

## *Pseudidiomarina marina* sp. nov. and *Pseudidiomarina tainanensis* sp. nov. and reclassification of *Idiomarina homiensis* and *Idiomarina salinarum* as *Pseudidiomarina homiensis* comb. nov. and *Pseudidiomarina salinarum* comb. nov., respectively

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Two Gram-negative strains of heterotrophic, aerobic, marine bacteria, designated PIM1<sup>T</sup> and PIN1<sup>T</sup>, were isolated from seawater samples collected from the shallow coastal region of An-Ping Harbour, Tainan, Taiwan. Cells grown in broth cultures were straight rods and non-motile. The two isolates required NaCl for growth and grew optimally at 30–35 °C and 2–5% NaCl. They grew aerobically and were not capable of anaerobic growth by fermentation of glucose or other carbohydrates. The cellular fatty acids were predominantly iso-branched, with iso-C<sub>15:0</sub> (17.0–21.4%), iso-C<sub>17:0</sub> (18.2–21.0%) and iso-C<sub>17:1ω9c</sub> (15.7–16.6%) as the most abundant components. The predominant isoprenoid quinone was Q-8 (95.2–97.1%). Strains PIM1<sup>T</sup> and PIN1<sup>T</sup> had DNA G+C contents of 46.6 and 46.9 mol%, respectively. Phylogeny based on 16S rRNA gene sequences and DNA–DNA hybridization, together with data from physiological, morphological and chemotaxonomic characterizations, indicated that the two isolates should be classified as representatives of two novel species of the genus *Pseudidiomarina* of the family *Idiomarinaceae*, for which the names *Pseudidiomarina marina* sp. nov. (type strain PIM1<sup>T</sup>=BCRC 17749<sup>T</sup>=JCM 15083<sup>T</sup>) and *Pseudidiomarina tainanensis* sp. nov. (type strain PIN1<sup>T</sup>=BCRC 17750<sup>T</sup>=JCM 15084<sup>T</sup>) are proposed. In addition, based on the characterization data obtained in this study, it is proposed that *Idiomarina homiensis* and *Idiomarina salinarum* should be reclassified as *Pseudidiomarina homiensis* comb. nov. and *Pseudidiomarina salinarum* comb. nov., respectively.

The family *Idiomarinaceae* (Ivanova *et al.*, 2004; Jean *et al.*, 2006), in the class *Gammaproteobacteria*, comprises halophilic, aerobic, Gram-negative, rod-shaped bacteria that have a high content of iso-branched cellular fatty acids. This family is currently comprised of the genera *Idiomarina* (Ivanova *et al.*, 2000) and *Pseudidiomarina* (Jean *et al.*, 2006). At the time of writing, the genus *Idiomarina* contained nine recognized species, *Idiomarina*

*abyssalis* (Ivanova *et al.*, 2000), *I. baltica* (Brettar *et al.*, 2003), *I. fontislapidosi* (Martínez-Cánovas *et al.*, 2004), *I. homiensis* (Kwon *et al.*, 2006), *I. loihensis* (Donachie *et al.*, 2003), *I. ramblicola* (Martínez-Cánovas *et al.*, 2004), *I. salinarum* (Yoon *et al.*, 2007), *I. seosinensis* (Choi & Cho, 2005) and *I. zobellii* (Ivanova *et al.*, 2000), whereas the genus *Pseudidiomarina* contained only two recognized species, *Pseudidiomarina taiwanensis* (Jean *et al.*, 2006) and *P. sediminum* (Hu & Li, 2007). These bacteria were isolated from saline habitats with a wide range of salinities, such as coastal and oceanic waters, coastal sediments, submarine hydrothermal fluids, solar salterns and inland hypersaline

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains PIM1<sup>T</sup> and PIN1<sup>T</sup> are EU423908 and EU423907, respectively.

wetlands. Members of *Idiomarina* and *Pseudidiomarina* have been defined as possessing signature nucleotides (numbering by comparison with *Escherichia coli* sequence AE000471) 662 (A), 682 (A), 830 (T) and 856 (A). Members of *Idiomarina* have an additional signature nucleotide C at position 143, whereas members of *Pseudidiomarina* have a signature nucleotide A instead of C at this sequence position (Jean *et al.*, 2006).

In the present study, two bacterial isolates were recovered from seawater samples collected from the shallow coastal region of An-Ping Harbour, Tainan, Taiwan, during a survey of the diversity of phenanthrene-degrading bacteria. Data from a polyphasic characterization indicated that the two isolates could be classified as representing two novel species of the genus *Pseudidiomarina*.

