Pseudidiomarina taiwanensis gen. nov., sp. nov., a marine bacterium isolated from shallow coastal water of An-Ping Harbour, Taiwan, and emended description of the family *Idiomarinaceae*

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Two strains of heterotrophic, aerobic, marine bacteria, designated strains PIT1^T and PIT2, were isolated from sea-water samples collected at the shallow coastal region of An-Ping Harbour, Tainan, Taiwan. Both strains were Gram-negative. Cells grown in broth cultures were straight rods that were non-motile, lacking flagella. Both strains required NaCl for growth and exhibited optimal growth at 30–35 °C, 1–4 % NaCl and pH 8. They grew aerobically and were incapable of anaerobic growth by fermentation of glucose or other carbohydrates. Cellular fatty acids were predominantly iso-branched, with $C_{15:0}$ iso and $C_{17:0}$ iso representing the most abundant components. The DNA G+C contents of strains PIT1^T and PIT2 were 49·3 and 48·6 mol%, respectively. Phylogeny based on 16S rRNA gene sequences, together with data from phenotypic and chemotaxonomic characterization, revealed that the two isolates could be assigned to a novel genus in the family *Idiomarinaceae*, for which the name *Pseudidiomarina* gen. nov. is proposed. *Pseudidiomarina taiwanensis* sp. nov. is the type species of the novel genus (type strain PIT1^T = BCRC 17465^T = JCM 13360^T).

Alteromonas-like bacteria belonging to the class *Gammaproteobacteria* comprise a large group of marine, heterotrophic, Gram-negative rods that are mainly strictly aerobic and motile by means of a single polar flagellum. Identification of these bacteria is impeded at the species level and even at the genus level, due to similar phenotypic characteristics. A recent phylogenetic study based on 16S rRNA gene sequences, however, revealed that the *Alteromonas*-like bacteria could be divided into the families *Alteromonadaceae*, *Pseudoalteromonadaceae*, *Psychromonadaceae*, *Colwelliaceae*, *Ferrimonadaceae*, *Idiomarinaceae*, *Moritellaceae* and *Shewanellaceae* and the genera *Oceanimonas* and *Oceanisphaera* (Ivanova *et al.*, 2004). The taxonomic affiliation of the genera *Oceanimonas* and *Oceanisphaera* at the family level remains undetermined and the recognition of a family must await more sequence data (Ivanova *et al.*, 2004).

Members of the family *Idiomarinaceae* are unique among the *Alteromonas*-like bacteria in possessing a high content of iso-branched cellular fatty acids (Ivanova *et al.*, 2004). They are also distinguished from other *Alteromonas*-like bacteria by their poor ability to use carbohydrates as sole sources of carbon and energy (Ivanova *et al.*, 2004). The genus *Idiomarina*, the only genus of the family *Idiomarinaceae*, was originally established to accommodate two species, *Idiomarina abyssalis* (the type species) and *Idiomarina zobellii* (Ivanova *et al.*, 2000). Both species were recovered from deep-sea water samples taken from the north-western Pacific Ocean. Five more species have been described in recent years, including *Idiomarina baltica* (Brettar *et al.*, 2003), *Idiomarina loihiensis* (Donachie *et al.*, 2003), *Idiomarina fontislapidosi* (Martínez-Cánovas *et al.*, 2004), *Idiomarina ramblicola* (Martínez-Cánovas *et al.*, 2004) and *Idiomarina seosinensis* (Choi & Cho, 2005). These species were isolated from surface water of the central Baltic Sea, hydrothermal fluid from a submarine volcano in Hawaii, soil from a hypersaline wetland in Spain, water from a hypersaline rambla in Spain and water from a solar saltern in Korea, respectively.

During a survey of the diversity of heterotrophic marine bacteria, two bacterial strains were recovered from sea-water samples collected at the shallow coastal region of An-Ping Harbour, Tainan, Taiwan. The data presented in this study show that the two isolates can be classified as representatives

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Abbreviation: PHB, poly-β-hydroxybutyrate.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain PIT^T is DQ118948.

of a novel species in the family *Idiomarinaceae* and deserve a novel genus rank.

