Idiomarina maris sp. nov., a marine bacterium isolated from sediment

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A protease-producing marine bacterium, designated CF12-14^T, was isolated from sediment of the South China Sea. Phylogenetic analysis of the 16S rRNA gene sequence revealed that strain CF12-14^T formed a separate lineage within the genus *Idiomarina* (*Gammaproteobacteria*). The isolate showed the highest 16S rRNA gene sequence similarity with *Idiomarina salinarum* ISL-52^T (94.7%), *Idiomarina seosinensis* CL-SP19^T (94.6%) and other members of the genus *Idiomarina* (91.9–94.6%). Cells were Gram-negative, aerobic, flagellated, straight or slightly curved, and often formed buds and prosthecae. Strain CF12-14^T grew at 4–42 °C (optimum 30–35 °C) and with 0.1–15% (w/v) NaCl (optimum 2–3%). The isolate reduced nitrate to nitrite and hydrolysed DNA, but did not produce acids from sugars. The predominant cellular fatty acids were iso-C_{15:0} (27.4%), iso-C_{17:0} (16.0%) and iso-C_{17:1ω9c} (15.8%). The major polar lipids were phosphatidylethanolamine, diphosphatidylglycerol and phosphatidylglycerol. The major respiratory quinone was ubiquinone 8. The DNA G + C content was 50.4 mol%. The phylogenetic, phenotypic and chemotaxonomic data supported the conclusion that CF12-14^T represents a novel species of the genus *Idiomarina*, for which the name *Idiomarina maris* sp. nov. is proposed. The type strain is CF12-14^T (=CCTCC AB 208166^T=KACC 13974^T).

The family *Idiomarinaceae*, class *Gammaproteobacteria*, was proposed by Ivanova *et al.* (2004) according to the phylogenetic relationships of marine *Alteromonas*-like bacteria. The family formerly contained two genera, *Idiomarina* and *Pseudidiomarina*, which were proposed by Ivanova *et al.* (2000) and Jean *et al.* (2006), respectively. Recently, the genera *Idiomarina* and *Pseudidiomarina* were combined into a single genus *Idiomarina*, on the basis of the absence of distinguishing phenotypic and chemotaxonomic characteristics (Taborda *et al.*, 2009, 2010). At the time of writing, there were 18 species in the genus: *Idiomarina abyssalis* (type species) and *I. zobellii* (Ivanova *et al.*, 2000), *I. baltica* (Brettar *et al.*, 2003), *I. loihiensis* (Donachie *et al.*,

2003), *I. fontislapidosi* and *I. ramblicola* (Martínez-Cánovas *et al.*, 2004), *I. seosinensis* (Choi & Cho, 2005), *I. homiensis* (Kwon *et al.*, 2006), *I. taiwanensis* (Jean *et al.*, 2006), *I. sediminum* (Hu & Li, 2007), *I. salinarum* (Yoon *et al.*, 2007), *I. donghaiensis* and *I. maritima* (Wu *et al.*, 2009), *I. marina* and *I. tainanensis* (Jean *et al.*, 2009), *I. insulalsae* (Taborda *et al.*, 2009), *I. aestuarii* (Park *et al.*, 2010) and *I. xiamenensis* (Wang *et al.*, 2011). All members of the genus *Idiomarina* possess characteristically high contents of iso-branched cellular fatty acids and have been isolated from saline environments (Ivanova *et al.*, 2004; Jean *et al.*, 2006; Taborda *et al.*, 2009). Specifically, *I. salinarum* was isolated from a marine solar saltern, *I. seosinensis* was isolated from hypersaline water of a solar saltern and *I. abyssalis* was isolated from deep seawater (NW Pacific Ocean).

In the course of screening protease-producing bacteria from marine samples, a member of the family *Idiomarinaceae*, strain CF12-14^T, was isolated from a deep-sea sediment sample of the South China Sea (Zhou *et al.*, 2009). The sample was collected using a core sampler at site CF12

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Abbreviation: ASW, artificial seawater.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain CF12-14^T is EU723884.

