Idiomarina homiensis sp. nov., isolated from seashore sand in Korea

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A halophilic, aerobic bacterium, designated PO-M2^T, was isolated from seashore sand, from Pohang, Korea and characterized on the basis of physiological and biochemical features. Phylogenetic analysis of 16S rRNA gene sequences revealed a clear affiliation of the novel strain with members of the genus *Idiomarina*. Sequence similarities between strain PO-M2^T and the type strains of species belonging to the genus *Idiomarina* ranged from 94.3 to 95.5 %. Cells of strain PO-M2^T were straight or slightly curved rods and formed light-yellow colonies on marine agar medium. The major isoprenoid quinone was ubiquinone (Q-8) and the predominant cellular fatty acids were C_{15:0} iso (19.3 %), C_{17:1}ω9c iso (11.9 %), C_{17:0} iso (10.9 %), C_{18:1}ω7c (10.4 %), C_{16:0} (9.0 %) and C_{16:1}ω7c and/or C_{15:0} iso 2-OH (7.2 %). The G + C content of the DNA was 45.1 mol%. Based on physiological, biochemical and chemotaxonomic traits and comparative 16S rRNA gene sequence analysis, it is demonstrated that the isolate represents a novel species of the genus *Idiomarina*, for which the name *Idiomarina homiensis* sp. nov. is proposed. The type strain is PO-M2^T (=KACC 11514^T = DSM 17923^T).

The genus *Idiomarina* was first proposed by Ivanova *et al.* (2000) to accommodate two marine bacteria, *Idiomarina abyssalis* and *Idiomarina zobellii*. Since the description of the genus, the names of the species *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) have been validly published. Features that distinguish members of the genus *Idiomarina* from other marine bacteria are their high content of iso-branched fatty acids and their physiological properties, in particular their ability to grow within a broad range of temperatures, pH values and NaCl concentrations (Martínez-Cánovas *et al.*, 2004). In this study, we report on the taxonomic characterization of a novel *Idiomarina* bacterial strain, PO-M2^T, which was isolated from Homi Cape, near Pohang, Korea.

Strain PO-M2^T was isolated from a seashore sand sample by using a dilution plating technique on marine agar 2216 (MA; Difco). Colonial properties were observed on MA medium. Cell morphology was observed using light microscopy and transmission electron microscopy (TEM)

(Fig. 1). Flagellum type was also determined by TEM. Gram staining, KOH test and L-alanine aminopeptidase assay were performed according to the manufacturer's instructions by using a Gram stain kit (Difco), 3 % (w/v) KOH (Buck, 1982) and Bactident aminopeptidase (Merck), respectively. Phenotypic tests were performed using standard procedures (Smibert & Krieg, 1994). Hydrolysis of carboxymethylcellulose (0.1 %; Sigma), alginate (0.5 %, w/v), chitin from crab shells (1 %, w/v), pectin (0.5 %, w/v) and tyrosine (0.5 %, w/v) was also tested. Growth at different salinities was tested in 0, 1, 3, 5, 7, 10, 15, 20 and 25 % NaCl in half-strength marine broth. The temperature range for growth was assessed at 4, 10, 15, 20, 25, 30, 35, 40, 45, 50 and 55 °C on MA. Marine broth adjusted to initial pH values of 4, 5, 6, 7, 8, 9 and 10 with citrate/phosphate buffer or Tris/HCl buffer (Breznak & Costilow, 1994) was used to test the ability of the strain to grow at different pH values. API 20NE, API 50 CH and API ZYM test strips (bioMérieux) were used according to the manufacturer's instructions. To investigate the utilization of carbon substrates, Biolog GN2 plates were used with both 0.85 % NaCl solution and artificial seawater for cell suspension.

The following chemotaxonomic characteristics were analysed: isoprenoid quinone (as described by Groth *et al.*, 1996), fatty acids (according to the standard protocol of the

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain PO-M2^T is DQ342238.

Fig. 1. Electron micrograph of a negatively stained cell of PO-M2^T. Bar, 500 nm.

