Pseudomonas marincola sp. nov., isolated from marine environments

Lyudmila A. Romanenko,¹ Masataka Uchino,² Bradley M. Tebo,³
Naoto Tanaka,⁴ Galina M. Frolova¹ and Valery V. Mikhailov¹

Correspondence

Lyudmila A. Romanenko
lro@piboc.dvo.ru

¹Pacific Institute of Bioorganic Chemistry, Far-Eastern Branch, Russian Academy of Sciences, Prospect 100 Let Vladivostoku 159, Vladivostok 690022, Russia

²Laboratory of Food Science and Technology, Department of Applied Biology and Chemistry, Tokyo University of Agriculture, 1-1-1 Sakuragaoka, Setagaya-ku, Tokyo 156-8502, Japan

³Department of Environmental & Biomolecular Systems, OGI School of Science & Engineering, Oregon Health & Science University, 20000 NW Walker Road, Beaverton, OR 97006, USA

⁴NODAI Culture Collection Center, Tokyo University of Agriculture, 1-1-1 Sakuragaoka, Setagaya-ku, Tokyo 156-8502, Japan

An aerobic, Gram-negative, motile, non-pigmented bacterium, strain KMM 3042^T, isolated from a deep-sea brittle star in the Fiji Sea, was subjected to a polyphasic taxonomic study. Phylogenetic analysis based on 16S rRNA gene sequences revealed marine Mn(II)-oxidizing isolate S185-2B as the closest neighbour of strain KMM 3042^T (99.9% sequence similarity). The two strains formed a distinct lineage within the genus *Pseudomonas* adjacent to the members of the *Pseudomonas borbori* cluster, sharing highest sequence similarity of 97.4 and 97.0%, respectively, with *P. borbori* DSM 17834^T and *Pseudomonas flavescens* DSM 12071^T. The DNA–DNA hybridization value (71%) between strains KMM 3042^T and S185-2B confirmed their assignment to the same species. On the basis of phylogenetic analysis, DNA–DNA hybridization and physiological and biochemical characterization, strains KMM 3042^T and S185-2B should be assigned to a novel species of the genus *Pseudomonas*, for which the name *Pseudomonas marincola* sp. nov. is proposed. The type strain is KMM 3042^T (=NRIC 0729^T =JCM 14761^T).

Pseudomonas-like bacteria are considered to be common members of microbial communities in marine environments. Relatively few species belonging to the genus *Pseudomonas* (*sensu stricto*) have been recovered from seawater or marine sediments, including *Pseudomonas stutzeri* (Baumann *et al.*, 1983; Rosselló-Mora *et al.*, 1993), *P. alcaligenes*, *P. pseudocaligenes* (Palleroni, 1984), *P. balearica* (Bennasar *et al.*, 1996) and *P. alcaliphila* (Yumoto *et al.*, 2001). At the same time, little is known about well-defined *Pseudomonas* species associated with deep-sea invertebrates. Recently, we have proposed two novel *Pseudomonas* species, *Pseudomonas pachastrellae*, associated with a deep-sea sponge *Pachastrella* sp. (Romanenko *et al.*, 2005a), and *Pseudomonas xanthomarina*, isolated from an ascidian specimen (*Halocynthia aurantium*). Strain KMM 235, also isolated from an

ascidian (*Didemnum* sp.), was characterized as a novel genomovar of *P. stutzeri* (Romanenko *et al.*, 2005b).