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

(19° 44.94' N 114° 44.95' E) in the South China Sea at a water depth of 1153 m in August 2007. Isolation and purification of protease-producing strains were done as described previously (Zhou *et al.*, 2009). Strain CF12-14^T was routinely cultivated at 30 °C in TYS broth, containing 0.5 % tryptone (Oxoid), 0.1 % yeast extract (Oxoid) and artificial seawater (ASW: containing 2.75 % NaCl, 0.5 % MgCl₂, 0.2 % MgSO₄, 0.05 % CaCl₂, 0.1 % KCl, 0.0001 % FeSO₄; distilled water; pH 7.0; Smibert & Krieg, 1994), or on TYS agar (1.5 % agar), unless otherwise indicated, and was stored at -80 °C in TYS broth supplemented with 16 % (v/v) glycerol. *I. salinarum* DSM 21900^T, *I. seosinensis* DSM 21922^T and *I. abyssalis* DSM 15222^T were obtained from the DSMZ (Braunschweig, Germany) and used as reference strains in some experiments. The reference strains were routinely cultivated in TYS broth or on TYS agar at 30 °C (*I. salinarum* DSM 21900^T and *I. seosinensis* DSM 21922^T) or 25 °C (*I. abyssalis* DSM 15222^T).

The Gram-reaction was examined by the non-staining method (Buck, 1982). Cellular morphology and flagella production were observed using transmission electron microscopy (JEM-100 CXII; JEOL). Cells were prepared in broth medium containing 1 % tryptone, 0.5 % yeast extract and ASW (pH 7.0) at 30 °C for 24 h and negatively stained with 2 % phosphotungstic acid. Growth at 4–45 °C was determined in TYS broth. Growth at pH 4.0–13.0 was determined in broth medium containing 0.5 % tryptone, 0.1 % yeast extract, 1 % NaCl and distilled water, with the pH adjusted with 1 M HCl or 1 M NaOH. Growth with 0–18 % (w/v) NaCl was determined in TYS broth with ASW replaced by distilled water and appropriate amounts of NaCl. Growth under anaerobic conditions was determined in marine broth 2216 medium (Difco) at 28 °C for 20 days by using an anaerobic chamber (Forma 1029; Thermo Electron).

Accumulation of poly- β -hydroxyalkanoate was determined by light microscopy after staining with Sudan Black B. Oxidase activity was determined using commercial oxidase test strips (Merck). Catalase activity was detected by bubble production in 3 % (v/v) hydrogen peroxide. DNase activity was tested by using DNase test agar (Oxoid) prepared with ASW. Amylase and lipase activities were tested on TYS agar supplemented with 0.2 % (w/v) soluble starch and 1 % (v/v) Tween 40 or 80. Physiological and biochemical characteristics, including endospore formation, glucose oxidation and fermentation, and hydrolysis of casein, carboxymethyl-cellulose and chitin, were examined following standard methods (Smibert & Krieg, 1994). Susceptibility to antimicrobial agents was tested using the disc-diffusion method on TYS agar (Bauer *et al.*, 1966). Inhibition zones were observed after 2 days at 30 °C. Other enzyme activities and biochemical characteristics were detected using the API ZYM, API 20E and API 20NE systems (bioMérieux) according to the manufacturer's instructions except that cells were suspended in ASW. The GN2 MicroPlate system (Biolog) was used to determine utilization of various carbon substrates; ASW was used to prepare the inocula.

PCR amplification and sequencing of the 16S rRNA gene of strain CF12-14^T were performed as described by Hu & Li (2007). The obtained 16S rRNA gene sequence (1502 bp) was aligned with related sequences retrieved from GenBank using MEGA version 4.0 (Tamura *et al.*, 2007). Phylogenetic trees were generated with the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Fitch, 1971) methods using MEGA. Evolutionary distances were calculated according to the model of Jukes & Cantor (1969). Bootstrap analysis based on 1000 replications was used to estimate the confidence level of the tree topologies.

The genomic DNA G+C content was determined by HPLC (Mesbah *et al.*, 1989) by the Identification Service of the DSMZ. Respiratory lipoquinone analysis was carried out by the Identification Service and B. J. Tindall (DSMZ).