MIDI/Hewlett Packard microbial identification system; Sasser, 1990) and G+C content of the DNA (Mesbah *et al.*, 1989).

The 16S rRNA gene sequence of strain PO-M2^T was analysed as described by Kwon *et al.* (2003). Phylogenetic analysis was performed by using MEGA, version 3.1 (Kumar *et al.*, 2004), after multiple alignment of the data using CLUSTAL W (Thompson *et al.*, 1994). Distances were obtained using options according to the Kimura two-parameter model (Kimura, 1980) and clustering was performed by using neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Fitch, 1971) methods. Bootstrap values with 1000 replications were used to determine the confidence level of the branches (Felsenstein, 1985).

Strain PO-M2^T had a 16S rRNA gene sequence similarity range of 94.3–95.5 % with sequences of the type strains of species within the genus *Idiomarina*, with the highest sequence similarities to *I. loihiensis* L2-TR^T (95.5 %) and *I. fontislapidosi* F23^T (95.5 %). The phylogenetic tree clearly showed that strain PO-M2^T formed a robust clade separate from other related genera, which was supported by a high bootstrap value (Fig. 2). A maximum-parsimony tree also confirmed the stable positioning of strain PO-M2^T within the genus *Idiomarina* (data not shown).

Cells of strain PO-M2^T were aerobic, Gram-negative, motile, straight or slightly curved rods, 0.4–0.6 µm in width and 0.7–2.0 µm in length. Colonies formed on MA medium were light-yellow and convex. The strain was oxidase- and catalase-positive. It did not grow anaerobically. Strain PO-M2^T grew at 4–45 °C, with optimum growth occurring at 25–30 °C. The pH range for growth was 6.0–9.0. Strain PO-M2^T was also positive for the KOH test and hydrolysis of tyrosine, gelatin, DNA and Tween 80. H₂S was produced from cysteine. Growth occurred in MA medium containing 15 % (w/v) NaCl. Details of various characteristics that differentiated strain PO-M2^T from phylogenetically related species are given in Table 1; other characteristics determined are given in the species description.

The major respiratory lipoquinone of strain PO-M2^T (Q-8) supported the affiliation of the strain to the genus *Idiomarina*. The fatty acid profile and DNA G+C content of strain PO-M2^T were similar to those of other species of the genus *Idiomarina*. The predominant fatty acids of strain PO-M2^T were iso-branched fatty acids, which is characteristic of the genus *Idiomarina* (Table 2). The detailed fatty acid profile was C_{15:0} iso (19.3 %), C_{17:1ω9c} iso (11.9 %), C_{17:0} iso (10.9 %), C_{18:1ω7c} (10.4 %), C_{16:0} (9.0 %) and C_{16:1ω7c} and/or C_{15:0} iso 2-OH (7.2 %). The G+C content of the genomic DNA was 45.1 mol%. The chemotaxonomic results supported the phylogenetic analysis, indicating that strain PO-M2^T belongs to the genus *Idiomarina*.

Fig. 2. Neighbour-joining tree showing the position of strain PO-M2^T and some other related taxa on the basis of 16S rRNA gene sequences. Numbers at branch points indicate bootstrap confidence values (%) of 1000 resamplings; only values greater than 60 % are shown. Bar, 0.01 substitutions per nucleotide position.

Table 1. Characteristics that distinguish *Idiomarina homiensis* sp. nov. from other *Idiomarina* species

Strains: 1, strain PO-M2^T; 2, *I. seosinensis* CL-SP19^T; 3, *I. abyssalis* KMM 227^T; 4, *I. zobellii* KMM 231^T; 5, *I. baltica* OS145^T; 6, *I. loihiensis* L2-TR^T; 7, *I. fontislapidosi* F23^T; 8, *I. ramblicola* R22^T. Data from Choi & Cho (2005), Martínez-Cánovas *et al.* (2004), Brettar *et al.* (2003), Ivanova *et al.* (2000) and this study. ND, No data available; +, positive response; –, negative response; w, weak response.