An-Ping Harbour is located in the south-west of Taiwan. Sea-water samples were collected from a shallow coastal region of the harbour in the morning at low tide. Each sample was decimally diluted with sterile NaCl/Tris buffer (30 g NaCl and 0·24 g Tris in 1 l deionized water, pH 8·0). Aliquots (0·1 ml) of the decimal dilutions (10^2–10^4 times) were spread on polypeptone-yeast extract (PY) plate medium (Shieh *et al.*, 2000). The plates were incubated at 25 °C in the dark for 7 days under aerobic conditions. Individual colonies appearing on the plates were picked off and purified by successive streaking on PY plates. PY stab cultures of the isolates were maintained at 25 °C under aerobic conditions. Two of the isolates, designated strains PIT1^T and PIT2, were used for the present study.

PY broth cultures of strains PIT1^T and PIT2 were incubated aerobically at 30 °C in the dark for 3 days. The cultures were centrifuged to harvest the cells. Total genomic DNA was extracted and purified from the cells by using a Puregene DNA isolation kit (Gentra Systems) in accordance with the manufacturer's instructions. Solutions of the purified DNA samples were prepared at concentrations of 200–700 μg ml^{-1} in sterile distilled water. The DNA solutions were used for PCR amplification. PCR amplification of each bacterial 16S rRNA gene was conducted using a universal primer pair at positions 8–27 and 1488–1511 of the *Escherichia coli* numbering system (Shieh *et al.*, 2003a). Accessory PCR amplification was performed using either of the primer pairs at positions 8–27 and 518–534 (Muyzer *et al.*, 1993) or positions 907–926 and 1488–1511 (Muyzer & Smalla, 1998). The PCR mixture contained 2·5 μl DNA solution, 1 μl mixture of one of the primer pairs (10 μM each primer), 1 μl mixture of the four deoxyribonucleoside 5′-triphosphates (2·5 mM; Gene Teks Bioscience), 5 μl 10× *Taq* buffer (Gene Teks Bioscience), 5 μl 10× BSA (Promega) and 0·5 μl (2 U) *Taq* DNA polymerase (Gene Teks Bioscience). Each sample was made up to 50 μl with sterile distilled water. PCR amplification was performed in a GeneAmp PCR System 2700 (Applied Biosystems) with the following temperature profile: an initial denaturation at 94 °C for 10 min; 35 cycles of denaturation (1 min at 92 °C), annealing (1·5 min at 52 °C) and extension (1·5 min at 72 °C); and a final extension at 72 °C for 4 min. Amplified DNAs were checked for size and purity by electrophoresis at 100 V for 30 min on 1 % agarose gels in Tris/acetate/EDTA (TAE) buffer (MDBio) with 5 μl aliquots of PCR products. The gels were stained with ethidium bromide (1 μg ml^{-1}) for 5 min in TAE buffer and the DNA bands that appeared on the gels were examined under an image analysing system consisting of a UV transilluminator (Spectroline), a dark box (EDAS 290; Kodak) and a zoom digital camera (DC290; Kodak).

Sequencing reactions of 16S rRNA gene samples, alignment and comparison of the resulting sequences and reference sequences available in the GenBank database, calculation of the distance matrices for the aligned sequences and reconstruction of a phylogenetic tree by the neighbour-joining method were performed as described previously by Shieh *et al.* (2004). Phylogenetic trees were also reconstructed by using maximum-parsimony (Fitch, 1971) and maximum-likelihood (Felsenstein, 1981) methods. The GenBank accession numbers for the sequences used to reconstruct the phylogenetic tree are shown in Fig. 1. Bootstrap confidence values (Felsenstein, 1985) were obtained with 1000 resamplings with an option of stepwise addition.

Fatty acids in whole cells grown on PY plate medium at 30 °C for 3 days were extracted, saponified and esterified, followed by GC analysis of the fatty acid methyl esters according to the instructions of the MIDI system (Sasser, 1997). This work was performed at the Bioresources Center for Research and Collection (BCRC), Food Industry Research and Development Institute, Taiwan. Genomic DNA G+C content was determined by HPLC analysis (Shieh & Liu, 1996), which was also performed at the BCRC.

