Idiomarina salinarum sp. nov., isolated from a marine solar saltern in Korea

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A Gram-negative, motile, rod-shaped, *Idiomarina*-like bacterial strain, ISL-52^T, was isolated from a marine solar saltern of the Yellow Sea in Korea and was subjected to a polyphasic taxonomic investigation. Strain ISL-52^T grew optimally at pH 7.0–8.0 and at 30–37 °C. It contained Q-8 as the predominant ubiquinone. The major fatty acids (>10% of total fatty acids) were iso-C_{15:0}, iso-C_{17:0} and iso-C_{17:1ω9c}. The DNA G+C content was 53.9 mol%. A phylogenetic analysis based on 16S rRNA gene sequences showed that strain ISL-52^T fell within the genus *Idiomarina*, joining the type strain of *Idiomarina homiensis* at a bootstrap resampling value of 100%. Strain ISL-52^T exhibited 16S rRNA gene sequence similarity values of 94.9–96.7% with respect to the type strains of eight recognized *Idiomarina* species. The differential phenotypic properties of ISL-52^T, together with its phylogenetic distinctiveness, demonstrated that this strain is distinguishable from the recognized *Idiomarina* species. On the basis of phenotypic, phylogenetic and genetic data, therefore, strain ISL-52^T represents a novel species of the genus *Idiomarina*, for which the name *Idiomarina salinarum* sp. nov. is proposed. The type strain is ISL-52^T (=KCTC 12971^T=CCUG 54359^T).

The genus *Idiomarina* was created by Ivanova *et al.* (2000) with the description of *Idiomarina abyssalis* and *Idiomarina zobellii*. Subsequently, six further *Idiomarina* species, *Idiomarina baltica* (Brettar *et al.*, 2003), *Idiomarina loihiensis* (Donachie *et al.*, 2003), *Idiomarina fontislapidosi* and *Idiomarina ramblicola* (Martínez-Cánovas *et al.*, 2004), *Idiomarina seosinensis* (Choi & Cho, 2005) and *Idiomarina homiensis* (Kwon *et al.*, 2006), have been described. In this study, we report on the taxonomic characterization of an *Idiomarina*-like bacterial strain, ISL-52^T, isolated from a marine solar saltern of the Yellow Sea in Korea.

Strain ISL-52^T was isolated by means of the dilution plating technique at 30 °C on marine agar 2216 (MA; Difco) supplemented with 8% (w/v) NaCl. The morphological, physiological and biochemical characteristics of strain ISL-52^T were investigated using routine cultivation on MA at 37 °C. The cell morphology was examined by light microscopy (E600; Nikon) and transmission electron microscopy. Flagellation was determined by using a Philips CM-20 transmission electron microscope with cells from exponentially growing cultures: for this purpose, the cells were negatively stained with 1% (w/v) phosphotungstic acid and the grids were examined after being air-dried. Growth under anaerobic conditions was determined after incubation in a Forma anaerobic chamber on MA and on MA supplemented with nitrate, both of which had been

prepared anaerobically using nitrogen. The pH range for growth was determined in marine broth 2216 (MB; Difco) that had been adjusted to various pH values (pH 4.5–9.5, using increments of 0.5 pH units). Growth in the absence of NaCl was investigated using trypticase soy broth prepared according to the formula of the Difco medium except that NaCl was excluded. Growth at various NaCl concentrations was investigated in MB or trypticase soy broth (Difco). Growth at various temperatures (4–45 °C) was measured on MA. Catalase and oxidase activities and hydrolysis of casein, starch and Tweens 20, 40, 60 and 80 were determined as described by Cowan & Steel (1965). DNase activity was examined by using DNase test agar with methyl green (Difco) supplemented with 3% (w/v) NaCl. Hydrolysis of hypoxanthine, tyrosine and xanthine was tested on MA using the substrate concentrations described by Cowan & Steel (1965). Hydrolysis of aesculin, gelatin and urea and reduction of nitrate were investigated as described previously (Lanyi, 1987) with the modification that artificial seawater was used for preparation of the media. The artificial seawater contained the following (l⁻¹ distilled water): 23.6 g NaCl, 0.64 g KCl, 4.53 g MgCl₂·6H₂O, 5.94 g MgSO₄·7H₂O and 1.3 g CaCl₂·2H₂O (Bruns *et al.*, 2001). H₂S production was tested as described previously (Bruns *et al.*, 2001). Susceptibility to antibiotics was investigated on MA plates by using antibiotic discs with the following concentrations (µg unless indicated otherwise): polymyxin B, 100 U; streptomycin, 50; penicillin G, 20 U; chloramphenicol, 100;