The mineral/phenanthrene (MP) liquid medium used for enrichment cultivation of phenanthrene-degrading bacteria contained the following (per litre deionized water): 0.54 g NH<sub>4</sub>Cl, 30 g NaCl, 3 g MgCl<sub>2</sub>·6H<sub>2</sub>O, 2 g K<sub>2</sub>SO<sub>4</sub>, 0.2 g K<sub>2</sub>HPO<sub>4</sub>, 0.01 g CaCl<sub>2</sub>, 0.006 g FeCl<sub>3</sub>·6H<sub>2</sub>O, 0.005 g Na<sub>2</sub>MoO<sub>4</sub>·7H<sub>2</sub>O, 0.004 g CuCl<sub>2</sub>·2H<sub>2</sub>O, 6 g Tris and 1 g phenanthrene. The medium was adjusted to pH 8.0. Other culture media used were as described by Shieh *et al.* (2000).

An-Ping Harbour is located on the south-west coast of Taiwan. Seawater samples were collected from the shallow coastal region of this harbour in the morning at low tide. Aliquots (1 ml) of the seawater samples were transferred to culture bottles containing MP medium (50 ml). All culture bottles were incubated aerobically at 30 °C in the dark for 2–3 weeks. Cultures in bottles that developed visible turbidity were streaked on polypeptone-yeast (PY) plate medium (Shieh *et al.*, 2000). Individual colonies that appeared 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 PIM1<sup>T</sup> and PIN1<sup>T</sup>, were deposited in the Bioresource Collection and Research Center (BCRC) and the Japan Collection of Micro-organisms (JCM) as lyophilized cultures, and were used in this study. It should be noted that strains PIM1<sup>T</sup> and PIN1<sup>T</sup> were not able to grow in MP medium as pure cultures, in spite of the fact that they were isolated from enrichment cultures with phenanthrene as the sole carbon source. However, it was unclear whether these isolates had grown in the initial enrichment cultures by utilizing metabolite(s) produced by co-existing phenanthrene-degrading bacteria, or whether the isolates had lost their phenanthrene-degrading ability after being grown on the non-selective PY plate medium.

Growth and other phenotypic properties used for the morphological and physiological characterization of strains PIM1<sup>T</sup> and PIN1<sup>T</sup> were determined following the established procedures described by Jean *et al.* (2006).

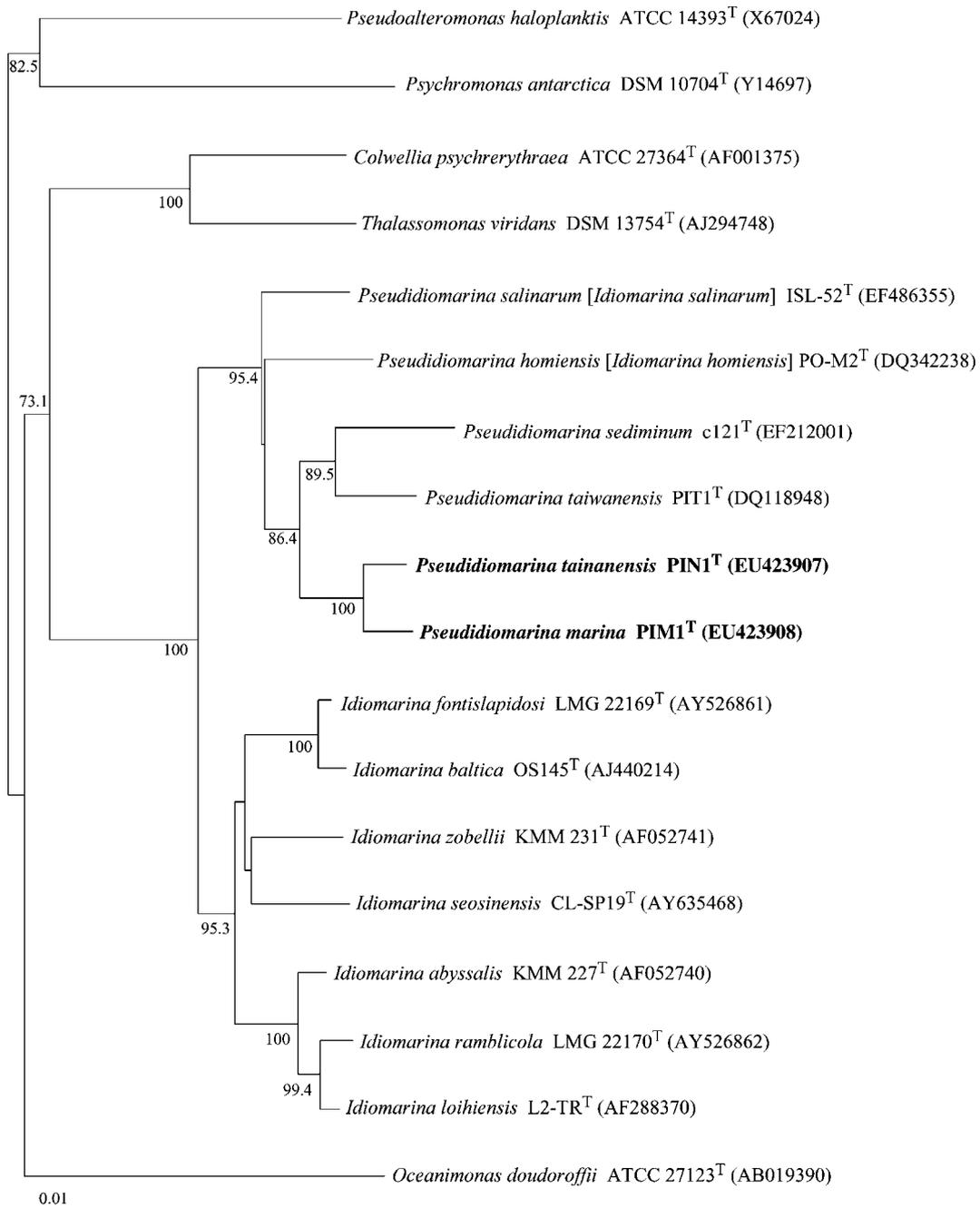
Fatty acids of whole cells grown on PY plate medium at 30 °C for 3 days were extracted, saponified and esterified.