Growth and phenotypic characteristics of strains PIT1^T and PIT2 were examined according to the methods of Shieh *et al.* (2000), with modifications and additional tests as described below. The ability to grow at different temperatures was determined in PY broth and recorded daily for up to 7 days at 20–45 °C and for 20 days at 4–15 °C, unless significant growth was observed. The ability to grow at various NaCl concentrations was determined in PY broth containing 0–15 % NaCl. Utilization of various carbohydrates as sole carbon and energy sources for growth was tested in carbohydrate/mineral (CM) media (Shieh *et al.*, 2004). Anaerobic growth in PY, polypeptone-yeast extract-glucose (PYG) and polypeptone-yeast extract-nitrate (PYN) broth media under argon gas was analysed as described by Shieh *et al.* (2004). Poly-β-hydroxybutyrate (PHB) accumulation and H_2S production from thiosulphate were tested according to the methods of Shieh *et al.* (1988) and Shieh *et al.* (2004), respectively. Tests for endospore formation and for activities of arginine dihydrolase and lysine and ornithine decarboxylases essentially followed the methods of Shieh *et al.* (2003b). Biolog GN2 microplates were used to determine the oxidation of carbohydrates, alcohols, organic acids, amino acids and nucleosides presented as single carbon sources. The API 50CH system (bioMérieux) was used to test either oxidation or fermentation of alcohols and carbohydrates. Constitutive enzyme activities were detected by using the API ZYM system (bioMérieux). Cell suspensions used for the API 50CH tests were prepared in PY broth supplemented with 0·18 g phenol red l^{-1}. For Biolog GN2 and API ZYM tests, samples were prepared by suspending cells in a mineral medium containing 0·54 g NH_4Cl, 30 g NaCl, 3 g $MgCl_2.6H_2O$, 2 g K_2SO_4, 0·2 g K_2HPO_4, 0·01 g $CaCl_2$, 0·006 g $FeCl_3.6H_2O$, 0·005 g $Na_2MoO_4.7H_2O$, 0·004 g $CuCl_2.2H_2O$ and 6 g Tris dissolved in 1 l deionized water and adjusted to pH 8·0. Antibiotic susceptibility tests were performed by disc diffusion methods as described in previous reports (Shieh *et al.*, 2003a, b). All the test cultures

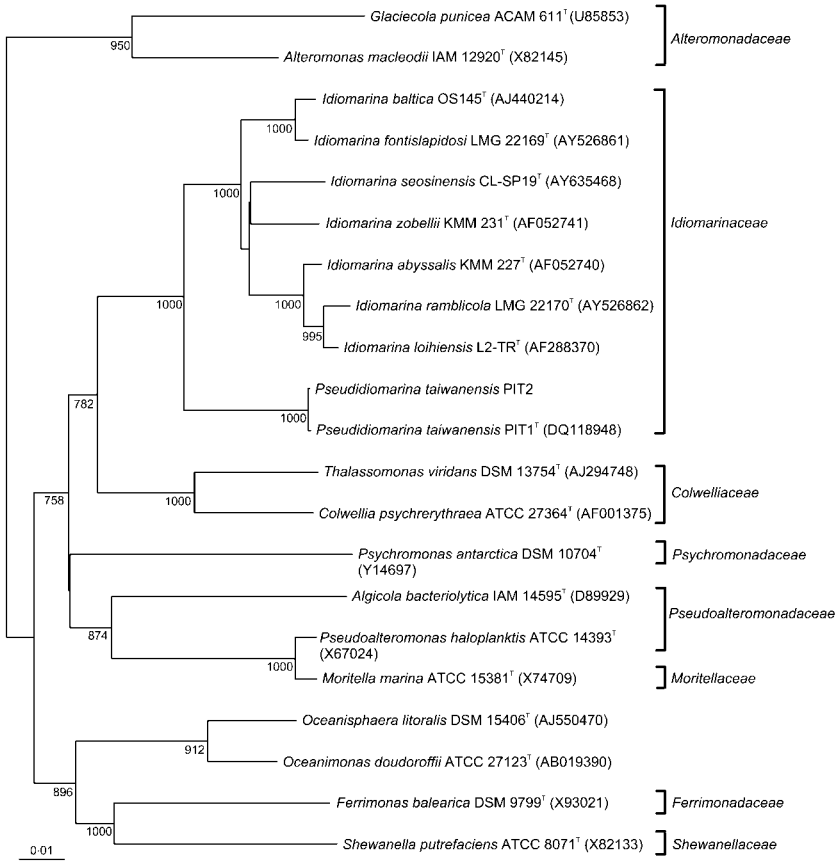

Fig. 1. Unrooted phylogenetic tree derived from neighbour-joining analysis of 16S rRNA gene sequences, showing the relationship between strains PIT1^T and PIT2 and *Idiomarina* species of the family *Idiomarinaceae* and other related taxa belonging to the *Alteromonas*-like bacteria. Bar, 0·01 substitutions per nucleotide position.

were incubated aerobically at 30 °C in the dark for 7 days, unless stated otherwise.