During a survey of the biodiversity of micro-organisms isolated from deep-sea invertebrates, *Pseudomonas*-like strain KMM 3042^T was isolated from an unidentified brittle-star specimen and subjected to taxonomic analysis. Based on 16S rRNA gene sequence analysis, strain KMM 3042^T was found to be closely related to *Pseudomonas* sp. strain S185-2B (99.9% sequence similarity). Strain S185-2B was isolated from a water sample from a seasonally anoxic fjord and characterized by Francis & Tebo (2001) as a Mn(II)-oxidizing bacterium. In comparison with named species, strain KMM 3042^T shared the highest 16S rRNA gene sequence similarity with *Pseudomonas borbori* DSM 17834^T (97.4%) and *Pseudomonas flavescens* DSM 12071^T (97.0%), and showed less than 97% similarity to other recognized *Pseudomonas* species. In the present study, the marine isolates KMM 3042^T and S185-2B were investigated to clarify their taxonomic position. Strains KMM 3042^T and S185-2B appeared to have similar phenotypic characteristics and to be closely related based on phylogenetic and DNA–DNA hybridization experiments, but clearly distinct from other recognized *Pseudomonas* species.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains KMM 3042^T and S185-2B are AB301071 and AF326382, respectively.

Thin-layer chromatograms of polar lipids, a maximum-likelihood tree and detailed fatty acid profiles are available as supplementary material with the online version of this paper.

A brittle star (ophiura) specimen (Ophiuroidea, Echinodermata) was collected from the Fiji Sea at a depth of 480 m in December 1991. The specimen was rinsed carefully with sterile seawater and cut aseptically. A small amount of tissue was crushed using a sterile mortar and pestle and an aliquot of the diluted homogenate was spread on seawater medium (SWM) agar plates, containing 5.0 g peptone, 2.5 g yeast extract, 1.0 g glucose, 0.2 g K_2HPO_4 , 0.05 g $MgSO_4$ and 15.0 g agar in 750 ml seawater/250 ml distilled water. Plates were incubated for 14 days at 28 °C. Each colony was picked and restreaked on agar before being processed further. Strain S185-2B was isolated from a seawater sample collected from a depth of 130 m at the oxic–anoxic interface from Saanish Inlet, British Columbia, Canada, by Francis & Tebo (2001). Strains KMM 3042^T and S185-2B grew aerobically on SWM, tryptic soya agar, (TSA), tryptic soya broth, (TSB), marine agar 2216 (MA) and marine broth 2216 (MB) (all from Difco) at 25–28 °C, and were stored at –80 °C in MB supplemented with 30 % (v/v) glycerol.

The type strains of *P. borbori* (DSM 17834^T), *P. flavescens* (DSM 12071^T) and *Pseudomonas peli* (DSM 17833^T) were kindly provided by the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ; Braunschweig, Germany). Motility was observed by the hanging drop method. Production of pyocyanin and formation of fluorescent pigments were tested on King A and King B medium, respectively (King *et al.*, 1954). The Gram-reaction, oxidase and catalase reactions, production of H_2S and indole and hydrolysis of casein, gelatin, starch, chitin, Tween 80 and Tween 40 were tested according to standard methods described by Smibert & Krieg (1994). Hydrolysis of starch was determined after 2 days incubation on nutrient agar containing 0.2 % (w/v) soluble starch by flooding the plates with a 1 % (w/v) iodine solution. Formation of H_2S from thiosulfate was tested using a lead acetate paper strip. The oxidation/fermentation medium of Leifson (1963) was used for testing acid production from carbohydrates with 1 % (w/v) of each compound. Growth at different temperatures, pH and salinities as well as substrate utilization were studied as described previously (Romanenko *et al.*, 2005a, b). In addition, biochemical tests were carried out using API 20NE, API ID32 GN and API 50 CH test kits (bioMérieux) as described by the manufacturer. API test results were read after 24 and 48 h of incubation at 28 °C.

For polar lipid and fatty acid analyses, strains KMM 3042^T and S185-2B were cultivated on TSA at 28 °C for 3 days and lipids were extracted using the chloroform/methanol extraction method of Bligh & Dyer (1959). Polar lipids were analysed as described by Vaskovsky & Terekhova (1979). Two-dimensional TLC of polar lipids was carried out using chloroform/methanol/benzene/28 % NH_4OH (65:30:10:6, by vol.) for the first direction and chloroform/methanol/benzene/acetone/acetic acid/water (70:30:10:5:4:1, by vol.) for the second direction. Non-specific detection of lipids on the TLC was conducted with