The fatty acid methyl esters were analysed by using GC, according to the protocol of the MIDI system (Sasser, 1997). This work was performed at the BCRC. Determination of the DNA G+C content by HPLC analysis (Shieh & Liu, 1996) was also performed at the BCRC. Isoprenoid quinones were extracted, purified and analysed by using an HPLC apparatus equipped with a reversed-phase column (Shieh *et al.*, 2008).

DNA–DNA hybridization was performed between strains PIM1<sup>T</sup> and PIN1<sup>T</sup>. Bacterial DNA was isolated and extracted using the method of Smibert & Krieg (1994). The DNA was blotted on a Hybond-N<sup>+</sup> membrane (Amersham Pharmacia Biotech) using a Bio-Dot micro-filtration apparatus (Bio-Rad Laboratories). The dot-blot hybridization was carried out at 68 °C (Fesefeldt *et al.*, 1998). Total genomic DNA of strain PIM1<sup>T</sup> labelled using a DIG DNA labelling kit (Roche Diagnostics) was used as the probe. Hybridization was determined by using an enzyme immunoassay and enzyme-catalysed colour reaction with a DIG nucleic acid detection kit (Boehringer Mannheim). The DNA–DNA relatedness between strains PIM1<sup>T</sup> and PIN1<sup>T</sup> was estimated according to the hybridization results obtained using 1D Image Analysis software (Kodak).

Cells grown in PY broth at 30 °C for 3 days were harvested by centrifugation. The methods described by Jean *et al.* (2006) were followed for the extraction and purification of total genomic DNA and for PCR amplification of the 16S rRNA genes. Sequencing of the 16S rRNA genes, alignment and comparison of the resulting sequences with reference sequences available in the GenBank database, calculation of distance matrices for the aligned sequences and reconstruction of phylogenetic trees using the neighbour-joining, maximum-parsimony and maximum-likelihood methods were performed as described previously (Shieh *et al.*, 2004; Jean *et al.*, 2006). The stability of clusters was evaluated by using a bootstrap analysis with 1000 resamplings.

Nearly complete 16S rRNA gene sequences were determined for strains PIM1<sup>T</sup> and PIN1<sup>T</sup> (1465 nt for each). The two sequences were aligned and compared with bacterial sequences available in the GenBank database. The two sequences had 98.6% sequence similarity (21 out of 1465 nt positions). Phylogeny based on a neighbour-joining analysis of the 16S rRNA gene sequences revealed that strains PIM1<sup>T</sup> and PIN1<sup>T</sup> were members of the family *Idiomarinaceae* in the class *Gammaproteobacteria* and that the two novel isolates formed a robust cluster (bootstrap value, 95.4%) with *P. taiwanensis* PIT1<sup>T</sup> and *P. sediminum* c121<sup>T</sup>, their closest neighbours, together with *I. homiensis* PO-M2<sup>T</sup> and *I. salinarum* ISL-52<sup>T</sup> (Fig. 1). Similar results were obtained using the maximum-likelihood and maximum-parsimony algorithms (data not shown). Strains PIM1<sup>T</sup> and PIN1<sup>T</sup> showed 94.7–96.5% 16S rRNA gene sequence similarities to their closest neighbours and 91.1–94.4% sequence similarities with other *Idiomarina* species. Bacterial species that were not members of the family



**Fig. 1.** Unrooted phylogenetic tree derived from a neighbour-joining analysis of 16S rRNA gene sequences, showing the relationship between strains PIM1<sup>T</sup> and PIN1<sup>T</sup> and recognized species of the family *Idiomarinaceae*, together with some other related taxa belonging to the *Gammaproteobacteria*. GenBank accession numbers are given in parentheses. Only bootstrap values greater than 70% are shown at branch nodes (based on 1000 replications). Bar, 0.01 substitutions per nucleotide position.

