Idiomarina seosinensis sp. nov., isolated from hypersaline water of a solar saltern in Korea

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A halophilic γ -proteobacterium, designated CL-SP19^T, was isolated from hypersaline water from a solar saltern located in Seosin, Korea. Analysis of the 16S rRNA gene sequence revealed an affiliation with the genus *Idiomarina*. The sequence similarities between CL-SP19^T and type strains of the genus *Idiomarina* ranged from 95.9 to 96.9%. Cells were straight or slightly curved rods and were motile by means of a single polar flagellum. The major fatty acids were C_{15:0} iso (17.1%) and C_{17:0} iso (15.2%). Three fatty acids, C_{19:0}ω8c cyclo (3.5%), C_{14:1}ω5c (1.4%) and C_{18:3}ω6c (1.2%), were found in minor quantities, but uniquely in CL-SP19^T among *Idiomarina* species. The DNA G + C content was 45.0 mol%. On the basis of its physiology, fatty acid composition and 16S rRNA gene sequence, strain CL-SP19^T could be assigned to the genus *Idiomarina* but distinguished from the recognized species of the genus. Strain CL-SP19^T, therefore, represents a novel species, for which the name *Idiomarina seosinensis* sp. nov. is proposed, with CL-SP19^T (=KCTC 12296^T =JCM 12526^T) as the type strain.

The genus *Idiomarina* was first described by Ivanova *et al.* (2000). Six species, *Idiomarina abyssalis* (type species) and *Idiomarina zobellii* (Ivanova *et al.*, 2000), *Idiomarina baltica* (Brettar *et al.*, 2003), *Idiomarina loihiensis* (Donachie *et al.*, 2003), *Idiomarina fontislapidosi* and *Idiomarina ramblicola* (Martínez-Cánovas *et al.*, 2004), have been isolated from sea water, i.e. from deep waters of the north-western Pacific Ocean, from surface water of the central Baltic Sea, from hydrothermal fluids from a submarine volcano in Hawaii and from hypersaline habitats in Spain, respectively.

A hypersaline water sample (318 practical salinity units) from a solar saltern in Seosin, Korea, was spread on a plate containing marine agar 2216 (MA; Difco); the plate was incubated at 30 °C for 2 weeks. Strain CL-SP19^T, appearing as a slightly yellowish colony, was isolated from the plate and subsequently purified four times on MA at 30 °C. The strain was maintained both on MA at 4 °C and in marine broth (MB; Difco) supplemented with 30% (v/v) glycerol at –80 °C.

The 16S rRNA gene was amplified from a single colony by a PCR with *Taq* DNA polymerase (Bioneer) and primers 27F and 1492R (Lane, 1991). The PCR product was purified

by using the AccuPrep PCR purification kit (Bioneer) and cloned using the pCR2.1 TOPO TA cloning kit (Invitrogen). Sequencing of the 16S rRNA gene was performed with an Applied Biosystems automatic sequencer (ABI3730XL) at Macrogen (Korea). The sequence of strain CL-SP19^T was compared with 16S rRNA gene sequences available in GenBank, using a BLAST (Altschul *et al.*, 1990) search. The sequence of strain CL-SP19^T was manually aligned with six species of the genus *Idiomarina* and type species of genera belonging to the family *Alteromonadaceae* obtained from the GenBank and Ribosomal Database Project (Cole *et al.*, 2003) databases using known 16S rRNA secondary structure information. 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). The robustness of tree topologies was assessed by bootstrap analyses based on 1000 replications for neighbour-joining and maximum-parsimony methods and 100 replications for the maximum-likelihood method. Alignment and phylogenetic analyses were carried out using the PHYLIT program (version 3.2, available at <http://plaza.snu.ac.kr/~jchun/phydit/>) and PAUP*, version 4.0 (Swofford, 1998). Likelihood parameters were estimated by the hierarchical ratio tests in MODELTEST, version 3.04 (Posada & Crandall, 1998). The almost-complete 16S rRNA gene sequence of strain CL-SP19^T (1463 bp) was obtained. Sequence similarity indicated that the closest relatives of strain CL-SP19^T were *I. fontislapidosi* (96.9%), *I. zobellii* (96.8%), *I. abyssalis*

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The GenBank/EMBL/DBJ accession number for the 16S rRNA gene sequence of strain CL-SP19^T is AY635468.

Additional phenotypic data are available as supplementary material in IJSEM Online.

Fig. 1. Neighbour-joining tree showing the relationship between strain CL-SP19^T and members of *Idiomarina* and other related genera belonging to the *Alteromonadaceae*. Only those bootstrap values above 70% are shown (1000 resamplings) at the branching points. Solid circles indicate that the corresponding nodes are also recovered in maximum-likelihood and maximum-parsimony trees. *Burkholderia cepacia* (M22518) was used as an outgroup (not shown). Bar, 0.1 nucleotide substitution per site.