10 % H_2SO_4 in methanol at 180 °C. Amino-group-containing lipids were detected with ninhydrin, phospholipids with molybdate reagent, glycolipids with anthrone spray and choline lipids with Dragendorff's reagent. Fatty acid methyl esters (FAMES) were obtained by alkaline methanolysis (15 % $NaOH$ /methanol). The resultant FAMES were extracted by hexane and analysed using a GLC-MS Hewlett Packard model 6890 gas chromatograph equipped with a HP 5 MS 5 % phenyl methyl siloxane capillary column (30 m × 250 μm × 0.25 μm) and connected to a Hewlett Packard model 5973 mass spectrometer. DNA base composition was determined as described by Marmur & Doty (1962) and Owen *et al.* (1969). The photobiotin-labelled DNA probe microplate method of Ezaki *et al.* (1989) was used to determine DNA–DNA relatedness of strains KMM 3042^T and S185-2B and selected type strains *P. borbori* DSM 17834^T, *P. flavescens* DSM 12071^T and *P. peli* DSM 17833^T.

The 16S rRNA gene sequence of strain KMM 3042^T (1438 nt) was determined as described by Shida *et al.* (1997); the sequence of strain S185-2B was retrieved from GenBank having been deposited by Francis & Tebo (2001). These sequences were compared with 16S rRNA gene sequences retrieved from the EMBL/GenBank/DBJ databases by using the FASTA program (Pearson & Lipman, 1988). Distances were calculated according to the method of Jukes & Cantor (1969). Phylogenetic trees were constructed by the neighbour-joining method of Saitou & Nei (1987) with the CLUSTAL_X program (version 1.8; Thompson *et al.*, 1997) and by the maximum-likelihood method using the BioEdit program (Hall, 1999). Bootstrap analysis was applied with the programs implemented in the PHYLIP package (Felsenstein, 1993) using 1000 resamplings of the dataset.

Phylogenetic analysis based on the 16S rRNA gene sequences revealed 99.9 % sequence similarity between strains KMM 3042^T and S185-2B. Different algorithms placed isolates KMM 3042^T and S185-2B in the genus *Pseudomonas* as a separate lineage adjacent to the cluster of *P. borbori* DSM 17834^T/*P. flavescens* DSM 12071^T (Fig. 1; see also Supplementary Fig. S1, available in IJSEM Online). 16S rRNA gene sequence similarities of less than 97.0 % were obtained for type strains of other *Pseudomonas* species (96.9 % to *Pseudomonas anguilliseptica* DSM 12111^T, 96.8 % to *Pseudomonas straminea* IAM 1587^T, 96.7 % to both *P. peli* DSM 17833^T and *Pseudomonas argentinensis* LMG 22563^T), which clearly demonstrated that the two isolates could be considered to be members of an independent species of the genus *Pseudomonas*.

The DNA–DNA relatedness between strains KMM 3042^T and S185-2B was 71 %. DNA–DNA relatedness values between KMM 3042^T and S185-2B and three type strains of phylogenetically related *Pseudomonas* species were 7–21 % (Table 1). These values confirmed that strains KMM 3042^T and S185-2B belong to the same species and represent a novel *Pseudomonas* species according to the value of 70 %

Fig. 1. Neighbour-joining tree based on 16S rRNA gene sequences showing relationships of isolates KMM 3042^T, S185-2B and *Pseudomonas* species. Numbers indicate bootstrap values greater than 700 from 1000 resamplings. Bar, 0.01 substitutions per nucleotide position. A sequence from an unidentified strain of *Escherichia coli* was used as the outgroup.