*Idiomarinaceae* had sequence similarities of less than 90.5% with the two novel isolates. The 16S rRNA gene-based phylogeny indicated that strains PIM1<sup>T</sup> and PIN1<sup>T</sup> could be classified as representing novel members of the genus *Pseudidiomarina*. Moreover, the data also suggested that *I. homiensis* PO-M2<sup>T</sup> and *I. salinarum* ISL-52<sup>T</sup> should

be reclassified as belonging to the genus *Pseudidiomarina*, as they clustered with species of *Pseudidiomarina* rather than with those of *Idiomarina*. The finding that strains PO-M2<sup>T</sup> and ISL-52<sup>T</sup> had nucleotide A, instead of C, at position 143 further indicated that they should be reclassified as *Pseudidiomarina* species.

Strains PIM1<sup>T</sup> and PIN1<sup>T</sup>, as for species of the genera *Pseudidiomarina* and *Idiomarina* in the family *Idiomarinaceae*, contained iso-C<sub>15:0</sub> (17.0–21.4%) and iso-C<sub>17:0</sub> (18.2–21.0%) as the major cellular fatty acids (Table 1). However, the fatty acid profiles of the two novel isolates could be distinguished from those of other species of the family *Idiomarinaceae*. This was shown by differences in the levels of the fatty acids iso-C<sub>19:0</sub> (3.5–3.6% versus 0%) and iso-C<sub>17:1</sub>ω9c (15.7–16.6% versus 0–11.9%). Other quantitative differences in the fatty acids that serve to differentiate the two novel isolates from other *Idiomarinaceae* species are given in Table 1. The fatty acid profiles of strains PIM1<sup>T</sup> and PIN1<sup>T</sup> were similar. However, only strain PIM1<sup>T</sup> contained

trace amounts of C<sub>10:0</sub>, C<sub>16:1</sub>ω9c and C<sub>19:0</sub>ω8c cyclo, whereas only strain PIN1<sup>T</sup> contained trace amounts of anteiso-C<sub>15:0</sub> and 11-methyl C<sub>18:1</sub>ω7c. Strains PIM1<sup>T</sup> and PIN1<sup>T</sup> contained Q-8 as the predominant isoprenoid quinone (95.2% for PIM1<sup>T</sup> and 97.1% for PIN1<sup>T</sup>) and Q-7 as a minor one (4.8% for PIM1<sup>T</sup> and 2.9% for PIN1<sup>T</sup>). The DNA G+C contents of strains PIM1<sup>T</sup> (46.6 mol%) and PIN1<sup>T</sup> (46.9 mol%) fell within the range of values reported for known *Idiomarinaceae* species (45.0–53.9 mol%), but were slightly lower than those of *P. taiwanensis* (48.6–49.3 mol%) and *P. sediminum* (50.0 mol%). DNA–DNA hybridization results showed that strains PIM1<sup>T</sup> and PIN1<sup>T</sup> had a relatedness value of 18.4%.

**Table 1.** Cellular fatty acid contents (%) of strains PIM1<sup>T</sup> and PIN1<sup>T</sup> and type strains of recognized *Idiomarinaceae* species

Strains: 1, PIM1<sup>T</sup> (*P. marina* sp. nov.; data from this study); 2, PIN1<sup>T</sup> (*P. tainanensis* sp. nov.; this study); 3, *P. sediminum* c121<sup>T</sup> (Hu & Li, 2007); 4, *P. taiwanensis* PIT1<sup>T</sup> and PIT2 (Jean *et al.*, 2006); 5, *I. salinarum* ISL-52<sup>T</sup> (Yoon *et al.*, 2007); 6, *I. homiensis* PO-M2<sup>T</sup> (Kwon *et al.*, 2006); 7, *I. baltica* OS145<sup>T</sup> (Brettar *et al.*, 2003); 8, *I. loihensis* L2-TR<sup>T</sup> (Donachie *et al.*, 2003); 9, *I. fontislapidosi* L23<sup>T</sup> (Martínez-Cánovas *et al.*, 2004); 10, *I. seosinensis* CL-SP19<sup>T</sup> (Choi & Cho, 2005); 11, *I. ramblicola* R22<sup>T</sup> (Martínez-Cánovas *et al.*, 2004); 12, *I. abyssalis* KMM 227<sup>T</sup> (Ivanova *et al.*, 2000); 13, *I. zobellii* KMM 231<sup>T</sup> (Ivanova *et al.*, 2000). –, Not detected/not reported; tr, trace amount detected (<1%).