(96.8%), *I. baltica* (96.4%), *I. loihiensis* (96.3%) and *I. ramblicola* (95.9%). Phylogenetic analyses based on the 16S rRNA gene sequence showed that strain CL-SP19^T formed a robust cluster with species of the genus *Idiomarina* (Fig. 1). Thus, it is clear that our isolate belongs to the genus *Idiomarina*. However, low similarities (95.9–96.9%) between the 16S rRNA gene sequence of the novel isolate and those of previously described species of the genus *Idiomarina* indicated that strain CL-SP19^T represents a novel species in the genus (Stackebrandt & Goebel, 1994; Rosselló-Mora & Amann, 2001).

Subsequently, morphological and physiological tests were also performed. Gram-staining was performed as described by Smibert & Krieg (1994). Cell morphology and motility were examined by using phase-contrast microscopy (BX60 microscope; Olympus) with cells grown at 30 °C in MB. The presence of a flagellum was observed using a scanning electron microscope (JSM-6700, FE-SEM; JEOL) (Fig. 2). Anaerobic growth was checked on MA using the GasPak anaerobic system (BBL). Catalase and oxidase activities were determined according to the protocols described by Smibert & Krieg (1994), while gelatinase, amylase, DNase

Fig. 2. Scanning electron micrograph of negatively stained cells of strain CL-SP19^T.

and nitrate reductase activities were examined as described by Hansen & Sørheim (1991).

The temperature range for growth was determined on the basis of colony formation on MA plates that were incubated at 4–46 °C. Optimum temperature (a range of 25–40 °C) and pH range (a range of pH 5–10) for growth were determined by observing changes in OD₆₀₀ values over time in MB. The final pH was adjusted using NaOH and HCl solutions. The sea salt concentrations allowing growth of CL-SP19^T were determined using synthetic ZoBell broth [Bacto peptone (Difco), 5 g; yeast extract, 1 g; ferric citrate, 0.1 g; distilled water, 1 litre] with various concentrations of sea salt (0, 0.5, 1, 3, 5, 7, 10, 15, 20 and 25 %, w/v). Indole production, acid production from glucose, arginine dihydrolase, urease, hydrolysis of aesculin, and gelatinase and β -galactosidase were tested using the API 20NE kit (bioMérieux) according to the manufacturer's instructions, except that cell suspensions were prepared using artificial sea water (NaCl, 24 g; MgCl₂, 5.1 g; Na₂SO₄, 4 g; CaCl₂, 1.1 g; KCl, 0.7 g; NaHCO₃, 0.2 g; KBr, 0.1 g; H₃BO₃, 0.027 g; SrCl₂, 0.024 g; NaF, 0.003 g; distilled water, 1 litre; Yi & Chun, 2004) as the suspension medium. Other enzyme activities were also assayed, using the API ZYM kit (bioMérieux) and artificial sea water as the suspension medium. The utilization of single carbon sources was investigated using Biolog GN plates and using artificial sea water for cell suspension; the results were read visually after incubation for 1, 2, 3 and 5 days. For the analyses of API 20NE, API ZYM and Biolog, *I. loihiensis* L2-TR^T, *I. baltica* OS145^T, *I. abyssalis* KMM 227^T and *I. zobellii* KMM

231^T were employed as reference strains. The results of morphological, biochemical and physiological tests are given in Table 1 and Table 2 and in the species description. The DNA G + C content was determined by HPLC analysis (Tamaoka & Komagata, 1984) at the Korean Culture Centre of Microorganisms. The results are shown in Table 1 and in the species description.

The fatty acid methyl esters in whole cells grown at 30 °C for 2 days on MA were analysed by GC, according to the instructions of the Microbial Identification System (MIDI), at the Korean Culture Centre of Microorganisms. The profile for strain CL-SP19^T shows a predominance of iso-branched fatty acids, which is characteristic of the genus *Idiomarina* (Table 3). However, in CL-SP19^T, the proportion of 15:0 iso fatty acids (17.1 %) is obviously lower than those of other *Idiomarina* species (24.7–40.6 %), whilst the proportion of 17:0 iso fatty acids is slightly higher in strain CL-SP19^T (15.2 %) than in *Idiomarina* species (8.8–12.9 %). Three fatty acids, C_{19:0}ω8c cyclo (3.5 %), C_{14:1}ω5c (1.4 %) and C_{18:3}ω6c (1.2 %), were found in minor quantities, but uniquely in CL-SP19^T among *Idiomarina* species. Therefore, the fatty acid pattern of strain CL-SP19^T differs distinctly from those of previously described species of *Idiomarina*.

Phylogenetic analyses based on 16S rRNA gene sequences, fatty acid profiles and physiological features suggest that strain CL-SP19^T represents a novel species of the genus *Idiomarina*, for which the name *Idiomarina seosinensis* sp. nov. is proposed.