proposed by Wayne *et al.* (1987) for the purposes of species discrimination. The fatty acid composition of strains studied was found to be similar and contained major fatty acids C_{16:0}, C_{16:1} and C_{18:1}. A more detailed fatty acid composition is given in the species description and in Supplementary Table S1. These results agree closely with data reported for related and more distant *Pseudomonas* species (Uchino *et al.*, 2001; Peix *et al.*, 2005; Romanenko *et al.*, 2005a, b; Vanparys *et al.*, 2006). In comparison with related *Pseudomonas* species, strains KMM 3042^T and S185-2B contained somewhat larger amounts of C_{17:1} (3.7 and 7.6%, respectively) and small amounts of C_{19:1} (1.3 and 2.5%) (Supplementary Table S1). The polar lipid compositions of strains KMM 3042^T and S185-2B were similar in their major components, including phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol and aminophospholipid (Supplementary Fig. S2). A small amount of aminolipid was detected in the phospholipid composition of strain KMM 3042^T only. These data

Table 1. DNA–DNA relatedness (%) of strains KMM 3042^T and S185-2B and type strains of related *Pseudomonas* species

Strain	KMM 3042 ^T	S185-2B
KMM 3042 ^T	(100)	71
<i>P. peli</i> DSM 17833 ^T	21	16
<i>P. borbori</i> DSM 17834 ^T	19	13
<i>P. fluorescens</i> DSM 12071 ^T	ND	7

ND, Not done.

are in agreement with those published previously for *Pseudomonas* species (Wilkinson, 1988; Romanenko *et al.*, 2005a).

The phylogenetic distinction of strains KMM 3042^T and S185-2B was supported by their phenotypic characteristics. Physiological, biochemical and chemotaxonomic characteristics of strains KMM 3042^T and S185-2B are given in Table 2, Supplementary Table S1 and Supplementary Fig. S2 and in the species description. Strains KMM 3042^T and S185-2B mostly had similar phenotypic characteristics, but differed in hydrolysis of Tweens 80 and 40 and assimilation of phenylacetate. The differential phenotypic characteristics of the two strains and phylogenetically related *Pseudomonas* species are displayed in Table 2. The two isolates could be distinguished from their close relatives by the presence of arginine dihydrolase (except *P. anguilliseptica* strains, which have variable reaction for arginine dihydrolase activity) and in carbon assimilation patterns. Strains KMM 3042^T and S185-2B were most consistent with *P. peli* and *P. anguilliseptica* in their lack of carbohydrate assimilation, but differed from both of these species in being able to reduce nitrate to nitrite. They were distinct from *P. peli* by being able to assimilate caprate and citrate and from *P. anguilliseptica* in not being able to assimilate D-galactose. Based on the results obtained, it is proposed that strains KMM 3042^T and S185-2B represent a novel species in the genus *Pseudomonas*, with the name *Pseudomonas marincola* sp. nov.

Description of *Pseudomonas marincola* sp. nov.

Pseudomonas marincola (ma.rin'co.la. L. n. *mare* the sea; L. n. *incola* inhabitant; N.L. n. *marincola* inhabitant of the sea).

Aerobic, Gram-negative, non-pigmented, motile, rod-shaped cells, 1.8–2.5 µm long and 0.4–0.6 µm in diameter. Oxidase- and catalase-positive. Colonies are smooth, non-pigmented, whitish and translucent, 3–4 mm in diameter. No production of pyocyanin on King A medium or fluorescent pigments on King B medium. Strains produce brown diffusible pigments on peptone-enriched media. Growth is observed in 0–8% (w/v) NaCl and at 5–37 °C (optimum at 25–28 °C); weak growth occurs at 4 °C and no growth is observed above 38 °C. The pH range is 5.5–9.5 (optimum pH 6.5–8.5). Produces H₂S slowly. Acid is not produced from D-glucose, arabinose, mannose, rhamnose, galactose, maltose, fructose, lactose, D-xylose, mannitol or glycerol. Hydrolysis of Tweens 80 and 40 and assimilation of phenylacetate are strain-dependent; reactions of the type strain are positive. Gelatin, casein, starch, aesculin, DNA and chitin are not hydrolysed. Other phenotypic and biochemical tests are listed in Table 2. In addition, in API 20 NE test strips, the ONPG test is negative. According to ID32 GN, assimilation of itaconic acid, sodium malonate, sodium acetate, lactic acid, propionic acid, capric acid, trisodium citrate, L-alanine, L-proline, 3-hydroxybutyric acid and 4-hydroxybenzoic

