Idiomarina xiamenensis sp. nov., isolated from surface seawater, and proposal to transfer *Pseudidiomarina aestuarii* to the genus *Idiomarina* as *Idiomarina aestuarii* comb. nov.

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A taxonomic study was carried out on strain 10-D-4^T, which was isolated from a crude oil-degrading consortium enriched from surface seawater collected around Xiamen Island, PR China. Strain 10-D-4^T grew optimally at pH 7.0–8.0 and at 25 °C. The 16S rRNA gene sequence of strain 10-D-4^T showed the highest similarity to those of *Idiomarina salinarum* ISL-52^T (94.6%), *Idiomarina tainanensis* PIN1^T (94.2%) and *Idiomarina seosinensis* CL-SP19^T (94.1%), and showed lower similarity (92.3–94.0%) to other members of the genus *Idiomarina*. The major isoprenoid quinone was ubiquinone 8 (Q-8). The major fatty acids were iso-C_{13:0} (5.2%), iso-C_{15:0} (15.3%), C_{16:0} (14.3%), summed feature 3 (C_{16:1}ω6c and/or C_{16:1}ω7c) (6.6%), iso-C_{17:0} (15.4%) and C_{18:1}ω7c (13.5%). The G + C content of the chromosomal DNA was 50.4 mol%. Phylogenetic analysis based on 16S rRNA gene sequences, together with data from phenotypic and chemotaxonomic characterization, revealed that strain 10-D-4^T represents a novel species of the genus *Idiomarina*, for which the name *Idiomarina xiamenensis* sp. nov. is proposed. The type strain is 10-D-4^T (=CCTCC AB 209061^T =LMG 25227^T =MCCC 1A01370^T). We also propose the transfer of *Pseudidiomarina aestuarii*, described recently, to the genus *Idiomarina* as *Idiomarina aestuarii* comb. nov. (type strain KYW314^T =KCTC 22740^T =JCM 16344^T).

In an attempt to investigate crude oil-degrading bacteria from surface seawater around Xiamen Island, PR China, several bacterial strains were isolated and characterized taxonomically. This study focused on one of these isolates, designated strain 10-D-4^T. 16S rRNA gene sequence analysis indicated that strain 10-D-4^T formed a clade within the genus *Pseudidiomarina* (*Idiomarina*). The genus *Idiomarina* was first proposed by Ivanova *et al.* (2000), while the genus *Pseudidiomarina* was established later, by Jean *et al.* (2006). Both genera belong to the family *Idiomarinaceae* and, at the time of writing, nine species of *Idiomarina* and seven species of *Pseudidiomarina* had been described. However, it is not possible to distinguish the genera from each other by using the phenotypic or chemotaxonomic characteristics examined, except for differences in 16S rRNA gene sequence signature nucleotides, and Taborda *et al.* (2009) proposed

the transfer of the species classified in the genus *Pseudidiomarina* to the genus *Idiomarina*. Hence, the genus *Idiomarina* contained 16 species with validly published names. Since this proposal, another species, *Pseudidiomarina aestuarii*, has been described (Park *et al.*, 2010), which should also be transferred to the genus *Idiomarina* based on 16S rRNA gene sequence phylogeny and phenotypic properties, as proposed by Taborda *et al.* (2009). The aim of the present work was to determine the exact taxonomic position of strain 10-D-4^T by using a polyphasic method that included determination of phenotypic properties and a detailed phylogenetic analysis based on 16S rRNA gene sequences.

Surface seawater was sampled around Xiamen Island (24° 28' N 118° 11' E) in October 2005. Once collected, 150 ml seawater was subsampled into sterilized bottles. To start the enrichment, the seawater was supplemented with 1 ml sterilized crude oil (as a carbon source) and nitrogen, phosphorus and iron sources with concentrations as in NH medium (Wang *et al.*, 2010). Cultures were incubated at 25 °C with shaking (150 r.p.m.) for 10 days. Serial dilutions of enrichments were streaked on HLB agar plates (Liu & Shao, 2005) and incubated at 25 °C. Representative

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A supplementary figure and a supplementary table are available with the online version of this paper.

colonies were picked and restreaked onto HLB plates to obtain pure cultures. For morphological and biochemical characterization, strain 10-D-4^T was cultivated on marine agar 2216 (MA; BD Difco).

