Marinimicrobium locisalis sp. nov., isolated from a marine solar saltern, and emended description of the genus *Marinimicrobium*

Jung-Hoon Yoon, So-Jung Kang, Yong-Taek Jung and Tae-Kwang Oh

Correspondence
Jung-Hoon Yoon
jhyoon@kribb.re.kr

Korea Research Institute of Bioscience and Biotechnology (KRIIBB), PO Box 115, Yusong, Taejeon, Republic of Korea

A Gram-negative, motile and rod-shaped bacterial strain, designated ISL-43^T, was isolated from a marine solar saltern of the Yellow Sea, Korea, and its taxonomic position was investigated by means of a polyphasic study. Strain ISL-43^T grew optimally at pH 7.0–8.0 and 30 °C and in the presence of approximately 2 % NaCl. It contained Q-8 as the predominant ubiquinone and C_{16:0}, C_{19:0ω8c}, C_{16:1ω7c} and/or iso-C_{15:0} 2-OH, and C_{18:1ω7c} as the major fatty acids. The DNA G + C content was 58.4 mol%. Phylogenetic analyses based on 16S rRNA gene sequences showed that strain ISL-43^T fell within the genus *Marinimicrobium*, clustering with *Marinimicrobium agarilyticum* M18^T with a bootstrap value of 100 %. Strain ISL-43^T exhibited 16S rRNA gene sequence similarity values of 98.1 and 95.5 % to *M. agarilyticum* M18^T and *Marinimicrobium koreense* M9^T, respectively. Strain ISL-43^T exhibited DNA–DNA relatedness values of 17 and 10 % to *M. agarilyticum* KCTC 12357^T and *M. koreense* KCTC 12356^T, respectively. On the basis of phenotypic, phylogenetic and genetic data, strain ISL-43^T represents a novel species within the genus *Marinimicrobium*, for which the name *Marinimicrobium locisalis* sp. nov. is proposed. The type strain is ISL-43^T (=KCTC 22484^T=CCUG 56757^T).

The genus *Marinimicrobium* was created by Lim *et al.* (2006) with the description of two species, *Marinimicrobium koreense* and *Marinimicrobium agarilyticum*. Phylogenetic analyses based on 16S rRNA gene sequences showed that the genus *Marinimicrobium* falls within the class *Gammaproteobacteria* (Lim *et al.*, 2006). In this study, we report on the taxonomic characterization of a *Marinimicrobium*-like bacterial strain, ISL-43^T, which was isolated from a marine solar saltern in Korea.

Strain ISL-43^T was isolated by means of the standard dilution-plating technique on marine agar 2216 (MA; Difco) supplemented with 8 % (w/v) NaCl and incubated at 30 °C. *M. koreense* KCTC 12356^T and *M. agarilyticum* KCTC 12357^T, which were used as reference strains for DNA–DNA hybridization and fatty acid analysis, were obtained from the KCTC. The morphological, physiological and biochemical characteristics of strain ISL-43^T were investigated using routine cultivation on MA at 30 °C.

Cell morphology was examined by using light microscopy (Eclipse E600; Nikon) and transmission electron microscopy. Flagellation was determined using a Philips CM-20 transmission electron microscope with cells from exponentially growing cultures: for this purpose, 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 potassium nitrate (0.1 %, w/v), both of which had been prepared anaerobically in nitrogen gas. The pH range for growth was determined in marine broth 2216 (MB; Difco) that was adjusted to various pH values (pH 4.5–9.5, at intervals of 0.5 pH units) by the addition of HCl or Na₂CO₃. Growth in the absence of NaCl was investigated using trypticase soy broth (Difco) prepared without NaCl. Growth at various NaCl concentrations (0.5 and 1–20 %, w/v, at intervals of 1 %) was investigated in trypticase soy broth for 0.5 and 1 % or MB for >2 % NaCl. Growth at various temperatures (4, 10, 20, 25, 28, 30, 35, 37, 40 and 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). 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 nitrate reduction were investigated as described by Lányi (1987) with the modification that artificial seawater was used for media preparation. The artificial seawater contained (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

The GenBank/EMBL/DBJ accession number for the 16S rRNA gene sequence of strain ISL-43^T is EU874388.

CaCl₂·2H₂O (Bruns *et al.*, 2001). H₂S production was tested as described by Bruns *et al.* (2001). Susceptibility to antibiotics was investigated on MA plates with the following antibiotic discs (µg unless otherwise stated): polymyxin B (100 U), streptomycin (50), penicillin G (20 U), chloramphenicol (100), ampicillin (10), cephalothin (30), gentamicin (30), novobiocin (5), tetracycline (30), kanamycin (30), lincomycin (15), oleandomycin (15), neomycin (30) and carbenicillin (100). Acid production from carbohydrates was tested as described by Leifson (1963). 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 1% (v/v) vitamin solution (Staley, 1968). Enzyme activities were determined by using the API ZYM system (bioMérieux).

