Pseudidiomarina sediminum sp. nov., a marine bacterium isolated from coastal sediments of Luoyuan Bay in China

Zong-Yun Hu^{1,2} and Yun Li^{1,3}

Correspondence

Yun Li

yunlisun@ouc.edu.cn

¹Laboratory of Microbiology, Ocean University of China, 5 Yushan Road, Qingdao 266003, PR China

²Freshwater Fisher Science Institute of Liaoning Province, 103 Weiguo Road, Liaoyang 111000, PR China

³Laboratory of Molecular Medicine, Ocean University of China, 5 Yushan Road, Qingdao 266003, PR China

A strain of heterotrophic, facultatively anaerobic, marine bacterium, designated strain c121^T, was isolated from coastal sediment of Luoyuan Bay, in Fujian province, PR China. Analysis of the 16S rRNA gene sequence revealed an affiliation with the genus *Pseudidiomarina*; the sequence similarity between c121^T and *Pseudidiomarina taiwanensis* PIT1^T was 97 %. Cells of the novel strain were non-pigmented, Gram-negative rods, 0.3 µm wide and 1.2–1.8 µm long. Cells grown in broth cultures were non-motile, lacking flagella. Growth of the strain was observed at salinities ranging from 0.5 to 15 % NaCl, and the optimal concentration was about 1–8 %. The temperature range for growth was rather broad and was high for a marine bacterium: the strain grew at 13–42 °C, showed good growth at 20–40 °C and had an optimum between 30 and 40 °C. The major cellular fatty acids were iso-C_{15:0} (24.2 %), C_{16:1ω7c}/iso-C_{15:0} 2-OH (15.3 %) and iso-C_{17:1ω9c} (11.9 %). The DNA G + C content was 50.0 mol%. Phylogeny based on 16S rRNA gene sequences, together with data from phenotypic and chemotaxonomic characterization, revealed that strain c121^T could be classified within a novel species of the genus *Pseudidiomarina*, for which the name *Pseudidiomarina sediminum* sp. nov. is proposed, with the type strain c121^T (=CICC 10319^T =LMG 24046^T).

Since the genus *Idiomarina* was first proposed by Ivanova *et al.* (2000), the seven species *Idiomarina abyssalis* (the type species), *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) and *I. seosinensis* (Choi & Cho, 2005) have been described. A recent phylogenetic study based on 16S rRNA gene sequences placed the genus *Idiomarina* in the family *Idiomarinaceae* (Ivanova *et al.*, 2004), a member of the class *Gammaproteobacteria*. The genus *Pseudidiomarina*, a new member of the family *Idiomarinaceae*, was first proposed by Jean *et al.* (2006). At the time of writing, the genus *Pseudidiomarina* contained only one species, *Pseudidiomarina taiwanensis*, which was isolated from seawater samples collected from coastal water of An-Ping Harbour, Taiwan (Jean *et al.*, 2006).

During a study of the microbial diversity associated with the marine environment in April 2006, a sediment sample

was collected from a coastal region of Luoyuan Bay in Fujian province, PR China. Aliquots of the diluted sediment sample were spread on plates of medium 2216E (5 g Bacto peptone, 1 g Bacto yeast extract and 0.1 g FePO₄ in 1000 ml seawater, pH 7.6–7.8). The plates were incubated at 28 °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 2216E plates. Cultures of the isolates in medium 2216E were maintained at 28 °C under aerobic conditions. One of the isolates, designated strain c121^T, is characterized in the present study.

Strain c121^T was tested for a number of key characteristics, such as Gram reaction (Gram-staining, Gram string test), cell size and morphology (phase-contrast microscopy, electron microscopy after negative staining with 1 % uranyl acetate), using standard procedures (Gerhardt *et al.*, 1994). Motility was observed in a hanging-drop preparation under a ×100 objective lens with oil immersion after 24 h incubation in 2216E medium. Single colonies removed from 2216E plates were tested for catalase and

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain c121^T is EF212001.