Almost complete 16S rRNA gene sequences were determined for strains PIT1^T and PIT2 (1465 bp for each). The two sequences were identical except for one nucleotide difference (99·9 % similarity; C versus T at position 590, *E. coli* 16S rRNA gene numbering system). The sequences were aligned and compared with all bacterial sequences available in the GenBank database. Phylogeny based on 16S rRNA gene sequences revealed that strains PIT1^T and PIT2 were members of marine *Alteromonas*-like bacteria in the class *Gammaproteobacteria*. The phylogenetic positions of strains PIT1^T and PIT2 among selected representatives of *Alteromonas*-like bacteria are shown in Fig. 1. Similar results were obtained from maximum-parsimony and maximum-likelihood algorithms (not shown). Phylogenetic analyses showed that the strains formed a robust cluster with *Idiomarina* species in the family *Idiomarinaceae*, with gene sequence similarity levels of 93·3–94·8 % (76–98 differences out of 1465 positions). The gene sequence similarity levels between the two novel strains and *I. abyssalis*, *I. baltica*, *I. fontislapidosi*, *I. loihiensis*, *I. ramblicola*, *I. seosinensis* and *I. zobellii* were 94·0, 94·8, 94·0, 94·3, 93·3, 93·7 and 94·2 %, respectively. No other known bacterial species shared more than 91·5 % sequence similarity with the two novel strains. Phylogenetically, strains PIT1^T and PIT2 could be assigned to a novel genus in the family *Idiomarinaceae* since they did not share more than 95 % sequence similarity with any known species of *Idiomarina* and were clearly an outgroup with respect to these species (Stackebrandt & Goebel, 1994; Rosselló-Mora & Amann, 2001).

One eminent feature of the genus *Idiomarina* in the family *Idiomarinaceae* is its uniquely high content of iso-branched fatty acids (Ivanova *et al.*, 2000, 2004) which is atypical in the phylum *Proteobacteria*, with a few exceptions such as *Marinobacter lutaoensis* (Shieh *et al.*, 2003b) and the *Xanthomonas*-branch bacteria (Finkmann *et al.*, 2000). Strains PIT1^T and PIT2, like species of the genus *Idiomarina*, contained $C_{15:0}$ iso (31·7 %) and $C_{17:0}$ iso (11·7 %) as the major cellular fatty acids (Table 1). However, the fatty acid profiles of the two novel strains were significantly different from those of other *Idiomarina* species. This was indicated by differences in the levels of the minor fatty acids $C_{15:1}$ isoF (5·7–5·9 % versus 0·6–2·3 %), $C_{13:0}$ iso (4·2–4·6 % versus 0–1·8 %) and $C_{18:1}\omega7c$ (1·9–3·4 % versus 5·5–9·3 %) (Table 1).

Strains PIT1^T and PIT2 grew in PY broth over a pH range of 7–9, with optimal growth at pH 8. No growth was observed at pH 5–6. Both strains could be considered mesophilic bacteria, since they grew in PY broth over a temperature range of 15–42 °C and grew most rapidly at 30–35 °C. Growth was absent at 4–10 or 45 °C. The novel strains were halophilic. They grew in PY broth at NaCl levels of 0·5–11 %, with optimal growth at 1–4 %. No growth was

Table 1. Cellular fatty acid content (%) of *Pseudidiomarina taiwanensis* sp. nov. and *Idiomarina* species

Strains: 1, *P. taiwanensis* sp. nov. PIT1^T and PIT2 (this study); 2, *I. baltica* OS145^T (Brettar *et al.*, 2003); 3, *I. loihiensis* L2-TR^T (Donachie *et al.*, 2003); 4, *I. fontislapidosi* F23^T (Martínez-Cánovas *et al.*, 2004); 5, *I. seosinensis* CL-SP19^T (Choi & Cho, 2005); 6, *I. ramblicola* R22^T (Martínez-Cánovas *et al.*, 2004); 7, *I. abyssalis* KMM 227^T (Ivanova *et al.*, 2000); 8, *I. zobellii* KMM 231^T (Ivanova *et al.*, 2000). −, Not detected; tr, less than 1 %.