Fatty acid	1	2	3	4	5	6	7	8	9	10	11	12	13
C <sub>10:0</sub>	tr	–	tr	tr	–	–	–	–	–	–	–	–	–
C <sub>10:0</sub> 3-OH	tr	tr	1.3	tr	–	1.2	1.2	tr	2.3	1.3	1.1	–	–
iso-C <sub>11:0</sub>	1.8	2.1	tr	2.2–2.4	4.2	1.8	2.5	2.0	2.8	3.2	3.4	–	–
iso-C <sub>11:0</sub> 3-OH	4.5	4.8	4.3	4.2–4.6	5.0	4.4	3.7	4.1	2.6	5.0	5.6	–	–
C <sub>12:0</sub>	tr	tr	tr	tr	–	–	–	–	–	–	–	–	–
C <sub>12:0</sub> 3-OH	1.0	tr	1.3	tr	–	–	–	tr	–	1.2	–	–	–
C <sub>13:0</sub>	–	–	–	tr	–	–	–	–	–	–	–	–	–
iso-C <sub>13:0</sub>	1.3	1.7	1.8	4.2–4.6	1.6	–	tr	1.8	tr	–	1.5	1.0	1.1
iso-C <sub>13:0</sub> 3-OH	3.3	4.0	4.0	2.9–3.8	5.0	3.6	3.2	3.3	1.6	4.2	2.3	–	–
C <sub>14:0</sub>	tr	tr	1.1	tr to 1.2	–	–	–	tr	1.9	tr	tr	–	–
C <sub>14:1</sub> ω5c	–	–	tr	–	–	–	–	–	–	1.4	–	–	–
anteiso-C <sub>15:0</sub>	–	tr	–	tr	–	–	–	tr	tr	–	1.2	–	–
iso-C <sub>15:1</sub> F	3.4	2.7	2.5	5.7–5.9	3.9	1.7	1.5	1.3	1.5	tr	1.9	2.3	1.6
iso-C <sub>15:0</sub>	17.0	21.4	24.2	31.7–37.8	34.1	19.3	36.9	32.6	26.8	17.1	24.7	33.7	40.6
C <sub>15:1</sub> ω8c	–	–	–	–	–	–	–	–	–	–	–	1.3	1.1
iso-C <sub>15:0</sub> 3-OH	–	–	–	–	–	–	–	tr	–	1.2	–	–	–
C <sub>16:0</sub>	4.2	3.4	5.7	4.4–8.1	4.3	9.0	4.8	7.6	11.7	8.9	7.4	6.3	4.6
iso-C <sub>16:0</sub>	tr	tr	tr	–	1.3	–	–	–	–	–	–	–	–
C <sub>16:1</sub> ω7c/iso-C <sub>15:0</sub> 2-OH	3.7	3.6	15.3	4.9–5.9	–	7.2	8.4	6.0	11.3	2.5	5.2	7.0	8.3
C <sub>16:1</sub> ω9c	tr	–	1.0	–	–	–	–	–	–	–	–	–	–
C <sub>17:0</sub>	1.0	tr	tr	tr	–	–	–	tr	tr	tr	1.7	–	–
iso-C <sub>17:0</sub>	18.2	21.0	9.4	11.4–14.4	19.9	10.9	11.2	11.0	8.8	15.2	12.9	11.9	12.5
C <sub>17:1</sub> ω6c	–	–	–	–	–	–	–	–	–	–	–	1.5	3.4
C <sub>17:1</sub> ω8c	tr	tr	1.6	tr	–	–	tr	tr	tr	–	1.1	tr	1.1
iso-C <sub>17:1</sub> ω9c	16.6	15.7	11.9	7.9–8.3	11.8	11.9	10.0	11.9	4.0	8.8	11.0	–	–
C <sub>17:0</sub> cyclo	1.3	tr	tr	–	1.7	tr	tr	1.7	1.2	4.5	2.5	–	–
C <sub>18:0</sub>	7.9	4.7	tr	2.0–3.0	tr	4.8	tr	1.6	4.9	3.9	3.0	1.8	tr
C <sub>18:1</sub> ω7c	5.5	3.4	6.9	1.9–3.4	tr	10.4	6.0	5.5	9.3	8.7	5.9	6.7	5.9
C <sub>18:1</sub> ω9c	1.4	1.0	1.9	tr	–	2.5	tr	1.0	1.1	tr	1.2	1.4	tr
11-methyl C <sub>18:1</sub> ω7c	–	tr	–	–	–	–	1.8	–	tr	–	tr	–	–
C <sub>18:3</sub> ω6c (6,9,12)	–	–	–	–	–	–	–	–	–	1.2	–	–	–
iso-C <sub>19:0</sub>	3.5	3.6	–	–	–	–	–	–	–	–	–	–	–
C <sub>19:1</sub> ω6c	–	–	–	–	–	–	tr	tr	–	2.1	–	–	–
C <sub>19:0</sub> ω8c cyclo	tr	–	–	–	tr	–	–	–	–	3.5	–	–	–