Table 2. Differential phenotypic characteristics of strains KMM 3042^T and S185-2B and related *Pseudomonas* species

Strains/species: 1, KMM 3042^T; 2, S185-2B (data in columns 1 and 2 from this study); 3, *P. borbori*; 4, *P. peli* (data in columns 3 and 4 from Vanparrys *et al.*, 2006); 5, *P. flavescens* (Hildebrand *et al.*, 1994; Uchino *et al.*, 2000); 6, *P. straminea* (Uchino *et al.*, 2000, 2001); 7, *P. argentinensis* (Peix *et al.*, 2005); 8, *P. anguilliseptica* (Wakabayashi & Egusa, 1972; Euzéby, 2005). +, Positive; –, negative; v, variable reaction between strains (reaction of type strain in parentheses). All were positive for oxidase and catalase and negative for indole production, urease, aesculin hydrolysis and the assimilation of *N*-acetylglucosamine, adipate, erythritol, D-arabinose, L-xylose, D-adonitol, methyl β-D-xyloside, L-sorbose, L-rhamnose, dulcitol, inositol, D-sorbitol, methyl α-D-mannoside, methyl α-D-glucoside, amygdalin, arbutin, aesculin, salicin, cellobiose, D-lactose, melibiose, inulin, melezitose, raffinose, starch, glycogen, xylitol, gentiobiose, turanose, D-lyxose, D-tagatose, D-fucose, L-fucose and L-arabitol.

Characteristic	1	2	3	4	5	6	7	8
Production of:								
Yellow–orange water-insoluble pigments	–	–	–	–	+	+	+	–
Fluorescent pigments	–	–	–	–	+	–	+	–
Gelatin hydrolysis	–	–	–	–	–	–	–	v (+)
Arginine dihydrolase	+	+	–	–	–	–	–	v (–)
Nitrate reduction	+	+	+	–	+	+	+	–
Assimilation of:								
D-Glucose	–	–	+	–	+	+	+	–
L-Arabinose	–	–	–	–	–	+	+	–
D-Mannose	–	–	–	–	+	+	+	–
D-Mannitol	–	–	–	–	+	+	+	–
Maltose	–	–	+	–	–	–	–	–
Sucrose	–	–	v (+)	–	+	–	–	–
Trehalose	–	–	–	–	+	–	+	–
D-Galactose	–	–	–	–	+	+	+	+
D-Xylose	–	–	–	–	v (+)	–	v (+)	–
D-Ribose	–	–	–	–	–	v (+)	v (+)	–
Glycerol	–	–	–	–	+	+	v (+)	–
2-Ketogluconate	–	–	–	–	–	–	+	–
5-Ketogluconate	–	–	–	–	+	–	–	–
Gluconate	–	–	+	–	+	+	+	–
Caprate	+	+	v (+)	–	+	+	+	+
Malate	+	+	v (+)	+	–	–	+	+
Citrate	+	+	v (–)	–	+	+	+	+
Phenylacetate	+	–	–	–	–	–	–	–

acid is positive. Does not assimilate suberic acid, potassium 5-ketogluconate, 3-hydroxybenzoic acid, L-arabinose, L-serine, valeric acid, L-histidine or potassium 2-ketogluconate. Strains KMM 3042^T and S185-2B contain C_{16:0} (20.2 and 24.2 %, respectively), C_{16:1} (18.6 and 13.3 %) and C_{18:1} (32.1 and 25.6 %) as major fatty acids, as well as C_{12:0} (13.2 and 9.6 %), C_{10:0} 3-OH (5.7 and 6.4 %), C_{17:1} (3.7 and 7.6 %) and C_{19:1} (1.3 and 2.5 %). C_{14:0}, C_{15:0}, iso-C_{17:1}, C_{17:0}, C_{18:0} are present at ≤1 %. Contains the phospholipids phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol and aminophospholipid. Aminolipid is detected in some strains. The DNA G+C content of the type strain is 57.9 mol% (determined by the thermal denaturation method).