Genomic DNA was prepared according to the method of Ausubel *et al.* (1995) and the 16S rRNA gene was amplified by PCR using primers described previously (Liu & Shao, 2005). Identification of phylogenetic neighbours and calculation of pairwise 16S rRNA gene sequence similarities were achieved using the EzTaxon server (<http://www.eztaxon.org/>; Chun *et al.*, 2007). Sequences of related taxa were obtained from the GenBank database. Phylogenetic analysis was performed using MEGA version 4 (Tamura *et al.*, 2007) after multiple alignment of data by DNAMAN (version 5.1; Lynnon Biosoft). Distances (distance options according to Kimura's two-parameter model) and clustering with the neighbour-joining (Saitou & Nei, 1987) and minimum-evolution (Rzhetsky & Nei, 1992, 1993) methods were determined by using bootstrap values based on 1000 replications. As the result of the minimum-evolution analysis was similar to that obtained with the neighbour-joining method, the results are not shown.

A nearly full-length 16S rRNA gene sequence (1504 nt) of strain 10-D-4^T was obtained. Phylogenetic analysis indicated that strain 10-D-4^T belonged to the family *Idiomarinaceae*, forming a robust clade with members of the genus *Idiomarina* (Fig. 1). The closest related strain was *Idiomarina salinarum* ISL-52^T (94.6 % similarity), followed by *I. tainanensis* PIN1^T (94.2 %) and *I. seosinensis* CL-SP19^T (94.1 %), and lower similarities (92.3–94.0 %) were shown to other members of the genus *Idiomarina*. In general, 16S rRNA gene sequence divergence greater than 3 % is accepted as a criterion for delineating different species (Stackebrandt & Goebel, 1994). The 16S rRNA gene sequence divergence between strain 10-D-4^T and recognized species was >5.4 %, and the data therefore support the view that strain 10-D-4^T represents a novel species. In addition, [*Pseudidiomarina*]

aestuarii KYW314^T showed highest similarity (97.3 %) with *I. salinarum* ISL-52^T, and shared 93.7–96.7 % sequence identity with other *Idiomarina* species. As the species of the genus *Pseudidiomarina* were transferred to the genus *Idiomarina* by Taborda *et al.* (2009), [*Pseudidiomarina*] *aestuarii* should also be transferred to the genus *Idiomarina*. Differential characteristics and DNA–DNA hybridization results between [*Pseudidiomarina*] *aestuarii* KYW314^T and the closest type strains, *I. salinarum* ISL-52^T and *Idiomarina taiwanensis* PIT1^T, were shown by Park *et al.* (2010). As shown in Table 1, [*Pseudidiomarina*] *aestuarii* KYW314^T possesses the specific signature nucleotides of rRNA group 2 except for position 143. However, strain 10-D-4^T could not be assigned to either rRNA group, as half of the specific signature nucleotides differ from those of both rRNA groups, and we propose that it represents rRNA group 3 of the genus *Idiomarina*.

The Gram reaction, catalase, oxidase and lipase (Tween 80) activities, hydrolysis of aesculin and starch, optimal growth temperature and pH, tolerance of NaCl, antibiotic susceptibility and general cell morphology by electron microscopy were studied as described previously (Lai *et al.*, 2009). H₂S production was assessed according to Dong & Cai (2001). Other biochemical tests were carried out using API 20NE and API ZYM strips (bioMérieux) according to the manufacturer's instructions, except that the NaCl concentration was adjusted to 3.0 % in all tests. *I. salinarum* KCTC 12971^T and *I. tainanensis* PIN1^T were tested at the same time for comparison. These results are given in the species description and Table 2.

Analysis of the respiratory quinones of strain 10-D-4^T was carried out by the Identification Service of the DSMZ and Dr Brian Tindall (Braunschweig, Germany). The major quinone was ubiquinone 8 (Q-8; 97 %), in agreement with all members of the genus *Idiomarina*. Minor amounts of Q-7 (3 %) were also present. Fatty acids in whole cells grown on MA at 28 °C for 48 h were extracted, saponified

Fig. 1. Neighbour-joining tree showing the phylogenetic positions of strain 10-D-4^T and representatives of some other related taxa, based on 16S rRNA gene sequences. Bootstrap values (expressed as percentages of 1000 replications) are shown at branch points. Bar, 0.01 substitution rate (K_{nuc}) units.

Table 1. 16S rRNA gene sequence signature sites detected among strain 10-D-4^T and two groups found in the family *Idiomarinaceae* cluster

Signature sites for the family *Idiomarinaceae* are shown in bold; other entries indicate signature sites for each rRNA group. rRNA groups are numbered according to Taborda *et al.* (2009).