Fatty acid	1	2	3	4	5	6	7	8
$C_{10:0}$	tr	−	tr	−	−	−	−	−
$C_{10:0}$ 3-OH	tr	1·2	tr	2·3	1·3	1·1	−	−
$C_{11:0}$ iso	2·2–2·4	2·5	2·0	2·8	3·2	3·4	−	−
$C_{11:0}$ iso 3-OH	4·2–4·6	3·7	4·1	2·6	5·0	5·6	−	−
$C_{12:0}$	tr	−	tr	−	−	−	tr	tr
$C_{12:0}$ 3-OH	tr	−	tr	−	1·2	−	−	−
$C_{13:0}$	tr	−	−	−	−	−	tr	tr
$C_{13:0}$ iso	4·2–4·6	tr	1·8	tr	−	1·5	1·0	1·1
$C_{13:0}$ iso 3-OH	2·9–3·8	3·2	3·3	1·6	4·2	2·3	−	−
$C_{14:0}$	tr–1·2	−	tr	1·9	tr	tr	−	−
$C_{14:1}\omega5c$	−	−	−	−	1·4	−	tr	tr
$C_{15:0}$ anteiso	tr	−	tr	tr	−	1·2	tr	tr
$C_{15:1}$ isoF	5·7–5·9	1·5	1·3	1·5	tr	1·9	2·3	1·6
$C_{15:0}$ iso	31·7–37·8	36·9	32·6	26·8	17·1	24·7	33·7	40·6
$C_{15:1}\omega8c$	−	−	−	−	−	−	1·3	1·1
$C_{15:0}$ iso 3-OH	−	−	tr	−	1·2	−	−	−
$C_{16:0}$	4·4–8·1	4·8	7·6	11·7	8·9	7·4	6·3	4·6
$C_{16:1}\omega7c/C_{15:0}$ iso 2-OH	4·9–5·9	8·4	6·0	11·3	2·5	5·2	7·0	8·3
$C_{17:0}$	tr	−	tr	tr	tr	1·7	tr	tr
$C_{17:0}$ iso	11·4–14·4	11·2	11·0	8·8	15·2	12·9	11·9	12·5
$C_{17:1}\omega6c$	−	−	−	−	−	−	1·5	3·4
$C_{17:1}\omega8c$	tr	tr	tr	tr	−	1·1	tr	1·1
$C_{17:1}$ iso$\omega9c$	7·9–8·3	10·0	11·9	4·0	8·8	11·0	−	−
$C_{17:0}$ cyclo	−	tr	1·7	1·2	4·5	2·5	−	−
$C_{18:0}$	2·0–3·0	tr	1·6	4·9	3·9	3·0	1·8	tr
$C_{18:1}\omega7c$	1·9–3·4	6·0	5·5	9·3	8·7	5·9	6·7	5·9
$C_{18:1}\omega9c$	tr	tr	1·0	1·1	tr	1·2	1·4	tr
$C_{18:1}\omega7c$ 11-methyl	−	1·8	−	tr	−	tr	−	−
$C_{18:3}\omega6c$ (6,9,12)	−	−	−	−	1·2	−	−	−
$C_{19:1}\omega6c$	−	tr	tr	−	2·1	−	−	−
$C_{19:1}\omega8c$ cyclo	−	−	−	−	3·5	−	−	−

observed at 0 and 12–15 % NaCl. Substitution of KCl (1–5 %) for NaCl did not support growth, indicating that the strains required Na^+ for growth and that the Na^+ requirement was not for osmotic function. Both strains exhibited good growth in PY, PYN and PYG broth media (maximal OD_{600} >0·5) under aerobic conditions. Growth was relatively weak in PYN broth (maximal OD_{600} 0·13–0·15) and was negligible in PY and PYG broth (maximal OD_{600} 0·04–0·05) under anaerobic conditions. Anaerobic growth could be achieved by reduction of nitrate, but not by fermentation of glucose. Thus, the strains were considered as aerobes rather than facultative anaerobes in a strict sense. Strains PIT1^T and PIT2 did not grow in any of the CM media which contained glucose or other carbohydrates as sole carbon and energy sources, although these media have been shown to support the growth of various other marine bacteria (Shieh *et al.*, 2000, 2003a, b, 2004).