Table 1. Selected phenotypic characteristics of *Idiomarina seosinensis* sp. nov. and other *Idiomarina* species

Strains: 1, *I. seosinensis* CL-SP19^T (this study); 2, *I. abyssalis* KMM 227^T (Ivanova *et al.*, 2000); 3, *I. zobellii* KMM 231^T (Ivanova *et al.*, 2000); 4, *I. baltica* OS145^T (Brettar *et al.*, 2003); 5, *I. loihiensis* L2-TR^T (Donachie *et al.*, 2003); 6, *I. fontislapidosi* F23^T (Martínez-Cánovas *et al.*, 2004); 7, *I. ramblicola* R22^T (Martínez-Cánovas *et al.*, 2004). ND, No data available; +, positive response; –, negative response. All strains are Gram-negative.

Characteristic	1	2	3	4	5	6	7
Cell shape	Straight or slightly curved rod	Rod	Rod	Curved rod	Straight or slightly curved rod	Slightly curved rod	Slightly curved rod
Cell length (μm)	1.0–1.9	1–1.8	1–1.8	0.7–1.6	0.7–1.8	3–4	2–3
Cell diameter (μm)	0.3–0.6	0.7–0.9	0.7–0.9	0.4–0.7	0.35	0.75	0.75
Flagellum	Single, polar	Single, polar	Single, polar	Single, polar	Single, polar or subpolar	Single, polar	Single, polar
Growth on NaCl medium*	–	+	+	+	+	+	+
Salt range (% w/v)	1–20†	0.6–15‡	1.0–10‡	0.8–10‡	0.5–20‡	0.5–25‡	0.5–15‡
Salt optimum (% w/v)	7–10	3–6	3–6	3–6	7.5–10	3–5	3–5
Temperature range (°C)	4–40	4–30	4–30	8–46	4–46	4–45	15–40
Optimum temperature	30–35	20–22	20–22	30–40	ND	32	32
pH range	6–10	5.5–9.5	5.5–9.5	ND	ND	5–10	5–10
DNA G + C content (mol%)	45.0	50.4	48.0	49.7	47.4	46.0	48.7

*Medium supplemented with NaCl as sole salt.

†Sea salt concentration.

‡NaCl concentration.

Table 2. Selected phenotypic characteristics that differentiate *Idiomarina seosinensis* sp. nov. from other *Idiomarina* species

Strains: 1, *I. seosinensis* CL-SP19^T; 2, *I. abyssalis* KMM 227^T; 3, *I. zobellii* KMM 231^T; 4, *I. baltica* OS145^T; 5, *I. loihiensis* L2-TR^T. +, Positive response; –, negative response; w, weak response. Data for all strains in the table were obtained in this study. Additional phenotypic data are available as supplementary material in IJSEM Online.

Characteristic	1	2	3	4	5
API 20NE					
Nitrate reduction*	+	+	–	–	–
Aesculin hydrolysis	+	–	–	+	–
Gelatinase†	–	–	–	+	+
API ZYM					
Valine arylamidase	–	–	–	+	–
Cystine arylamidase	–	–	–	+	w
Trypsin	–	–	–	–	+
α-Chymotrypsin	–	–	w	+	w
Naphthol-phosphohydrolase	–	+	w	+	w
Biolog					
Glycogen	+	–	w	–	+
α-D-Glucose	–	–	+	–	+
Pyruvic acid methyl ester	–	+	w	w	+
Succinic acid mono-methyl-ester	w	–	–	–	–
Acetic acid	–	+	–	+	+
β-Hydroxybutyric acid	w	–	–	w	w
γ-Hydroxybutyric acid	–	–	–	–	+
Propionic acid	–	+	–	+	w
Succinic acid	+	w	+	–	w
D-Alanine	+	–	–	–	–
L-Asparagine	+	–	+	+	+
L-Aspartic acid	+	–	+	–	–
L-Glutamic acid	+	–	+	+	+
Glycyl-L-aspartic acid	–	–	+	+	+
L-Leucine	–	–	w	–	+
L-Proline	+	–	–	+	+
L-Serine	–	+	+	+	+
L-Threonine	+	–	+	+	+

*Test was also performed by Hansen & Sørheim (1991) and gave congruent results.

†Test was also performed by Hansen & Sørheim (1991) and showed positive response in all strains tested.

Description of *Idiomarina seosinensis* sp. nov.

Idiomarina seosinensis (se.o.sin.en'sis. N.L. fem. adj. *seosinensis* referring to the Seosin region in Korea, where the type strain was found).