For cellular fatty acid analysis, cells were prepared in TYS broth for 24 h at 30 °C (strain CF12-14^T, *I. salinarum* DSM 21900^T and *I. seosinensis* DSM 21922^T) or 25 °C (*I. abyssalis* DSM 15222^T). Cellular fatty acid methyl esters were obtained from lyophilized cells (approx. 140 mg) by saponification, methylation and extraction according to the protocol of the Sherlock Microbial Identification System (MIDI), separated by GC (Hewlett Packard 6890) and identified with Sherlock Microbial Identification System software (version 4.5 and the TSBA40 database) at the Institute of Microbiology and Epidemiology, Academy of Military Medical Sciences, Beijing, PR China.

Polar lipid analysis of strain CF12-14^T and *I. abyssalis* DSM 15222^T was performed by two-dimensional TLC. Polar lipids were extracted according to Komagata & Suzuki (1987) and separated on Kieselgel 60 F₂₅₄ plates (10 × 10 cm; Merck) (Collins & Jones, 1980). The solvent for the first dimension was chloroform/methanol/water (65:25:4, by vol.) and that for the second dimension was chloroform/methanol/acetic acid/water (80:12:15:4, by vol.). Lipid spots were visualized by spraying with 10 % molybdophosphoric acid in ethanol followed by heating at 110 °C for 15 min and further characterized by spraying with specific reagents, including ninhydrin (aminolipids), Zinzadze reagent (phospholipids) and anisaldehyde/sulfuric acid (glycolipids).

Phylogenetic analysis based on 16S rRNA gene sequences revealed that strain CF12-14^T belonged to the family *Idiomarinaceae* in the class *Gammaproteobacteria*. Strain CF12-14^T had highest 16S rRNA gene sequence similarities with *I. salinarum* ISL-52^T (94.7 %), *I. seosinensis* CL-SP19^T (94.6 %) and other members of the genus *Idiomarina* (91.9–94.6 %). Sequence similarities with members of genera outside the family *Idiomarinaceae* were <91.9 %. The neighbour-joining tree (Fig. 1) showed that strain CF12-14^T belonged to the cluster comprising the genus *Idiomarina* (100 % bootstrap support) and formed a separate lineage within the cluster. The maximum-parsimony tree exhibited a similar tree topology (data not shown). Thus, it is proposed that strain CF12-14^T represents a novel species within the genus *Idiomarina*. Furthermore, in accordance

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the phylogenetic position of strain CF12-14^T with members of the genus *Idiomarina* and some related genera in the class *Gammaproteobacteria*. Bootstrap values ($\geq 70\%$) based on 1000 replicates are shown at branch nodes. Bar, 0.01 substitutions per nucleotide position.

with its phylogenetic position, strain CF12-14^T displayed a distinctive pattern of signature nucleotides at various positions of the 16S rRNA gene sequence as compared with the three rRNA groups described for the genus *Idiomarina* (Taborda *et al.*, 2009; Wang *et al.*, 2011; Supplementary Table S1, available in IJSEM Online), and hence could be assigned to rRNA group 4 of the genus *Idiomarina* (Fig. 1).

The major cellular fatty acids of strain CF12-14^T were iso-C_{15:0} (27.4%), iso-C_{17:0} (16.0%), iso-C_{17:1ω9c} (15.8%), iso-C_{15:0} 3-OH (6.2%) and C_{18:1ω7c} (6.1%) (Table 1). This predominance of iso-branched fatty acids has been found in members of the genus *Idiomarina* (Ivanova *et al.*, 2000, 2004; Brettar *et al.*, 2003; Donachie *et al.*, 2003; Martínez-Cánovas *et al.*, 2004; Choi & Cho, 2005; Jean *et al.*, 2006; Kwon *et al.*, 2006; Hu & Li, 2007; Yoon *et al.*, 2007; Wu *et al.*, 2009; Taborda *et al.*, 2009; Jean *et al.*, 2009; Park *et al.*, 2010; Wang *et al.*, 2011); however, strain CF12-14^T could be differentiated from other members of the genus *Idiomarina* as it contained iso-C_{15:0} 3-OH (6.2%). Strain CF12-14^T possessed three major polar lipids, i.e. phosphatidylethanolamine, diphosphatidylglycerol and phosphatidylglycerol, which were in accordance with the genus *Idiomarina* (Taborda *et al.*, 2009). The isolate also contained minor amounts of three phospholipids and an unknown aminophospholipid (Supplementary Fig. S1, available in IJSEM Online). The DNA G+C content was 50.4 mol%, which was in the range (45.0–56.4 mol%) reported for the genus *Idiomarina*. The major respiratory quinone of strain CF12-14^T was ubiquinone 8, as observed for the genus *Idiomarina* (Taborda *et al.*, 2009). Overall, the above chemotaxonomic data confirmed the assignment of strain CF12-14^T to the genus *Idiomarina*.