Strains PIT1^T and PIT2 were Gram-negative. They produced circular, translucent and non-luminescent colonies on PY agar plates after 2–3 days incubation. Cells grown in PY broth appeared as straight rods that were non-motile, lacking flagella during the late exponential to early stationary phase of growth (Fig. 2). A few of them exhibited sac-like processes (Fig. 2c) or prosthecae (Fig. 2d) from the cell wall; such uncommon structures might possibly originate from extrusion of cytoplasm. Carbohydrate fermentation tests in polypeptone-yeast extract-carbohydrate (PYC) stab media (Shieh *et al.*, 2000) indicated that the strains did not ferment any of the test carbohydrates: D-arabinose, L-arabinose,

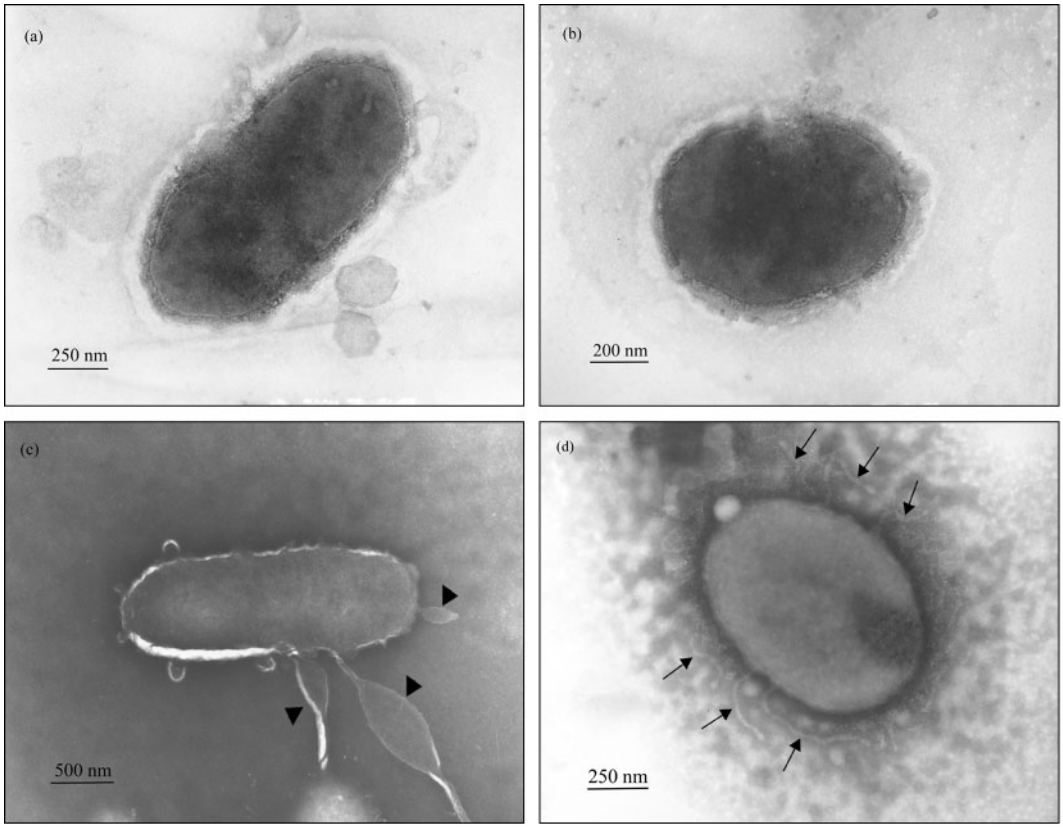

Fig. 2. Electron micrographs of strain PIT1^T. (a, b) Non-flagellated, rod-shaped cells. A few of the cells exhibited sac-like processes (c; shown by arrowheads) or prosthecae (d; shown by arrows) from the cell wall. Bars: (a), 250 nm; (b), 200 nm; (c), 500 nm; (d), 250 nm.

cellobiose, galactose, glucose, lactose, mannose, melibiose, sucrose, trehalose or xylose. PHB-like granules were not found as intracellular reserve products. Endospores were not found. Neither sporulation nor endospore-like structures were ever observed or confirmed by phase-contrast microscopy or spore staining in the present study. Oxidase and catalase tests were both positive. Indole was not produced. Tween 80 and gelatin were hydrolysed, but neither DNA nor starch was hydrolysed. Nitrate was reduced to nitrite, but not further to N_2O or N_2. Additional phenotypic characterization data are given below in the species description. Strains PIT1^T and PIT2 had DNA G+C contents of 49·3 and 48·6 mol%, respectively.