The type strain, KMM 3042^T (=NRIC 0729^T =JCM 14761^T), was isolated from an ophiuran specimen collected from the Fiji Sea at a depth of 480 m.

Acknowledgements

We would like to thank Professor Dr Erko Stackebrandt (DSMZ) for the gift of *Pseudomonas* type strains for DNA–DNA hybridization experiments and Miss Rei Suzuki for excellent technical assistance. This study was supported by a grant from the Russian Foundation for Basic Research (RFBR) and the Far-Eastern Branch of the Russian Academy of Sciences (06-04-96007) and by grant 06-III-A-06 183 from Presidium of the Far-Eastern Branch of the Russian Academy of Sciences.

References

- Baumann, P., Bowditch, R. D., Baumann, L. & Beaman, B. (1983). Taxonomy of marine *Pseudomonas* species: *P. stanieri* sp. nov.; *P. perfectamarina* sp. nov., nom. rev.; *P. nautica*; and *P. doudoroffii*. *Int J Syst Bacteriol* **33**, 857–865.
- Bennasar, A., Rosselló-Mora, R., Lalucat, J. & Moore, E. R. B. (1996). 16S rRNA gene sequence analysis relative to genomovars of

- Pseudomonas stutzeri* and proposal of *Pseudomonas balearica* sp. nov. *Int J Syst Bacteriol* **46**, 200–205.
- Bligh, E. G. & Dyer, W. J. (1959). A rapid method of total lipid extraction and purification. *Can J Biochem Physiol* **37**, 911–917.
- Euzéby, J. P. (2005). *Pseudomonas aguilliseptica*. In *Dictionnaire de Bactériologie Vétérinaire*. <http://www.bacterio.cict.fr/bacdico/pp/anguilliseptica.html> (in French).
- 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. (1993). PHYLIP (phylogeny inference package) version 3.5.1. Distributed by the author. Department of Genome Sciences, University of Washington, Seattle, USA.
- Francis, C. A. & Tebo, B. M. (2001). *cumA* multicopper oxidase genes from diverse Mn(II)-oxidizing and non-Mn(II)-oxidizing *Pseudomonas* strains. *Appl Environ Microbiol* **67**, 4272–4278.
- Hall, T. A. (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp Ser* **41**, 95–98.
- Hildebrand, D. C., Palleroni, N. J., Hendson, M., Toth, J. & Johnson, J. L. (1994). *Pseudomonas flavescentis* sp. nov., isolated from walnut blight cankers. *Int J Syst Bacteriol* **44**, 410–415.
- Jukes, T. H. & Cantor, C. R. (1969). Evolution of protein molecules. In *Mammalian Protein Metabolism*, vol. 3, pp. 21–132. Edited by H. N. Munro. New York: Academic Press.
- King, E. O., Ward, M. K. & Rainey, D. E. (1954). Two simple media for demonstration of pyocyanin and fluorescein. *J Lab Clin Med* **44**, 301–307.
- Leifson, E. (1963). Determination of carbohydrate metabolism of marine bacteria. *J Bacteriol* **85**, 1183–1184.
- Marmur, J. & Doty, P. (1962). Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature. *J Mol Biol* **5**, 109–118.
- Owen, R. J., Hill, L. R. & Lapage, S. P. (1969). Determination of DNA base composition from melting profiles in dilute buffers. *Biopolymers* **7**, 503–516.
- Palleroni, N. J. (1984). Genus I. *Pseudomonas* Migula 1984, 237^{AL}. In *Bergey's Manual of Systematic Bacteriology*, vol. 1, pp. 141–199. Edited by N. R. Krieg & J. G. Holt. Baltimore: Williams & Wilkins.
- Pearson, W. R. & Lipman, D. J. (1988). Improved tools for biological sequence comparison. *Proc Natl Acad Sci U S A* **85**, 2444–2448.