Group/strain	Position												
	143	206	213	241	285	653	662	658	682	748	830	856	1256
rRNA group 1	C	C	G	G	C	A	A	C	A	G	U	A	C
rRNA group 2	A	U	A	A	U	C	A	U	A (U)	A	U	A	A
Strain 10-D-4 ^T	T	U	A	A	U	A	G	C	A	G	U	A	C
[<i>P.</i>] <i>aestuarii</i> KYW314 ^T	T	U	A	A	U	C	A	U	A	A	U	A	A

and esterified; this was followed by GC analysis of the fatty acid methyl esters according to the instructions of the MIDI system (Sasser, 1997). *I. salinarum* KCTC 12971^T and *I. tainanensis* PIN1^T were analysed in parallel with

strain 10-D-4^T in this study. As shown in Supplementary Table S1 (available in IJSEM Online), the major fatty acids of strain 10-D-4^T were iso-C_{13:0} (5.2 %), iso-C_{15:0} (15.3 %), C_{16:0} (14.3 %), summed feature 3 (C_{16:1}ω6)

Table 2. Characteristics of strain 10-D-4^T and type strains of related *Idiomarina* species

Strains: 1, 10-D-4^T; 2, *I. salinarum* ISL-52^T (unless indicated, data from Yoon *et al.*, 2007); 3, *I. tainanensis* PIN1^T (unless indicated, data from Jean *et al.*, 2009); 4, [*P.*] *aestuarii* KYW314^T (Park *et al.*, 2010). Data on catalase and oxidase activities, API 20NE and API ZYM tests, tolerance of NaCl and antibiotic susceptibility were obtained in parallel for *I. salinarum* KCTC 12971^T and *I. tainanensis* PIN1^T in this study. In API 20NE tests, all strains were positive for gelatin hydrolysis and negative for indole production, D-glucose fermentation, β-glucosidase and β-galactosidase activities and utilization of all 12 carbon sources. In API ZYM tests, all strains were positive for alkaline phosphatase and α-chymotrypsin and negative for N-acetyl-β-glucosaminidase, α-fucosidase, α-galactosidase, α-glucosidase, α-mannosidase, β-galactosidase, β-glucosidase and β-glucuronidase. +, Positive; w, weakly positive; −, negative; ND, no data available.

Characteristic	1	2	3	4
Cell width (μm)	0.4–0.6	0.3–0.6	0.6–0.9	0.3
Cell length (μm)	1.2–1.7	0.8–3.5	2.5–3.2	0.7–1.4
Motility	+	+	−	−
Catalase	w	+	+	+
NaCl range (% w/v)	0–15	1–14	0.5–15	1–10
NaCl optimum (% w/v)	1–3	2–3	2–5	3
Temperature range (°C)	4–42	4–42	10–42	10–37
Temperature optimum (°C)	25	30–37	30–35	30
Nitrate reduction	−	+	−	−
Arginine dihydrolase	+	−	−	−
Urease	+	−	+	−
Hydrolysis of:				
Aesculin	+	−	−	+
Tween 80	−	+	−	+
API ZYM results				
Acid phosphatase, esterase lipase (C8), leucine aminopeptidase, naphthol-AS-BI-phosphoamidase	+	+	+	−
Cystine aminopeptidase, trypsin	w	+	+	−
Esterase (C4)	+	w	w	−
Lipase (C14), valine aminopeptidase	+	w	+	−
Susceptibility to:				
Cefobid, cephradine, gentamicin, minomycin	−	−	+	ND
Co-trimoxazole	−	+	+	ND
Vancomycin	−	+	+	−
Norfloxacin, ofloxacin	+	−	+	ND
Penicillin G	−	−	+	+
DNA G + C content (mol%)	50.4	53.9	46.9	56.4

and/or C_{16:1}ω7c (6.6%), iso-C_{17:0} (15.4%) and C_{18:1}ω7c (13.5%). This profile was similar to those of other members of the genus *Idiomarina*. However, strain 10-D-4^T can be differentiated from its closest relative *I. salinarum* KCTC 12971^T by the amounts of C_{16:0} (14.3 vs 7.8%), summed feature 8 (C_{18:1}ω7c and/or C_{18:1}ω6c) (13.5 vs 6.2%), C_{19:0} cyclo ω8c (4.0 vs 0%) and summed feature 9 (iso-C_{17:1}ω9c and/or 10-methyl C_{16:0}) (6.7 vs 23.5%). The proportions of most fatty acids in strain 10-D-4^T were within the ranges reported by Taborda *et al.* (2009) (Supplementary Table S1).