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ampicillin, 10; cephalothin, 30; gentamicin, 30; novobiocin, 5; tetracycline, 30; kanamycin, 30; lincomycin, 15; oleandomycin, 15; neomycin, 30; carbenicillin, 100. Acid production from carbohydrates was tested as described by Leifson (1963). The utilization of various substrates for growth was determined as described by Baumann & Baumann (1981), using supplementation with 2% (v/v) Hutner's mineral salts solution (Cohen-Bazire *et al.*, 1957) and a 1% (v/v) vitamin solution (Staley, 1968), and by Yurkov *et al.* (1994). Enzyme activities were determined by using the API ZYM system (bioMérieux).

Cell biomass for DNA extraction and for isoprenoid quinone analysis was obtained from cultivation in MB at 37 °C. Chromosomal DNA was isolated and purified according to the method described by Yoon *et al.* (1996), except that RNase T1 was used in combination with RNase A to minimize contamination of the RNA. The 16S rRNA gene was amplified by using a PCR with two universal primers, as described previously (Yoon *et al.*, 1998). Sequencing of the amplified 16S rRNA gene and phylogenetic analysis were performed as described by Yoon *et al.* (2003). Isoprenoid quinones were analysed as described by Komagata & Suzuki (1987), using reversed-phase HPLC. For cellular fatty acid analysis, cell mass of strain ISL-52^T was harvested from MA plates after cultivation for 3 days at 37 °C. The fatty acids were extracted and fatty acid methyl esters were prepared according to the standard protocol of the MIDI/Hewlett Packard Microbial Identification System (Sasser, 1990). The DNA G+C content was determined by using the method of Tamaoka & Komagata (1984), with the modification that the DNA was hydrolysed using nuclease P1 (Sigma) and the resultant nucleotides were analysed by reversed-phase HPLC.

Morphological, cultural, physiological and biochemical characteristics of strain ISL-52^T are given in the species description (see later) and in Table 1. The almost-complete 16S rRNA gene sequence of strain ISL-52^T determined in this study comprised 1497 nt, representing approximately 96% of the *Escherichia coli* 16S rRNA gene sequence. A comparative 16S rRNA gene sequence analysis revealed that strain ISL-52^T was phylogenetically most closely affiliated to members of the genus *Idiomarina*. In the phylogenetic tree based on the neighbour-joining algorithm, strain ISL-52^T joined *I. homiensis* PO-M2^T at a bootstrap confidence level of 100% (Fig. 1). The same tree topology was also found in trees based on the maximum-likelihood and maximum-parsimony algorithms. Strain ISL-52^T exhibited 16S rRNA gene sequence similarities of 96.7% and 94.9–95.8%, respectively, with *I. homiensis* PO-M2^T and the type strains of other *Idiomarina* species. The sequence similarities with respect to other species used in the phylogenetic analysis were less than 90.7%.

The predominant isoprenoid quinone detected in strain ISL-52^T was Q-8 (at a peak area ratio of approximately 89%), as for other *Idiomarina* species. The fatty acid profile of strain ISL-52^T consisted of the following (each constituting