cytochrome oxidase activities with 3 % hydrogen peroxide (Sigma) and tetramethyl-*p*-phenylenediamine, respectively. Nitrate reductase activity was tested in nitrate broth that contained 0, 3 or 7.5 % (w/v) NaCl. Amylase activity was tested on starch medium (Difco) with a range of NaCl concentrations from 0 to 7.5 % (w/v) by flooding plates with iodine after 7 days growth at 28 °C. DNA hydrolysis was determined on DNase test agar with methyl green (Difco) and gelatinase activity was checked in gelatinase nutrient medium (Difco), both with 2–7 % (w/v) NaCl. Poly- β -hydroxybutyrate accumulation and H₂S 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.* (2003). Furthermore, acid production from glucose, ribose and arabinose and hydrolysis of starch, gelatin, Tween 80 and casein were tested. Chitinase activity was tested as described by Cottrell *et al.* (2000). Growth at different temperatures was assessed at 4–60 °C. Growth at different salinities was tested at 0–20 % NaCl (w/v). Growth at different pH values was tested at pH 4–11; the final pH was adjusted using NaOH and HCl solutions. For these tests, half-strength marine broth (Difco; catalogue no. 2216) was used, except for the salinity test, where half-strength Caso medium (DSMZ catalogue no. 220) was supplemented with the appropriate amount of NaCl. Growth under anaerobic conditions was determined after inoculation in an anaerobic chamber using marine broth (Difco) and marine broth supplemented with nitrate at a final concentration of 10 mM in the dark for up to 18 days at 28 °C. As a positive control, *Escherichia coli* JM109, able to use the electron acceptors provided, was used for comparisons. Strain c121^T was additionally characterized by using the whole test spectrum of the identification systems API 20E and API 20NE (bioMérieux) at 20 °C and VITEK 2 GN (bioMérieux) at 28 °C.

Genomic DNA was extracted from 2216E broth cultures (after 24 h) by using the Genomic DNA kit (Qiagen). A

~1.4 kbp fragment of the 16S rRNA gene was amplified by PCR with DNA polymerase and primers 27F and 1492R (Mullis & Faloona, 1987; Lane, 1991). The PCR product corresponding to the 16S rRNA sequence of strain c121^T was purified by using a QIAquick PCR purification kit (Qiagen) and cloned with the pUCm-T vector kit (Sangon). Sequencing of the 16S rRNA gene was performed with an Applied Biosystems automatic sequencer (ABI3730XL) at Sangon (China). The resultant 16S rRNA gene sequence was aligned manually against sequences obtained from the GenBank database using a BLAST search. Phylogenetic trees were obtained by using neighbour-joining (Saitou & Nei, 1987), maximum-parsimony (Fitch, 1971) and maximum-likelihood (Felsenstein, 1981) methods. An evolutionary distance matrix for the neighbour-joining method was generated according to the model of Jukes & Cantor (1969).

Genomic DNA was extracted from 2216E broth cultures (after 24 h) by using the Genomic DNA kit (Qiagen). DNA G+C content was determined using the LightCycler as described by Xu *et al.* (2000).

Cellular fatty acid methyl esters in whole cells grown at 30 °C for 2 days on 2216E plates were analysed by GC, according to the instructions of the MIDI System (Sasser, 1990). This analysis was done at the Institute of Microbiology and Epidemiology, Academy of Military Medical Sciences, Beijing, PR China.

Cells of strain c121^T were Gram-negative rods, 0.3 μ m wide and 1.2–1.8 μ m long, without flagella (Fig. 1). No endospores were observed. Colonies on marine 2216 agar were non-pigmented, circular, smooth and convex with an entire edge. Cells grown in broth cultures were non-motile. The strain was negative in the oxidation/fermentation test. The strain was positive for oxidase and lipase and negative for catalase, aminopeptidase, DNase, amylase, chitinase and nitrate reductase activities. Growth was observed between 13 and 42 °C. The strain showed good growth between 20 and 40 °C and optimum growth between 30 and 40 °C. Growth of strain c121^T was observed in the

Fig. 1. Electron micrographs of negatively stained cells of strain c121^T. Bars, 200 nm (a) and 500 nm (b).

Table 1. Phenotypic characteristics useful for differentiating strain c121^T from *P. taiwanensis* PIT1^T and *Idiomarina* species

Strains: 1, c121^T (*Pseudidiomarina sediminum* sp. nov.; data from this study); 2, *P. taiwanensis* PIT1^T (data from Jean *et al.*, 2006); 3, *I. baltica* OS145^T (Brettar *et al.*, 2003); 4, *I. loihensis* L2-TR^T (Donachie *et al.*, 2003); 5, *I. fontislapidosi* F23^T (Martínez-Cánovas *et al.*, 2004); 6, *I. seosinensis* CL-SP19^T (Choi & Cho, 2005); 7, *I. ramblicola* R22^T (Martínez-Cánovas *et al.*, 2004); 8, *I. abyssalis* KMM 227^T (Ivanova *et al.*, 2000); 9, *I. zobellii* KMM 231^T (Ivanova *et al.*, 2000). +, Positive; –, negative; ND, no data available. All strains grow at 15–30 °C and in 1–10 % NaCl, are positive for oxidase and gelatinase activities and are negative for agarase and amylase activities.