The G+C content of the chromosomal DNA was determined according to the method described by Mesbah & Whitman (1989) by reversed-phase HPLC analysis. The DNA G+C content of strain 10-D-4^T was 50.4 mol%, within the range of values reported for known *Idiomarina* species (45.0–56.4 mol%).

Phylogenetic analysis based on 16S rRNA gene sequences, physiological features, respiratory quinone and fatty acid profiles suggest that strain 10-D-4^T belongs to the genus *Idiomarina* and represents a novel species of this genus. Physiological features that differentiate strain 10-D-4^T from *I. salinarum* KCTC 12971^T include differences in nitrate reduction, arginine dihydrolase, urease and hydrolysis of aesculin and Tween 80. The name *Idiomarina xiamenensis* sp. nov. is proposed. It is also proposed that *Pseudidiomarina aestuarii* Park *et al.* 2010 is reclassified as *Idiomarina aestuarii* comb. nov.

Description of *Idiomarina xiamenensis* sp. nov.

Idiomarina xiamenensis (xia.me.nen'sis. N.L. fem. adj. *xiamenensis* of Xiamen, a city in Fujian, PR China, where the type strain was isolated).

Cells are rod-shaped, 1.2–1.7 µm long and 0.4–0.6 µm wide, motile by one polar flagellum (Supplementary Fig. S1). Positive for oxidase, catalase (weak), urease, β-glucosidase (aesculin hydrolysis), gelatinase and arginine dihydrolase, but negative for Gram reaction, amylase, indole production, lipase (Tween 80), β-galactosidase, H₂S production and reduction of nitrate to nitrite. On MA, forms smooth, grey colonies with regular edges, 1–2 mm in diameter after 72 h of incubation at 28 °C. Grows in 0–15% NaCl (optimum 1–3%), at 4–42 °C (optimum 25 °C) and at pH 6–10 (optimum pH 7–8). Unable to ferment glucose. The major isoprenoid quinone is ubiquinone 8 (Q-8). Principal fatty acids are iso-C_{13:0}, iso-C_{15:0}, C_{16:0}, summed feature 3 (C_{16:1}ω6c and/or C_{16:1}ω7c), iso-C_{17:0} and C_{18:1}ω7c. Sensitive to (µg per disc unless indicated; Oxoid) ampicillin (10), carbenicillin (100), cefazolin (30), chloramphenicol (30), ciprofloxacin (5), erythromycin (15), kanamycin (30), norfloxacin (10), ofloxacin (5), polymyxin B (30 IU), rifampicin (5) and rocephin (30); resistant to cefalexin (30), cefobid (30), cephradine (30), clindamycin (2), co-trimoxazole (25), furazolidone (15), gentamicin (10), lincomycin (2g), metronidazole (5), minomycin (30), neomycin (10), oxacillin (1), penicillin G (10), piperacillin

(100), streptomycin (10), tetracycline (30), vancomycin (30) and vibramycin (30). In API ZYM tests, positive for acid phosphatase, alkaline phosphatase, cystine aminopeptidase, esterase (C4), esterase lipase (C8), leucine aminopeptidase, lipase (C14) (weak), naphthol-AS-BI-phosphoramidase, trypsin (weak), valine aminopeptidase and α-chymotrypsin; negative for *N*-acetyl-β-glucosaminidase, α-fucosidase, α-galactosidase, α-glucosidase, α-mannosidase, β-galactosidase, β-glucosidase and β-glucuronidase. Cannot utilize any of the 12 carbon sources included in the API 20NE strip. The DNA G+C content of the type strain is 50.4 mol%. Table 1 shows characteristics that can be used to distinguish the species from related species.

The type strain, 10-D-4^T (=CCTCC AB 209061^T =LMG 25227^T =MCCC 1A01370^T), was isolated from surface seawater around Xiamen Island, PR China.

Description of *Idiomarina aestuarii* comb. nov.

Idiomarina aestuarii (aes.tu.a'ri.i. L. gen. n. *aestuarii* of the shallow coast, from where the type strain was isolated).

Basonym: *Pseudidiomarina aestuarii* Park *et al.* 2010.

The description is identical to that given for *Pseudidiomarina aestuarii* by Park *et al.* (2010). The type strain is KYW314^T (=KCTC 22740^T =JCM 16344^T), isolated from seawater collected from the South Sea, Republic of Korea.

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