>0.5% of total fatty acids): branched fatty acids iso-C_{15:0} (34.1%), iso-C_{17:0} (19.9%), iso-C_{17:1ω9c} (11.8%), iso-C_{11:0} (4.2%), iso-C_{15:1} (3.9%), iso-C_{13:0} (1.6%), iso-C_{16:0} (1.3%) and anteiso-C_{17:0} (0.5%); hydroxyl fatty acids iso-C_{13:0} 3-OH (5.0%) and iso-C_{11:0} 3-OH (5.0%); straight-chain fatty acids C_{16:0} (4.3%) and C_{18:0} (0.8%); cyclo fatty acids cyclo-C_{17:0} (1.7%) and cyclo-C_{19:0ω8c} (0.6%); unsaturated fatty acid C_{18:1ω7c} (0.9%). This fatty acid profile was similar to those of *Idiomarina* species, although there are differences in the proportions of some fatty acids, perhaps because of differences in cultivation conditions and extraction procedures (Kwon *et al.*, 2006). The DNA G+C content of strain ISL-52^T was 53.9 mol%. There were no distinct phenotypic, particularly chemotaxonomic, properties to differentiate strain ISL-52^T from the genus *Idiomarina*. Accordingly, it seems reasonable to allocate strain ISL-52^T to the genus *Idiomarina*. Strain ISL-52^T differs from eight recognized *Idiomarina* species in several phenotypic characteristics, as shown in Table 1. The phylogenetic distinctiveness and differential phenotypic properties are sufficient to allocate strain ISL-52^T to a species that is separate from the recognized *Idiomarina* species (Stackebrandt & Goebel, 1994; Table 1). Therefore, on the basis of the data presented, strain ISL-52^T represents a novel species of the genus *Idiomarina*, for which the name *Idiomarina salinarum* sp. nov. is proposed.

Description of *Idiomarina salinarum* sp. nov.

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

Cells are Gram-negative and rod-shaped (0.3–0.6 × 0.8–3.5 µm). Colonies on MA are circular to slightly irregular, raised, smooth, glistening, pale yellow in colour and 1.5–2.0 mm in diameter after 3 days incubation at 37 °C. Growth occurs at 4 and 42 °C, but not at 43 °C. The optimal pH for growth is between 7.0 and 8.0; growth occurs at pH 6.0, but not at pH 5.5. Growth occurs in the presence of 14% (w/v) NaCl, but not in the absence of NaCl or in the presence of more than 15% (w/v) NaCl. Anaerobic growth does not occur on MA or on MA supplemented with nitrate. Tweens 20, 40 and 60 are hydrolysed, but hypoxanthine, xanthine and urea are not. D-Glucose, D-fructose, D-galactose, D-cellobiose, D-mannose, trehalose, D-xylose, sucrose, benzoate, L-malate, pyruvate, salicin, formate and L-glutamate are not utilized. Acid is not produced from the following substrates: L-arabinose, D-cellobiose, D-fructose, D-galactose, D-glucose, lactose, maltose, D-mannose, D-melezitose, melibiose, D-raffinose, L-rhamnose, D-ribose, sucrose, trehalose, D-xylose, D-sorbitol, D-mannitol and myo-inositol. Susceptible to chloramphenicol and polymyxin B and weakly susceptible to gentamicin; resistant to cephalothin, lincomycin, neomycin, novobiocin, oleandomycin, penicillin G and tetracycline. The predominant ubiquinone is Q-8. The major fatty acids (>10% of total fatty acids) are iso-C_{15:0}, iso-C_{17:0} and iso-C_{17:1ω9c}. The DNA G+C content is 53.9 mol% (determined by HPLC). Other phenotypic characteristics are given in Table 1.

Table 1. Differential phenotypic characteristics of strain ISL-52^T and *Idiomarina* species

Taxa: 1, strain ISL-52^T; 2, *I. abyssalis*; 3, *I. zobellii*; 4, *I. baltica*; 5, *I. loihiensis*; 6, *I. fontislapidosi*; 7, *I. ramblicola*; 8, *I. seosinensis*; 9, *I. homiensis*. Data are from Ivanova *et al.* (2000), Brettar *et al.* (2003), Martínez-Cánovas *et al.* (2004), Choi & Cho (2005) and Kwon *et al.* (2006). All are strictly aerobic, motile (by means of single polar or subpolar flagella) and positive for the following: oxidase, catalase and DNase (not determined for *I. baltica*), alkaline phosphatase, esterase (C4), esterase lipase (C8) and leucine arylamidase. All are negative for the following: Gram-stain, starch hydrolysis, lipase (C14), α -galactosidase, β -galactosidase, β -glucuronidase, α -glucosidase, β -glucosidase, *N*-acetyl- β -glucosaminidase and α -fucosidase. +, Positive reaction; –, negative reaction; w, weakly positive reaction; ND, not determined. Data in parentheses are for the type strain.