Characteristic	1	2	3	4	5	6	7	8	9
Monotrichous flagellation	–	–	+	+	+	+	+	+	+
Motility	–	–	+	+	+	+	+	+	+
Nitrate reduction	–	+	–	+	–	+	–	+	–
Production of H ₂ S	–	–	+	ND	+	ND	+	ND	ND
Acid from D-glucose	–	–	+	–	–	–	–	–	+
Catalase	–	+	+	+	+	+	+	+	+
Hydrolysis of:									
DNA	–	–	ND	+	+	+	+	+	+
Tween 80	+	+	+	+	+	+	+	–	–
Growth at:									
10 °C	–	–	+	+	+	+	–	+	+
40 °C	+	+	+	+	+	+	+	–	–
42 °C	+	+	+	+	+	–	–	–	–
45 °C	–	–	+	+	+	–	–	–	–
Optimum temperature (°C)	30–40	30–35	30–40	ND	32	30–35	32	20–22	20–22
Growth in 12–15 % NaCl	+	–	–	+	+	+	+	+	–
Optimum NaCl concentration (%)	1–8	1–4	3–6	7.5–10	3–5	7–10	3–5	3–6	3–6
DNA G + C content (mol%)	50.0	49.3	49.7	47.4	46.0	45.0	48.7	50.4	48.0

presence of 0.5–15 % NaCl, with optimum growth between 1 and 8 % NaCl. Growth occurred under anaerobic conditions in marine broth and marine broth supplemented with nitrate. Strain c121^T was able to grow in marine broth at pH 6.5–10.5, with optimum growth at pH 8.0–9.0. No growth was observed below pH 6.5 or above pH 11.0. Strain c121^T could not produce H₂S from cysteine. The strain hydrolysed gelatin and Tween 80. Acid production was not observed from D-glucose,

D-ribose or D-arabinose. Acid was produced from maltose, and maltose could be used by acidification. The strain could not ferment glucose. The strain was not able to use the substrates provided by the API 20NE system, except gelatin and *p*-nitrophenyl β-D-galactopyranoside. The strain showed a narrow spectrum of enzyme activities and was positive for β-galactosidase, protease (gelatinase) (API 20NE), phosphatase, lipase, glutamyl arylamidase, maltose assimilation, L-proline arylamidase, tyrosine

Fig. 2. Unrooted phylogenetic tree constructed from 16S rRNA gene sequences of strain c121^T, *P. taiwanensis* PIT1^T and representatives of related genera of the *Gammaproteobacteria*. Numbers at nodes are percentages of bootstrap support based on neighbour-joining analysis of 1000 replicates with Kimura's two-parameter distance correction. Bar, 0.01 substitutions per nucleotide position.

arylamidase, succinate alkalization, glycine arylamidase, Ala-Phe-Pro arylamidase and Glu-Gly-Arg arylamidase activities (VITEK 2 GN). Strain c121^T was negative for catalase and nitrate reductase activities, in contrast to *P. taiwanensis*, which was reported as positive for these activities. More detailed phenotypic characteristics which are useful for differentiating strain c121^T from *P. taiwanensis* and *Idiomarina* species are listed in Table 1.

General phylogenetic analysis based on the 16S rRNA gene sequence of the novel strain (Fig. 2) revealed that it was a member of the *Gammaproteobacteria*. More-detailed analyses showed that the novel strain formed a robust cluster

with *P. taiwanensis* PIT1^T. The 16S rRNA sequence similarity of strain c121^T to *P. taiwanensis* PIT1^T was 97 %, while similarity to species of the genus *Idiomarina* was less than 96 %. As strain c121^T was clearly an outgroup to the genus *Pseudidiomarina*, according to phylogenetic principles, it could be described as representing a novel species of the genus *Pseudidiomarina*. Phenotypic traits, especially the fatty acid profile, suggested that strain c121^T could be included in the genus *Pseudidiomarina*. The DNA G+C content determined for strain c121^T was 50.0 mol%.