- Peix, A., Berge, O., Rivas, R., Abril, A. & Velázquez, E. (2005). *Pseudomonas argentinensis* sp. nov., a novel yellow pigment-producing bacterial species, isolated from rhizospheric soil in Córdoba, Argentina. *Int J Syst Evol Microbiol* **55**, 1107–1112.
- Romanenko, L. A., Uchino, M., Falsen, E., Frolova, G. M., Zhukova, N. V. & Mikhailov, V. V. (2005a). *Pseudomonas pachastrellae* sp. nov., isolated from a marine sponge. *Int J Syst Evol Microbiol* **55**, 919–924.
- Romanenko, L. A., Uchino, M., Falsen, E., Lysenko, A. M., Zhukova, N. V. & Mikhailov, V. V. (2005b). *Pseudomonas xanthomarina* sp. nov., a novel bacterium isolated from marine ascidian. *J Gen Appl Microbiol* **51**, 65–71.
- Rosselló-Mora, R. A., García-Valdés, E. & Lalucat, J. (1993). Taxonomic relationship between *Pseudomonas perfectomarina* ZoBell and *Pseudomonas stutzeri*. *Int J Syst Bacteriol* **43**, 852–854.
- Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.
- Shida, O., Takagi, H., Kadowaki, K., Nakamura, L. K. & Komagata, K. (1997). Transfer of *Bacillus alginolyticus*, *Bacillus chondroitinus*, *Bacillus curdlanolyticus*, *Bacillus glucanolyticus*, *Bacillus kobensis*, and *Bacillus thiaminolyticus* to the genus *Paenibacillus* and emended description of the genus *Paenibacillus*. *Int J Syst Bacteriol* **47**, 289–298.
- Smibert, R. M. & Krieg, N. R. (1994). Phenotypic characterization. In *Methods for General and Molecular Bacteriology*, pp. 607–655. Edited by P. Gerhardt, R. G. E. Murray, W. A. Wood & N. R. Krieg. Washington, DC: American Society for Microbiology.
- 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.
- Uchino, M., Kosako, Y., Uchimura, T. & Komagata, K. (2000). Emendation of *Pseudomonas straminea* Iizuka and Komagata 1963. *Int J Syst Evol Microbiol* **50**, 1513–1519.
- Uchino, M., Shida, O., Uchimura, T. & Komagata, K. (2001). Recharacterization of *Pseudomonas fulva* Iizuka and Komagata 1963, and proposals of *Pseudomonas parafulva* sp. nov. and *Pseudomonas cremoricolorata* sp. nov. *J Gen Appl Microbiol* **47**, 247–261.
- Vanparys, B., Heylen, K., Lebbe, L. & De Vos, P. (2006). *Pseudomonas peli* sp. nov. and *Pseudomonas borbori* sp. nov., isolated from a nitrifying inoculum. *Int J Syst Evol Microbiol* **56**, 1875–1881.
- Vaskovsky, V. E. & Terekhova, T. A. (1979). HPTLC of phospholipid mixtures containing phosphatidylglycerol. *J High Resolut Chromatogr Chromatogr Commun* **2**, 671–672.
- Wakabayashi, H. & Egusa, S. (1972). Characteristics of a *Pseudomonas* sp. from an epizootic of pond-cultured eels (*Anguilla japonica*). *Bull Jpn Soc Sci Fish* **38**, 577–587.
- 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.
- Wilkinson, S. G. (1988). Gram-negative bacteria. In *Microbial Lipids*, vol. I, pp. 299–488. Edited by C. Ratledge & S. G. Wilkinson. London: Academic Press.
- Yumoto, I., Yamazaki, K., Hishinuma, M., Nodasaka, Y., Suemori, A., Nakajima, K., Inoue, N. & Kawasaki, K. (2001). *Pseudomonas alcaliphila* sp. nov., a novel facultatively psychrophilic alkaliphile isolated from seawater. *Int J Syst Evol Microbiol* **51**, 349–355.