Characteristic	1	2	3	4	5	6	7	8	9
Cell morphology	Straight or curved rods	Rods	Rods	Curved rods	Straight to slightly curved rods	Slightly curved rods	Slightly curved rods	Straight or slightly curved rods	Straight or slightly curved rods
Cell size (μm)	0.3–0.6 $\times 0.8$ –3.5	0.7–0.9 $\times 1.0$ –1.8	0.7–0.9 $\times 1.0$ –1.8	0.4–0.7 $\times 0.7$ –1.6	0.35–0.45 $\times 0.7$ –1.8	0.75 $\times$ 3–4	0.75 $\times$ 2–3	0.3–0.6 $\times 1.0$ –1.9	0.4–0.6 $\times 0.7$ –2.0
Maximum NaCl concn (% w/v) for growth	14	15	10	10	20	25	15	20*	1–15
Optimal NaCl concn (% w/v)	2–3	3–6	3–6	3–6	7.5–10	3–5	3–5	7–10*	3–5
Temperature range for growth ($^{\circ}\text{C}$)	4–42	4–30	4–30	10–46	4–46	4–45	15–40	4–40	4–45
Optimal temperature for growth ($^{\circ}\text{C}$)	30–37	20–22	20–22	30–40	ND	32	32	30–35	25–30
Acid production from glucose	–	–	–	+	–	–	–	–	–
Nitrate reduction	+	+	–	(–)	–	–	–	+	+
Hydrolysis of:									
Aesculin	–	–	–	(+)	–	+	+	+	+
Casein	–	ND	ND	ND	ND	+	+	ND	–
Tyrosine	+	+	+	ND	ND	–	–	ND	+
Gelatin	–	+	+	+	+	+	+	+	+
Tween 80	+	–	–	+	+	+	+	+	+
Growth on:									
L-Arabinose	–	–	–	+	–	–	–	–	–
Maltose	–	–	–	–	+	–	–	–	–
Acetate	–	+	–	+	+	–	–	–	–
Citrate	–	–	–	–	+	–	–	–	–
Succinate	–	+	+	–	–	–	–	+	–
Susceptibility to:									
Ampicillin	–	–	–	ND	ND	+	+	ND	ND
Carbenicillin	–	ND	ND	ND	ND	+	+	ND	ND
Kanamycin	–	–	–	ND	ND	+	–	ND	ND
Streptomycin	+	+	–	ND	ND	+	–	ND	ND
API ZYM tests									
Valine arylamidase	–	–	–	+	–	ND	ND	–	+
Cystine arylamidase	–	–	–	+	w	ND	ND	–	+
Trypsin	+	–	–	–	+	ND	ND	–	+
α -Chymotrypsin	+	–	w	+	w	ND	ND	–	+
Acid phosphatase	–	+	+	+	+	ND	ND	+	+
Naphthol-AS-BI-phosphohydrolase	–	+	w	+	+	ND	ND	–	+
α -Mannosidase	+	–	–	–	–	ND	ND	–	–
DNA G + C content (mol%)	53.9	50.4	48	49.4–49.9	47.4	46.0	48.7	45.0	45.1
Habitat	Marine solar saltern	Deep sea (NW Pacific Ocean)	Deep sea (NW Pacific Ocean)	Surface seawater (central Baltic Sea)	Hydrothermal vent (Hawaii)	Saline wetland soil (Spain)	Saline rambla water (Spain)	Hypersaline water of solar saltern (Korea)	Seashore sand (Korea)

*Sea-salt concentration.

Fig. 1. Neighbour-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the positions of strain ISL-52^T, *Idiomarina* species and some other related taxa. Bootstrap percentages (based on 1000 replications) >50% are shown at branch points. Filled circles indicate that the corresponding nodes were also recovered in trees generated with the maximum-likelihood and maximum-parsimony algorithms. *Pseudomonas aeruginosa* DSM 50071^T (GenBank accession no. X06684) was used as an outgroup. Bar, 0.01 substitutions per nucleotide position.

The type strain, ISL-52^T (=KCTC 12971^T=CCUG 54359^T), was isolated from a marine solar saltern of the Yellow Sea, Korea.

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