Cells are Gram-negative, strictly aerobic and straight or slightly curved rods that are approximately 0.3–0.6 µm wide and 1.0–1.9 µm long. Motile by means of a single polar flagellum. On MA solid medium, colonies are slightly yellowish, entire circular, convex, opaque and shiny. After

Table 3. Fatty acid compositions of *Idiomarina seosinensis* sp. nov. and other *Idiomarina* species

Strains: 1, *I. seosinensis* CL-SP19^T (this study); 2, *I. abyssalis* KMM 227^T (Ivanova *et al.*, 2000); 3, *I. zobellii* KMM 231^T (Ivanova *et al.*, 2000); 4, *I. baltica* OS145^T (Brettar *et al.*, 2003); 5, *I. loihiensis* L2-TR^T (Donachie *et al.*, 2003); 6, *I. fontislapidosi* F23^T (Martínez-Cánovas *et al.*, 2004); 7, *I. ramblicola* R22^T (Martínez-Cánovas *et al.*, 2004). –, Not detected or <1 %.

Fatty acid	1	2	3	4	5	6	7
C _{14:0}	0.5	–	–	–	0.8	1.9	0.6
C _{16:0}	8.9	6.3	4.6	4.8	7.6	11.7	7.4
C _{17:0}	0.8	–	–	–	0.8	0.6	1.7
C _{18:0}	3.9	1.8	0.8	0.9	1.6	4.9	3.0
C _{11:0} iso	3.2	–	–	2.5	2.0	2.8	3.4
C _{13:0} iso	–	1.0	1.1	0.8	1.8	0.9	1.5
C _{15:0} iso	17.1	33.7	40.6	36.9	32.6	26.8	24.7
C _{15:1} isoF	0.6	2.3	1.6	1.5	1.3	1.5	1.9
C _{17:0} iso	15.2	11.9	12.5	11.2	11.0	8.8	12.9
C _{15:0} anteiso	–	–	–	–	0.3	0.6	1.2
C _{14:1} ω5c	1.4	–	–	–	–	–	–
C _{15:1} ω8c	–	1.3	1.1	–	–	–	–
C _{16:1} ω7c	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} ω8c	–	0.8	1.1	0.7	0.9	0.3	1.1
C _{17:1} ω9c iso	8.8	–	–	10.0	11.9	4.0	11.0
C _{18:1} ω7c	8.7	6.7	5.9	6.0	5.5	9.3	5.9
C _{18:1} ω7c 11-methyl	–	–	–	1.8	–	0.9	0.2
C _{18:1} ω9c	0.8	1.4	0.9	0.9	1.0	1.1	1.2
C _{18:3} ω6c (6,9,12)	1.2	–	–	–	–	–	–
C _{19:1} ω6c	2.1	–	–	0.4	0.6	–	–
C _{10:0} 3-OH	1.3	–	–	1.2	0.8	2.3	1.1
C _{12:0} 3-OH	1.2	–	–	–	0.7	–	–
C _{11:0} iso 3-OH	5.0	–	–	3.7	4.1	2.6	5.6
C _{13:0} iso 3-OH	4.2	–	–	3.2	3.3	1.6	2.3
C _{15:0} iso 3-OH	1.2	–	–	–	0.5	–	–
C _{17:0} cyclo	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.

3 days on MA at 30 °C, colonies are approximately 2–3 mm in diameter. They grow within the temperature range 4–40 °C (optimum of 30–35 °C) and at pH values between 6 and 10. Growth occurs in sea salt concentrations of 1–20 % (w/v) (optimum of 5–10 %), whereas no growth occurs in media containing NaCl as the only salt. Positive for oxidase, catalase, DNase, gelatinase and nitrate reductase activities but negative for amylase activity. The major fatty acids are 15:0 iso (17.1 %) and 17:0 iso (15.2 %). Minor amounts of C_{19:0}ω8c cyclo (3.5 %), C_{14:1}ω5c (1.4 %) and C_{18:3}ω6c (1.2 %) are also found. In API 20NE tests, cells are positive for nitrate reductase activity and hydrolysis of aesculin, but negative for indole production, acid production from glucose, arginine dihydrolase, urease, gelatinase and β-galactosidase. In API ZYM tests, cells are positive for

alkaline phosphatase, esterase (C4 and C8) and leucine arylamidase activities, but negative for valine arylamidase, cysteine arylamidase, trypsin, α -chymotrypsin and naphthol-phosphohydrolase activities. In Biolog tests, the following substrates are utilized: glycogen, Tween 40, Tween 80, α -ketobutyric acid, α -ketovaleric acid, succinic acid, L-alaninamide, L-alanine, L-alanylglycine, L-asparagine, L-aspartic acid, L-glutamic acid, glycyl-L-glutamic acid, L-proline and L-threonine. The DNA G+C content is 45.0 mol%.

The type strain is CL-SP19^T (=KCTC 12296^T=JCM 12526^T), isolated from hypersaline water from a solar saltern located in Seosin, Korea.

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