Phylogeny based on 16S rRNA gene sequences, analyses of isoprenoid quinones and fatty acids and DNA–DNA hybridization data indicated that the two isolates described here could be assigned to the genus *Pseudidiomarina* as representing two novel species. The names *Pseudidiomarina marina* sp. nov. and *Pseudidiomarina tainanensis* sp. nov. are proposed, with PIM1<sup>T</sup> and PIN1<sup>T</sup>, respectively, as the type strains. It is also proposed that *I. homiensis* and *I. salinarum* should be reclassified as *Pseudidiomarina homiensis* comb. nov. and *Pseudidiomarina salinarum* comb. nov., respectively.

Strains PIM1<sup>T</sup> and PIN1<sup>T</sup> shared many physiological and morphological characteristics. However, the two strains could be differentiated from each other by different colony colours and by different reactions in the tests for hydrolysis of DNA and susceptibility to the antibiotics carbenicillin, chloramphenicol, cephalothin, gentamicin, erythromycin, nalidixic acid, neomycin, penicillin G, vancomycin and

tetracycline. The ability to grow at 10 °C and pH 6 and the inability to hydrolyse Tween 80 differentiated strains PIM1<sup>T</sup> and PIN1<sup>T</sup> from *P. taiwanensis* and *P. sediminum*. Cells of strains PIM1<sup>T</sup> and PIN1<sup>T</sup> were non-motile, which allowed the two novel species to be distinguished from the motile, polar-flagellated *Idiomarinaceae* species, including *I. abyssalis*, *I. baltica*, *I. fontislapidosi*, *P. homiensis* comb. nov. [*I. homiensis*], *I. loihiensis*, *I. ramblicola*, *I. seosinensis* and *I. zobellii*. Additional characteristics useful for differentiating strains PIM1<sup>T</sup> and PIN1<sup>T</sup> from other *Idiomarinaceae* species are given in Table 2. Details of the characterization data for strains PIM1<sup>T</sup> and PIN1<sup>T</sup> are given in the species descriptions.

**Description of *Pseudidiomarina marina* sp. nov.**

*Pseudidiomarina marina* (ma.ri'na. L. fem. adj. *marina* of the sea, marine).

**Table 2.** Characteristics that distinguish strains PIM1<sup>T</sup> and PIN1<sup>T</sup> from recognized species of the family *Idiomarinaceae*