Strains PIT1^T and PIT2 were phenotypically incompatible with the genus *Idiomarina* since they were non-motile and lacking flagella. Inability to grow at pH 6 and a negative DNase reaction also allowed the novel strains to be differentiated from most species of *Idiomarina*. More detailed phenotypic characteristics that are useful for differentiating strains PIT1^T and PIT2 from *Idiomarina* species are listed in Table 2.

Phylogeny based on 16S rRNA gene sequences, fatty acid profiles and phenotypic characteristics indicated that strains PIT1^T and PIT2 could be assigned to a novel genus in the family *Idiomarinaceae*. *Pseudidiomarina* gen. nov. is proposed to accommodate the two novel strains. The type species of this genus is proposed as *Pseudidiomarina taiwanensis* sp. nov. with strain PIT1^T as the type strain of the type species.

The inclusion of *Pseudidiomarina taiwanensis* gen. nov., sp. nov. into the family *Idiomarinaceae* requires an emended description of the family because the family *Idiomarinaceae* is currently defined as including only motile species that have been isolated from open- and deep-sea waters. The description that the family *Idiomarinaceae* includes aerobes and facultative anaerobes would be also inappropriate since no species of this family have been reported to be facultative anaerobes capable of fermentative metabolism. Moreover, the 16S rRNA gene sequences of the *Idiomarinaceae* species are defined as having a signature nucleotide C at position 143, whereas *Pseudidiomarina taiwanensis* sp. nov. has nucleotide A instead of C at this sequence position.

To date, *Pseudidiomarina taiwanensis* sp. nov. has only been found in shallow coastal water. The species may also occur in other saline habitats, since it can grow over a wide range of temperatures and salinities.

Table 2. Phenotypic characteristics useful for differentiating *Pseudidiomarina taiwanensis* sp. nov. from *Idiomarina* species

Strains: 1, *P. taiwanensis* sp. nov. PIT1^T and PIT2 (this study); 2, *I. baltica* OS145^T (Brettar *et al.*, 2003); 3, *I. loihiensis* L2-TR^T (Donachie *et al.*, 2003); 4, *I. fontislapidosi* F23^T (Martínez-Cánovas *et al.*, 2004); 5, *I. seosinensis* CL-SP19^T (Choi & Cho, 2005); 6, *I. ramblicola* R22^T (Martínez-Cánovas *et al.*, 2004); 7, *I. abyssalis* KMM 227^T (Ivanova *et al.*, 2000); 8, *I. zobellii* KMM 231^T (Ivanova *et al.*, 2000). +, Positive; −, negative; ND, no data available. All species grow at 15–30 °C and in 1–10 % NaCl, are positive for oxidase, catalase and gelatinase activities and are negative for agarase and amylase activities.

Characteristic	1	2	3	4	5	6	7	8
Monotrichous flagellation	−	+	+	+	+	+	+	+
Motility	−	+	+	+	+	+	+	+
Nitrate reduction	+	−	+	−	+	−	+*	−*
Production of H_2S	−	+	ND	+	ND	+	ND	ND
Acid from D-glucose	−	+	−	−	−	−	−	−
Hydrolysis of:								
DNA	−	ND	+	+	+	+	+	+
Tween 80	+	+	+	+	+	+	−	−
Growth at:								
10 °C	−	+	+	+	+	−	+	+
40 °C	+	+	+	+	+	+	−	−
42 °C	+	+	+	+	−	−	−	−
45 °C	−	+	+	+	−	−	−	−
Optimum temperature (°C)	30–35	30–40	ND	32	30–35	32	20–22	20–22
Growth in 12–15 % NaCl	−	−	+	+	+	+	+	−
NaCl optimum (%)	1–4	3–6	7·5–10	3–5	7–10	3–5	3–6	3–6

*Data from Choi & Cho (2005).

Emended description of *Idiomarinaceae* Ivanova *et al.* 2004

Idiomarinaceae (Idio.ma.ri.na′ce.ae. N.L. fem. n. *Idiomarina* type genus of the family; *-aceae* ending to denote a family; N.L. fem. pl. n. *Idiomarinaceae* the *Idiomarina* family).