Cells of strain CF12-14^T were Gram-negative, flagellated, straight or slightly curved, and able to produce buds and prosthecae (Fig. 2). Other phenotypic characteristics of strain CF12-14^T, including morphological, physiological

and biochemical data, are given in the species description and Table 2. CF12-14^T exhibited many phenotypic characteristics commonly exhibited by the genus *Idiomarina*, such

Table 1. Cellular fatty acid compositions of strain CF12-14^T and related *Idiomarina* species

Strain: 1, CF12-14^T; 2, *I. salinarum* DSM 21900^T; 3, *I. seosinensis* DSM 21922^T; 4, *I. abyssalis* DSM 15222^T. All data are from this study. Values <1% are not shown.

Fatty acid (%)	1	2	3	4
C _{14:0}	—	—	1.4	—
C _{15:0}	—	1.1	3.4	1.4
C _{16:0}	2.9	4.3	8.3	8.1
C _{17:0}	2.3	3.8	3.0	4.0
C _{18:0}	3.5	1.5	2.3	3.1
iso-C _{11:0}	—	3.1	2.0	1.9
iso-C _{13:0}	—	1.9	1.3	1.1
iso-C _{15:0}	27.4	18.3	34.0	23.7
iso-C _{16:0}	—	2.7	1.2	1.1
iso-C _{17:0}	16.0	15.8	8.4	12.2
C _{17:0} cyclo	—	2.1	5.5	3.1
iso-C _{11:0} 3-OH	5.0	5.4	3.5	3.5
iso-C _{13:0} 3-OH	—	3.9	3.7	4.2
iso-C _{15:0} 3-OH	6.2	—	—	—
iso-C _{17:1ω9c}	15.8	16.7	5.9	14.1
iso-C _{15:1} F	3.4	4.5	3.8	2.9
Summed feature 3*	3.4	2.6	1.6	1.3
C _{17:1ω8c}	—	2.1	—	1.4
C _{18:1ω7c}	6.1	3.5	1.6	4.5
11-Methyl C _{18:1ω7c}	—	—	1.7	—
C _{18:1ω9c}	2.0	—	—	—

*Summed features represent two or three fatty acids that cannot be separated by the Microbial Identification System. Summed feature 3 consisted of C_{16:1ω7c} and/or iso-C_{15:0} 2-OH.

Fig. 2. Transmission electron micrographs of negatively stained cells of strain CF12-14^T showing a polar flagellum (F), prosthecae (P) and buds (B). Bars: a, 0.5 μ m; b, 1 μ m.

as a poor ability to utilize carbohydrates, broad ranges of temperature and NaCl concentrations for growth, the requirement of NaCl for growth, the absence of arginine dihydrolase activity, and the presence of oxidase and catalase activities (Ivanova *et al.*, 2004; Jean *et al.*, 2006; Taborda *et al.*, 2009). However, the isolate also differed from the reference strains in a combination of some phenotypic characteristics, including production of buds and prosthecae, temperature range and optimum for growth, growth with 15 % (w/v) NaCl, hydrolysis of different substrates, enzyme production (API ZYM), carbon source utilization pattern (Biolog), susceptibility to kanamycin and genomic DNA G+C content.

On the basis of 16S rRNA gene sequence analysis, and chemotaxonomic and phenotypic characterization, strain CF12-14^T should be assigned to the genus *Idiomarina* as a representative of a novel species, for which the name *Idiomarina maris* sp. nov. is proposed.

Description of *Idiomarina maris* sp. nov.

Idiomarina maris (ma'ris. L. gen. n. *maris* of the sea, isolated from sediment of the South China Sea).