One distinctive feature of the genera *Pseudidiomarina* and *Idiomarina* in the family *Idiomarinaceae* is their uniquely

Table 2. Cellular fatty acid contents (%) of strain c121^T, *P. taiwanensis* PIT1^T and type strains of *Idiomarina* species

Strains: 1, c121^T (*P. sediminum* sp. nov.; data from this study); 2, *P. taiwanensis* PIT1^T (data from Jean *et al.*, 2006); 3, *I. baltica* OS145^T (Brettar *et al.*, 2003); 4, *I. loihiensis* L2-TR^T (Donachie *et al.*, 2003); 5, *I. fontislapidosi* F23^T (Martínez-Cánovas *et al.*, 2004); 6, *I. seosinensis* CL-SP19^T (Choi & Cho, 2005); 7, *I. rambicola* R22^T (Martínez-Cánovas *et al.*, 2004); 8, *I. abyssalis* KMM 227^T (Ivanova *et al.*, 2000); 9, *I. zobellii* KMM 231^T (Ivanova *et al.*, 2000). —, Not detected/not reported; tr, less than 1 %.

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

high content of iso-branched fatty acids (Ivanova *et al.*, 2000, 2004; Jean *et al.*, 2006). The profile for strain c121^T also shows a predominance of iso-branched fatty acids; strain c121^T contained iso-C_{15:0} (24.2%), C_{16:1}ω7c/iso-C_{15:0} 2-OH (15.3%) and iso-C_{17:1}ω9c (11.9%) as the major cellular fatty acids (Table 2). Some fatty acids, i.e. iso-C_{15:1} I/C_{13:0} 3-OH, C_{15:1}ω6c, C_{15:0}, iso-C_{16:0} and C_{16:1}ω9c, that were found in minor quantities were unique to strain c121^T among *Pseudidiomarina* and *Idiomarina* species (Table 2). This observation may have been caused by different experimental conditions, e.g. cultivation conditions or analytical equipment. However, the fatty acid pattern of strain c121^T differs distinctly from those of previously described species of *Pseudidiomarina* and *Idiomarina*.

Phylogenetic analysis based on 16S rRNA gene sequences, physiological features and fatty acid profiles suggests that strain c121^T belongs to the genus *Pseudidiomarina* and represents a novel species of this genus. The name *Pseudidiomarina sediminum* sp. nov. is proposed for the novel species. Physiological features that differentiate *P. sediminum* from *P. taiwanensis* are differences in enzyme activities, such as catalase and nitrate reductase, and the spectrum of utilizable substrates. *P. sediminum* can grow over wider ranges of temperature and salinity than can *P. taiwanensis*. All species of the *Idiomarinaceae* have comparable patterns of fatty acids dominated by iso-branched fatty acids, with *P. sediminum* showing a similar profile.

Description of *Pseudidiomarina sediminum* sp. nov.

Pseudidiomarina sediminum (se.di.mi'num. L. gen. pl. n. *sediminum* of sediments, pertaining to source of isolation of the type strain).

Cells are Gram-negative, facultatively anaerobic and rod-shaped, approximately 0.3 µm wide and 1.2–1.8 µm long. They are non-motile, lacking flagella. Growth occurs under anaerobic conditions in marine broth and marine broth supplemented with nitrate. No endospores are formed. Colonies on marine 2216 agar are non-pigmented, circular, smooth and convex with an entire edge. Organic growth factors are not required. Growth occurs at 0.5–15% NaCl but there is no growth at 20% NaCl. Growth temperature ranges from 13 to 42 °C, with optimum growth at 30–40 °C. No growth is detected at 45 °C. pH range for growth is 6.3–10.5, with optimum growth at pH 8.0–9.0. Positive for oxidase, gelatinase and lipase and negative for catalase, DNase, amylase, caseinase, chitinase and agarase. Able to utilize a limited range of carbohydrates. Of the tests in the VITEK 2 GN system, the type strain produces phosphatase, lipase, glutamyl arylamidase, L-proline arylamidase, tyrosine arylamidase, succinate alkalization, glycine arylamidase, Ala-Phe-Pro arylamidase and Glu-Gly-Arg arylamidase activities. Maltose is utilized. The main cellular fatty acids are iso-C_{15:0} (24.2%), C_{16:1}ω7c/iso-C_{15:0} 2-OH (15.3%) and iso-C_{17:1}ω9c (11.9%). Belongs to the class *Gammaproteobacteria*, based on 16S rRNA gene sequences. The DNA G+C content of the type strain is 50.0 mol%.

The type strain is c121^T (=CICC 10319^T =LMG 24046^T), isolated from coastal sediment.

Acknowledgements

We thank Mr J. T. Jia, from the Qingdao Branch of the China Import and Export Commodity Inspection Bureau, for help during this work. This work was supported by the 863 program 2004AA626090 funded by MOST, PR China.

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