonas marina* sp. nov., a marine bacterium isolated from tidal flats of the Yellow Sea, and reclassification of *Pseudoalteromonas sagamiensis* as *Algicola sagamiensis* comb. nov. *Int J Syst Evol Microbiol* **57**, 12–18.

Park, Y.-D., Baik, K. S., Yi, H., Bae, K. S. & Chun, J. (2005). *Pseudoalteromonas byunsanensis* sp. nov., isolated from tidal flat sediment in Korea. *Int J Syst Evol Microbiol* **55**, 2519–2523.

Romanenko, L. A., Zhukova, N. V., Lysenko, A. M., Mikhailov, V. V. & Stackebrandt, E. (2003a). Assignment of '*Alteromonas marinoglutinosa*' NCIMB 1770 to *Pseudoalteromonas mariniglutinosa* sp. nov., nom. rev., comb. nov. *Int J Syst Evol Microbiol* **53**, 1105–1109.

Romanenko, L. A., Zhukova, N. V., Rohde, M., Lysenko, A. M., Mikhailov, V. V. & Stackebrandt, E. (2003b). *Pseudoalteromonas agarivorans* sp. nov., a novel marine agarolytic bacterium. *Int J Syst Evol Microbiol* **53**, 125–131.

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

Sawabe, T., Tanaka, R., Iqbal, M. M., Tajima, K., Ezura, Y., Ivanova, E. P. & Christen, R. (2000). Assignment of *Alteromonas elyakovii* KMM 162^T and five strains isolated from spot-wounded fronds of *Laminaria japonica* to *Pseudoalteromonas elyakovii* comb. nov. and the extended description of the species. *Int J Syst Evol Microbiol* **50**, 265–271.

Tamura, K., Dudley, J., Nei, M. & Kumar, S. (2007). MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Mol Biol Evol* **24**, 1596–1599.

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.

Venkateswaran, K. & Dohmoto, N. (2000). *Pseudoalteromonas peptidolytica* sp. nov., a novel marine mussel-thread-degrading bacterium isolated from the Sea of Japan. *Int J Syst Evol Microbiol* **50**, 565–574.

Ventosa, A., Quesada, E., Rodriguez-Valera, F., Ruiz-Berraquero, F. & Ramos-Cormenzana, A. (1982). Numerical taxonomy of moderately halophilic Gram-negative rods. *J Gen Microbiol* **128**, 1959–1968.

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, X.-W., Wu, Y.-H., Wang, C.-S., Oren, A., Zhou, P.-J. & Wu, M. (2007a). *Haloferax larsenii* sp. nov., an extremely halophilic archaeon from a solar saltern. *Int J Syst Evol Microbiol* **57**, 717–720.

Xu, X.-W., Wu, Y.-H., Zhou, Z., Wang, C.-S., Zhou, Y.-G., Zhang, H.-B., Wang, Y. & Wu, M. (2007b). *Halomonas saccharevitans* sp. nov., *Halomonas arcis* sp. nov. and *Halomonas subterranea* sp. nov., halophilic bacteria isolated from hypersaline environments of China. *Int J Syst Evol Microbiol* **57**, 1619–1624.