Cells are Gram-negative, straight or slightly curved rods. Cells can produce buds and prosthecae. Motile with a polar flagellum. Oxidase- and catalase-positive. Colonies are circular, slightly convex, white to light yellow and about 1.0–1.5 mm in diameter after 48 h at 30 °C. Cells are approximately 0.3–0.6 μ m wide and 0.8–2.4 μ m long. No

Table 2. Differential characteristics of strain CF12-14^T and closely related members of the genus *Idiomarina*

Strain: 1, *Idiomarina maris* sp. nov. CF12-14^T; 2, *I. salinarum* DSM 21900^T (Yoon *et al.*, 2007); 3, *I. seosinensis* DSM 21922^T (Choi & Cho, 2005); 4, *I. abyssalis* DSM 15222^T (Ivanova *et al.*, 2000). All strains are Gram-negative and oxidase-positive, hydrolyse DNA and reduce nitrate to nitrite. None of the strains hydrolyse starch or produce arginine dihydrolase. +, Positive; w, weakly positive; –, negative.

Characteristic	1	2	3	4
Buds and prosthecae	+	–	–	–
Temperature for growth (°C):				
Range	4–42	4–42	4–40	4–30
Optimum	30–35	30–37	30–35	20–22
Growth with 15 % (w/v) NaCl*	+	–	+	+
Hydrolysis of:*				
Casein	+	–	–	+
Aesculin	–	–	+	–
Gelatin	+	–	+	+
API ZYM*				
Trypsin	+	+	–	–
Acid phosphatase	w	+	+	+
Naphthol-AS-BI-phosphohydrolase	w	+	+	+
Biolog*				
D-Glucuronic acid	–	+	–	–
L-Alanine	–	–	–	+
L-Glutamic acid	–	–	+	–
L-Leucine	+	+	+	–
Glycerol	–	–	–	+
Susceptibility to kanamycin*	+	–	–	–
DNA G+C content (mol%)	50.4	53.9	45.0	50.4

*Data from this study.

endospores are formed. Accumulation of poly- β -hydroxy-alkanoate is not observed. Growth occurs at 4–42 °C, good growth is observed at 25–40 °C and optimum growth is observed at 30–35 °C. Growth occurs with 0.1–15.0 % (w/v) NaCl (optimum 2.0–3.0 %), but not with 0 or 18.0 % NaCl, and at pH 6.0–11.5 (optimum pH 8.0–9.5). Anaerobic growth is not observed. Hydrolyses casein, DNA and Tween 40 but not aesculin (API 20NE), carboxymethyl-cellulose, chitin, starch or Tween 80. Nitrate is reduced to nitrite (API 20NE). Negative for Voges–Proskauer (API 20E) and glucose oxidation/fermentation tests. Indole and H₂S are not produced (API 20E). With API ZYM and API 20E, positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, trypsin, α -chymotrypsin, acid phosphatase (weak), naphthol-AS-BI-phosphohydrolase (weak) and gelatinase, but negative for lipase (C14), valine arylamidase, cystine arylamidase, α - and β -galactosidases, β -glucuronidase, α - and β -glucosidases, *N*-acetyl- β -glucosaminidase, α -mannosidase, α -fucosidase, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, tryptophan deaminase and urease. With API 20E, does

not produce acid from glucose, mannitol, inositol, sorbitol, rhamnose, sucrose, melibiose, amygdalin or arabinose and does not utilize citrate. With API 20NE, does not assimilate glucose, arabinose, mannose, mannitol, *N*-acetylglucosamine, maltose, gluconate, decanoate, adipic acid, malic acid, citrate or phenylacetic acid. With the GN2 MicroPlate system, utilizes only L-leucine (30 °C for 24 h). Sensitive to (µg per disc) carbenicillin (100), chloramphenicol (30), ampicillin (10), cefazolin (30), gentamicin (10), kanamycin (30), streptomycin (10) and nalidixic acid (30), but resistant to vancomycin (30), lincomycin (2), doxycycline hydrochloride (30), tetracycline (30) and minocycline (30). Ubiquinone 8 is the major respiratory quinone. The major fatty acids are iso-C_{15:0}, iso-C_{17:0} and iso-C_{17:1ω9c}. The major polar lipids are phosphatidylethanolamine, diphosphatidylglycerol and phosphatidylglycerol.

The type strain is CF12-14^T (=CCTCC AB 208166^T =KACC 13974^T), isolated from deep-sea sediment of the South China Sea. The DNA G+C content of the type strain is 50.4 mol%.

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