Gram-negative, rod-shaped bacteria belonging to the class *Gammaproteobacteria*. Motile or non-motile. Do not form endospores or microcysts. Chemo-organotrophs capable of respiratory, but not fermentative, metabolism. Arginine dihydrolase activity is absent. Sodium ions are required for growth. The major cellular fatty acids are $C_{15:0}$ iso and $C_{17:0}$ iso. Members of the family have been isolated from saline habitats with a wide range of salinities, such as coastal and oceanic waters, submarine hydrothermal fluids, solar salterns and inland hypersaline wetlands. The 16S rRNA gene sequences have the following signature nucleotide positions (numbering by comparison with *E. coli* sequence AE000471): 143 (C or A), 662 (A), 682 (A), 830 (T), 856 (A). The type genus is *Idiomarina* Ivanova *et al.* 2000.

Description of *Pseudidiomarina* gen. nov.

Pseudidiomarina (Pseud′i.di.o.ma.ri′na. Gr. adj. *pseudes* false; N.L. fem. n. *Idiomarina* a name of a bacterial genus; N.L. fem. n. *Pseudidiomarina* false *Idiomarina*).

Members are Gram-negative rods belonging to the family *Idiomarinaceae* in the class *Gammaproteobacteria*. Cells grown in broth cultures are non-motile, lacking flagella. Microcysts or endospores are absent. Sodium ions are required for growth. PHB is not accumulated as an intracellular reserve product. Oxidase- and catalase-positive. Chemo-organotrophs capable of respiratory, but not fermentative, metabolism. Molecular oxygen is a universal electron acceptor while in some cases nitrate can be used as an alternative electron acceptor under anaerobic conditions. Mesophilic, grow at 20–30 °C, but not at 4 or 45 °C. Cellular fatty acids are predominantly iso-branched with 15 and 17 carbons. The genus has the following signature nucleotide positions (numbering by comparison with *E. coli* sequence AE000471): 143 (A), 662 (A), 682 (A), 830 (T), 856 (A). The type species is *Pseudidiomarina taiwanensis*. The DNA G+C content of the type strain of the type species, PIT1^T (=BCRC 17465^T=JCM 13360^T), is 49·3 mol%.

Description of *Pseudidiomarina taiwanensis* sp. nov.

Pseudidiomarina taiwanensis (tai.wan.en′sis. N.L. fem. adj. *taiwanensis* pertaining to Taiwan, where the type strain was isolated).

Description is as for the genus with the following additional characteristics. During the late exponential to early stationary phase of growth, cells in broth cultures are straight rods that measure approximately 0·8–2·4 μm by 0·6–0·8 μm. A small number of the cells produce prosthecae or sac-like processes from the cell wall. Colonies produced on agar plates are circular, translucent, non-pigmented and non-luminescent. Swarming does not occur. Capable of anaerobic growth by reducing nitrate as the terminal electron acceptor, but not by fermenting glucose or other carbohydrates as substrates. Grows at 15–42 °C, with optimal growth at 30–35 °C. Grows at NaCl levels of 0·5–11 %, with optimal growth at 1–4 % and no growth at 0 and 12 % NaCl. Grows optimally at about pH 8, no growth at pH 5–6. Gelatinase and lipase tests are positive, but agarase, amylase, caseinase, DNase, arginine dihydrolase and lysine and ornithine decarboxylase tests are negative. Nitrate is reduced to nitrite, but not further to N_2O or N_2. Indole is not produced from tryptophan. H_2S is not produced from thiosulphate. The following constitutive enzyme activities are detected in API ZYM tests: alkaline phosphatase, esterase (C4), esterase lipase (C8), lipase (C14), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, acid phosphatase and naphthol-phosphohydrolase. Oxidative or fermentative acid production was not detected from carbohydrates using the API 50CH system. According to Biolog GN2 tests, L-alanine, L-glutamic acid, L-proline, D-serine, L-serine, uridine, glycogen, succinic acid and glycyl L-glutamic acid are oxidized. Resistant to the vibriostatic agent O/129 at 10–150 μg. Susceptible to ampicillin (10 μg), carbenicillin (100 μg), cephalothin (30 μg), chloramphenicol (30 μg), colistin (10 μg), gentamicin (10 μg), erythromycin (15 μg), lincomycin (2 μg), nalidixic acid (30 μg), novobiocin (30 μg), penicillin G (10 U), polymyxin B (300 U), tetracycline (30 μg) and vancomycin (30 μg). Resistant to clindamycin (2 μg), kanamycin (30 μg), neomycin (30 μg), oxacillin (1 μg) and streptomycin (10 μg).

The type strain, PIT1^T (=BCRC 17465^T=JCM 13360^T), was isolated from shallow coastal water of An-Ping Harbour, Taiwan.

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