Characteristic	1	2	3	4	5	6	7	8
Cell morphology	Straight or slightly curved rod	Straight or slightly curved rod	Rod	Rod	Curved rod	Straight or slightly curved rod	Slightly curved rod	Slightly curved rod
Cell size (µm)	0.7–2.0 × 0.4–0.6	1.0–1.9 × 0.3–0.6	1–1.8 × 0.7–0.9	1–1.8 × 0.7–0.9	0.7–1.6 × 0.4–0.7	0.7–1.8 × 0.35	3–4 × 0.75	2–3 × 0.75
Flagellum	Single, polar	Single, polar	Single, polar	Single, polar	Single, polar	Single, polar or subpolar	Single, polar	Single, polar
NaCl range (% w/v)*	1–15 ^a	1–20 ^b	0.6–15 ^a	1.0–10 ^a	0.8–10 ^a	0.5–20 ^a	0.5–25 ^a	0.5–15 ^a
NaCl optimum (% w/v)	3–5	7–10	3–6	3–6	3–6	7.5–10	3–5	3–5
Temperature range (°C)	4–45	4–40	4–30	4–30	8–46	4–46	4–45	15–40
Temperature optimum (°C)	25–30	30–35	20–22	20–22	30–40	ND	32	32
pH range	6–9	6–10	5.5–9.5	5.5–9.5	ND	ND	5–10	5–10
Acid from D-glucose	–	–	–	–	W	–	–	–
Nitrate reduction	+	+	+	–	–	–	–	–
Hydrolysis of:								
Aesculin	+	+	–	–	+	–	+	+
Casein	–	ND	ND	ND	ND	ND	+	+
Tyrosine	+	ND	+	+	ND	ND	–	–
Starch	–	ND	–	–	–	ND	–	–
Gelatin	+	–	–	–	+	+	+	+
DNA	+	+	+	+	ND	ND	+	+
Urea	–	–	ND	ND	ND	ND	–	–
Tween 80	+	+	–	–	+	+	+	+
H ₂ S production from cysteine	+	ND	ND	ND	+	ND	+	+
Growth on:								
L-Arabinose	–	ND	–	–	+	ND	–	–
Maltose	–	ND	–	–	–	+	–	–
Acetate	–	–	+	–	+	+	–	–
Citrate	–	ND	–	–	–	+	–	–
Lactate	–	ND	–	–	–	+	–	–
Malonate	–	ND	–	–	–	+	–	–
Propionate	–	–	+	–	–	+	–	–
Succinate	–	+	+	+	–	–	–	–
Glycerol	–	ND	+	–	–	+	–	–
L-Alanine	–	+	+	+	–	+	–	–
L-Serine	–	–	–	–	–	+	–	–
DNA G + C content (mol%)	45.1	45.0	50.4	48.0	49.7	47.4	46.0	48.7
Habitat	Seashore sand, Korea	Hypersaline water, Korea	NW Pacific Ocean (depth)	NW Pacific Ocean (depth)	Central Baltic Sea (surface)	Hydrothermal vent, Hawaii	Saline wetland (soil), Spain	Saline rambla (water), Spain

*a, NaCl concentration; b, sea salt concentration.

Table 2. Fatty acid composition of *Idiomarina homiensis* sp. nov. and other *Idiomarina* species

Strains: 1, strain PO-M2^T (data from this study); 2, *I. seosinensis* CL-SP19^T (Choi & Cho, 2005); 3, *I. abyssalis* KMM 227^T (Ivanova *et al.*, 2000); 4, *I. zobellii* KMM 231^T (Ivanova *et al.*, 2000); 5, *I. baltica* OS145^T (Brettar *et al.*, 2003); 6, *I. loihiensis* L2-TR^T (Donachie *et al.*, 2003); 7, *I. fontislapidosi* F23^T (Martínez-Cánovas *et al.*, 2004); 8, *I. ramblicola* R22^T (Martínez-Cánovas *et al.*, 2004). Values are percentages of total fatty acids. –, Not detected or <1%.