Strains: 1, PIM1<sup>T</sup> (*P. marina* sp. nov.; data from this study); 2, PIN1<sup>T</sup> (*P. tainanensis* sp. nov.; this study); 3, *P. sediminum* c121<sup>T</sup> (Hu & Li, 2007); 4, *P. taiwanensis* PIT1<sup>T</sup> and PIT2 (Jean *et al.*, 2006); 5, *I. salinarum* ISL-52<sup>T</sup> (Yoon *et al.*, 2007); 6, *I. homiensis* PO-M2<sup>T</sup> (Kwon *et al.*, 2006); 7, *I. baltica* OS145<sup>T</sup> (Brettar *et al.*, 2003); 8, *I. loihiensis* L2-TR<sup>T</sup> (Donachie *et al.*, 2003); 9, *I. fontislapidosi* L23<sup>T</sup> (Martínez-Cánovas *et al.*, 2004); 10, *I. seosinensis* CL-SP19<sup>T</sup> (Choi & Cho, 2005); 11, *I. ramblicola* R22<sup>T</sup> (Martínez-Cánovas *et al.*, 2004); 12, *I. abyssalis* KMM 227<sup>T</sup> (Ivanova *et al.*, 2000); 13, *I. zobellii* KMM 231<sup>T</sup> (Ivanova *et al.*, 2000). +, Positive; –, negative; ND, no data available. All species are non-fermentative, Gram-negative rods that require NaCl for growth and grow at 15–30 °C.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13
Motility	–	–	–	–	+	+	+	+	+	+	+	+	+
Monotrichous flagellation	–	–	–	–	ND	+	+	+	+	+	+	+	+
Nitrate reduction	–	–	–	+	+	+	–	+	–	+	–	+	–
Production of H <sub>2</sub> S	–	–	–	–	ND	+	+	ND	+	ND	+	ND	ND
Hydrolysis of:													
Aesculin	–	–	ND	+	–	+	+	–	+	+	+	–	–
Casein	–	–	–	–	–	–	ND	ND	+	ND	+	ND	ND
DNA	+	–	–	–	+	+	ND	ND	+	+	+	+	+
Gelatin	+	+	+	+	–	+	+	+	+	–	+	–	–
Tween 80	–	–	+	+	+	+	+	+	+	+	+	–	–
Growth at:													
4 °C	–	–	–	–	+	+	–	+	+	+	–	+	+
10 °C	+	+	–	–	+	+	+	+	+	+	–	+	+
42 °C	+	+	+	+	+	+	+	+	+	–	–	–	–
45 °C	–	–	–	–	–	+	+	+	+	–	–	–	–
Temperature optimum (°C)	30–35	30–35	30–40	30–35	30–37	25–30	30–40	ND	32	30–35	32	20–22	20–22
Growth in 15% NaCl	+	+	+	–	–	+	–	+	+	+	+	+	–
NaCl optimum (%)	2–5	2–5	1–8	1–4	2–3	3–5	3–6	7.5–10	3–5	7–10	3–5	3–6	3–6
Growth at pH 6	+	+	–	–	+	+	ND	ND	+	+	+	+	+
Susceptibility to:													
Ampicillin	+	+	ND	+	–	ND	ND	ND	+	ND	+	–	–
Carbenicillin	–	+	ND	+	–	ND	ND	ND	+	ND	+	–	–
Chloramphenicol	+	–	ND	+	+	ND	ND	ND	+	ND	+	ND	ND
Cephalothin	+	–	ND	+	–	ND	ND	ND	ND	ND	ND	ND	ND
Novobiocin	–	–	ND	+	–	ND	ND	ND	ND	ND	ND	ND	ND
DNA G + C content (mol%)	46.6	46.9	50.0	48.6–49.3	53.9	45.1	49.7	47.4	46.0	45.0	48.7	50.4	48.0

Cells are Gram-negative, rod-shaped, approximately 2.5–3.0 µm long and 0.5–0.9 µm wide, and non-motile. Colonies on PY agar plates at 30 °C for 7 days are approximately 4–6 mm in diameter, off-white, circular, convex and non-luminescent, with entire edges. Endospores are absent. Poly-β-hydroxybutyrate is not accumulated as an intracellular reserve product. Nitrate is not reduced to nitrite. Does not grow anaerobically by fermentation of glucose or other carbohydrates as substrates. NaCl is required for growth; growth occurs at 0.5–15% NaCl, with optimum growth at 2–5%. Growth occurs at 10–42 °C, with optimum growth at 30–35 °C; does not grow at 4 or 45 °C. Growth occurs at pH values between 6 and 10, with optimum growth at pH 7–8; does not grow at pH 5. Oxidase- and catalase-positive, but negative for arginine dihydrolase, lysine decarboxylase and ornithine decarboxylase. H<sub>2</sub>S is not produced from thiosulphate. DNA and gelatin are hydrolysed, but aesculin, agar, alginate, casein, starch, Tween 80 and urea are not. The following constitutive enzyme activities are positive in API ZYM tests: acid phosphatase, alkaline phosphatase, esterase (C4), esterase lipase (C8), lipase (C14), leucine arylamidase, valine arylamidase, cystine arylamidase, α-chymotrypsin, naphthol-AS-BI-phosphohydrolase (weakly positive) and trypsin. No reactions are observed in API 50 CH and Biolog GN2 tests. Isoprenoid quinones comprise Q-8 (95.2%) and Q-7 (4.8%). Cellular fatty acids present at levels of more than 3% include iso-C<sub>17:0</sub>, iso-C<sub>15:0</sub>, iso-C<sub>17:1ω9c</sub>, C<sub>18:0</sub>, C<sub>18:1ω7c</sub>, iso-C<sub>11:0</sub> 3-OH, C<sub>16:0</sub>, C<sub>16:1ω7c</sub> and/or iso-C<sub>15:0</sub> 2-OH, iso-C<sub>19:0</sub>, iso-C<sub>15:1</sub> F and iso-C<sub>13:0</sub> 3-OH. Susceptible to ampicillin (10 µg), cephalothin (30 µg), chloramphenicol (30 µg), colistin (10 µg), nalidixic acid (30 µg), and polymyxin B (300 U), and intermediately susceptible to penicillin G (10 U), tetracycline (30 µg) and vancomycin (30 µg). Resistant to carbenicillin (100 µg), clindamycin (2 µg), erythromycin (15 µg), gentamicin (10 µg), kanamycin (30 µg), lincomycin (2 µg), neomycin (30 µg), novobiocin (30 µg), oxacillin (1 µg) and streptomycin (10 µg).