Fatty acid	1	2	3	4	5	6	7	8
C _{16:0}	9.0	8.9	6.3	4.6	4.8	7.6	11.7	7.4
C _{18:0}	4.8	3.9	1.8	0.8	0.9	1.6	4.9	3.0
C _{11:0} iso	1.8	3.2	–	–	2.5	2.0	2.8	3.4
C _{15:0} iso	19.3	17.1	33.7	40.6	36.9	32.6	26.8	24.7
C _{15:1} isoF	1.7	0.6	2.3	1.6	1.5	1.3	1.5	1.9
C _{17:0} iso	10.9	15.2	11.9	12.5	11.2	11.0	8.8	12.9
C _{16:1} ω7c	7.2*	2.5*	7.0	8.3	8.4	6.0	11.3*	5.2
C _{17:1} ω6c	–	–	1.5	3.4	–	–	–	–
C _{17:1} ω9c iso	11.9	8.8	–	–	10.0	11.9	4.0	11.0
C _{18:1} ω7c	10.4	8.7	6.7	5.9	6.0	5.5	9.3	5.9
C _{18:1} ω9c	2.5	0.8	1.4	0.9	0.9	1.0	1.1	1.2
C _{19:1} ω6c	–	2.1	–	–	0.4	0.6	–	–
C _{10:0} 3-OH	1.2	1.3	–	–	1.2	0.8	2.3	1.1
C _{11:0} iso 3-OH	4.4	5.0	–	–	3.7	4.1	2.6	5.6
C _{13:0} iso 3-OH	3.6	4.2	–	–	3.2	3.3	1.6	2.3
C _{17:0} cyclo	0.7	4.5	–	–	0.6	1.7	1.2	2.5
C _{19:0} ω8c cyclo	–	3.5	–	–	–	–	–	–

*C_{16:1}ω7c/C_{15:0} iso 2-OH.

On the basis of phylogenetic, biochemical and physiological data, we propose that isolate PO-M2^T should be classified as representing the type strain of a novel species, *Idiomarina homiensis* sp. nov.

Description of *Idiomarina homiensis* sp. nov.

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

Cells are aerobic, straight or slightly curved rods, approximately 0.4–0.6 µm in width and 0.7–2.0 µm in length. Motile by means of a single polar flagellum. On MA solid medium, colonies are light-yellowish, round and convex. Growth occurs within the temperature range 4–45 °C (optimum growth at 25–30 °C) and at pH values between 6 and 9. Growth occurs in 1–15% (w/v) NaCl (optimum growth at 3–5%). Does not grow anaerobically. Cells are Gram-negative by Gram-stain test, KOH test and amino-peptidase test. Positive for oxidase, catalase, KOH test, production of H₂S from cysteine and hydrolysis of tyrosine, DNA, gelatin and Tween 80. Negative for indole production, Voges–Proskauer test, phenylalanine deamination and hydrolysis of casein, starch, chitin, lecithin, cellulose,

pectin and urea. Does not degrade alginic acid. In API 20NE tests, cells are positive for nitrate reductase activity and hydrolysis of aesculin, but negative for other reactions. In API ZYM tests, cells are positive for acid and alkaline phosphatase, esterase (C4 and C8), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin and naphthol phosphohydrolase activities, but negative for α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase activities. No reactions are observed in API 50 CH and Biolog tests. The DNA G+C content is 45.1 mol%. Ubiquinone-8 is the main respiratory lipoquinone. Major fatty acids are C_{15:0} iso (19.3%), C_{17:1}ω9c iso (11.9%), C_{17:0} iso (10.9%), C_{18:1}ω7c (10.4%), C_{16:0} (9.0%) and C_{16:1}ω7c and/or C_{15:0} iso 2-OH (7.2%) when grown on MA for 48 h.

The type strain is PO-M2^T (=KACC 11514^T=DSM 17923^T), which was isolated from seashore sand in Pohang, Korea.

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