The type strain, PIM1<sup>T</sup> (=BCRC 17749<sup>T</sup>=JCM 15083<sup>T</sup>), was isolated from shallow coastal seawater of An-Ping Harbour, Tainan, Taiwan. The DNA G+C content of the type strain is 46.6 mol%.

#### Description of *Pseudidiomarina tainanensis* sp. nov.

*Pseudidiomarina tainanensis* (tai.nan.en'sis. N.L. fem. adj. *tainanensis* pertaining to Tainan, Taiwan, where the type strain was isolated).

Cells are Gram-negative, rod-shaped, approximately 2.5–3.2 µm long and 0.6–0.9 µm wide, and non-motile. Colonies on PY agar plates at 30 °C for 7 days are approximately 3–6 mm in diameter, yellow in colour, circular, convex and non-luminescent, with entire edges. Endospores are absent. Poly-β-hydroxybutyrate is not accumulated as an intracellular reserve product. Nitrate is not reduced to nitrite. Does not grow anaerobically by

fermentation of glucose or other carbohydrates as substrates. NaCl is required for growth; growth occurs at 0.5–15% NaCl, with optimum growth at 2–5%. Growth occurs at 10–42 °C, with optimum growth at 30–35 °C; does not grow at 4 or 45 °C. Growth occurs at pH values between 6 and 10, with optimum growth at pH 7–8; does not grow at pH 5. Oxidase- and catalase-positive, but negative for arginine dihydrolase, lysine decarboxylase and ornithine decarboxylase. H<sub>2</sub>S is not produced from thiosulphate. Gelatin is hydrolysed, but aesculin, agar, alginate, casein, DNA, starch, Tween 80 and urea are not. The following constitutive enzyme activities are positive in API ZYM tests: acid phosphatase, alkaline phosphatase, esterase (C4), esterase lipase (C8), lipase (C14), leucine arylamidase, valine arylamidase, cystine arylamidase, α-chymotrypsin, naphthol-AS-BI-phosphohydrolase (weakly positive) and trypsin. No reactions are observed in API 50 CH and Biolog GN2 tests. Isoprenoid quinones comprise Q-8 (97.1%) and Q-7 (2.9%). Cellular fatty acids present at levels of more than 3% include iso-C<sub>15:0</sub>, iso-C<sub>17:0</sub>, iso-C<sub>17:1ω9c</sub>, iso-C<sub>11:0</sub> 3-OH, C<sub>18:0</sub>, iso-C<sub>13:0</sub> 3-OH, iso-C<sub>19:0</sub>, C<sub>16:1ω7c</sub> and/or iso-C<sub>15:0</sub> 2-OH, C<sub>16:0</sub> and C<sub>18:1ω7c</sub>. Susceptible to ampicillin (10 µg), carbenicillin (100 µg), colistin (10 µg) and polymyxin B (300 U), and intermediately susceptible to erythromycin (15 µg), gentamicin (10 µg), nalidixic acid (30 µg) and neomycin (30 µg). Resistant to cephalothin (30 µg), chloramphenicol (30 µg), clindamycin (2 µg), kanamycin (30 µg), lincomycin (2 µg), novobiocin (30 µg), oxacillin (1 µg), penicillin G (10 U), streptomycin (10 µg), tetracycline (30 µg) and vancomycin (30 µg).

The type strain, PIN1<sup>T</sup> (=BCRC 17750<sup>T</sup>=JCM 15084<sup>T</sup>), was isolated from shallow coastal seawater of An-Ping Harbour, Tainan, Taiwan. The DNA G+C content of the type strain is 46.9 mol%.

#### Description of *Pseudidiomarina homiensis* (Kwon *et al.* 2006) comb. nov.

*Pseudidiomarina homiensis* (ho.mi.en'sis. N.L. fem. adj. *homiensis* referring to the Homi Cape in Korea, where the type strain was isolated).

Basonym: *Idiomarina homiensis* Kwon *et al.* 2006.

The description is the same as that given for *Idiomarina homiensis* by Kwon *et al.* (2006). The type strain is PO-M2<sup>T</sup> (=DSM 17923<sup>T</sup>=KACC 11514<sup>T</sup>).

#### Description of *Pseudidiomarina salinarum* (Yoon *et al.* 2007) comb. nov.

*Pseudidiomarina salinarum* (sa.li.na'rum. L. gen. pl. *salinarum* of salt-works).

Basonym: *Idiomarina salinarum* Yoon *et al.* 2007.

The description is the same as that given for *Idiomarina salinarum* by Yoon *et al.* (2007). The type strain is ISL-52<sup>T</sup> (=CCUG 54359<sup>T</sup>=KCTC 12971<sup>T</sup>).

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