Cell biomass for DNA extraction and for isoprenoid quinone analysis was obtained from cultures grown in MB at 30 °C. Chromosomal DNA was isolated and purified according to the method described by Yoon *et al.* (1996), with the addition that RNase T1 was used in combination with RNase A to minimize the contamination of RNA. The 16S rRNA gene sequence was amplified by PCR using two universal primers as described previously (Yoon *et al.*, 1998). Sequencing and phylogenetic analysis were performed as described previously (Yoon *et al.*, 2003). Isoprenoid quinones were analysed as described by Komagata & Suzuki (1987) using reversed-phase HPLC and a YMC-Pack ODS-A (250 × 4.6 mm) column (YMC). For cellular fatty acid analysis, cell mass was harvested from MA plates after cultivation for 5 days at 30 and 35 °C, for strain ISL-43^T and from MA plates after cultivation for 5 days at 35 °C for *M. koreense* KCTC 12356^T and *M. agarilyticum* KCTC 12357^T. The fatty acids were extracted and the fatty acid methyl esters were prepared according to the standard protocol of the Microbial Identification System (Sasser, 1990). The DNA G+C content was determined by using the method of Tamaoka & Komagata (1984) with the modification that DNA was hydrolysed using nuclease P1 (Sigma) and the resultant nucleotides were analysed by reversed-phase HPLC. DNA–DNA hybridization was performed fluorometrically by the method of Ezaki *et al.* (1989) using photobiotin-labelled DNA probes and micro-dilution wells. Five replicate hybridizations were conducted for each sample. The highest and lowest values from each sample were excluded and the remaining three were used to calculate mean DNA–DNA relatedness values.

Morphological, cultural, physiological and biochemical characteristics of strain ISL-43^T are given in the species description and in Table 1. The almost-complete 16S rRNA gene sequence of strain ISL-43^T comprised 1486 nucleotides. In the phylogenetic tree based on the neighbour-joining algorithm, strain ISL-43^T clustered with *M. agarilyticum* M18^T and *M. koreense* M9^T (Fig. 1). The same topology was also observed in the trees constructed using the maximum-likelihood and maximum-parsimony

Table 1. Differential phenotypic characteristics of *Marinimicrobium* species

Strains: 1, ISL-43^T (*Marinimicrobium locisalis* sp. nov.); 2, *M. koreense* M9^T (data from Lim *et al.*, 2006); 3, *M. agarilyticum* M18^T (Lim *et al.*, 2006). All species are motile by means of a single flagellum and positive for hydrolysis of aesculin, starch and Tween 20, acid production from L-arabinose, D-glucose, lactose, maltose, D-mannose and melibiose, and activities of alkaline phosphatase, esterase (C4) and esterase lipase (C8). All strains are negative for nitrate reduction, hydrolysis of gelatin, tyrosine and xanthine, acid production from inositol and mannitol, and activities of lipase (C14), cystine arylamidase, trypsin, α-chymotrypsin, acid phosphatase, β-glucuronidase, β-glucosidase, α-mannosidase and α-fucosidase. +, Positive; w, weakly positive; –, negative.

Characteristic	1	2	3
Cell morphology	Short rods or rods	Short rods	Short rods
Colony colour on MA	Yellow-green	Cream	Cream
Catalase	+	–	–
Oxidase	+	–	+
Hydrolysis of:			
Agar	–	–	+
Casein	+	–	–
Tween 80	–	–	+
Acid production from:			
D-Fructose	–	+	+
Raffinose	–	–	+
D-Ribose	–	+	+
Sucrose	–	+	+
Trehalose	–	+	+
D-Xylose	–	+	+
Enzyme activity (API ZYM)			
Leucine arylamidase	w	+	+
Valine arylamidase	–	+	+
Naphthol-AS-BI-phospho-hydrolase	–	+	+
α-Galactosidase	–	+	–
β-Galactosidase	–	–	+
α-Glucosidase	+	+	–
N-Acetyl-β-glucosaminidase	–	+	+
DNA G + C content (mol%)	58.4	57	58

algorithms (Fig. 1). Strain ISL-43^T exhibited 16S rRNA gene sequence similarity values of 98.1 and 95.5% to *M. agarilyticum* M18^T and *M. koreense* M9^T, respectively, and less than 92.3% to the other strains used in the phylogenetic analysis.

The predominant ubiquinone detected in strain ISL-43^T was Q-8 (peak area ratio of approximately 91%). The cellular fatty acid profiles of strain ISL-43^T and the type strains of the two other *Marinimicrobium* species are shown in Table 2. Strain ISL-43^T had large amounts of straight-chain, cyclo, unsaturated and hydroxy fatty acids: the major components (>10% of total fatty acids) were C_{16:0}, C_{19:0}ω8c, C_{16:1}ω7c and/or iso-C_{15:0} 2-OH, and

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the relative positions of *Marinimicrobium locisalis* sp. nov. ISL-43^T, *Marinimicrobium* species and some other related taxa. Bootstrap values (>50%) based on 1000 resamplings are shown at branch nodes. Filled circles indicate that the corresponding nodes were also recovered in the trees generated with the maximum-likelihood and maximum-parsimony algorithms. *Escherichia coli* ATCC 11775^T was used as the outgroup. Bar, 0.01 substitutions per nucleotide position.

C_{18:1}ω7c. This is similar to those of the two recognized *Marinimicrobium* species, although there are differences in the proportions of some fatty acids, particularly C_{18:1}ω7c (Table 2). The DNA G+C content of strain ISL-43^T was 58.4 mol%. The results obtained from these chemotaxonomic analyses were consistent with the results from the phylogenetic analysis that suggest that strain ISL-43^T may belong to the genus *Marinimicrobium*.

Strain ISL-43^T exhibited mean DNA–DNA relatedness values of 17 and 10% to *M. agarilyticum* KCTC 12357^T and *M. koreense* KCTC 12356^T, respectively. Strain ISL-43^T was distinguishable from recognized *Marinimicrobium* species through differences in several phenotypic characteristics. The phylogenetic and genetic distinctiveness and the differential phenotypic properties are sufficient to allocate strain ISL-43^T to a novel *Marinimicrobium* species (Wayne *et al.*, 1987; Stackebrandt & Goebel, 1994), for which the name *Marinimicrobium locisalis* sp. nov. is proposed.

Description of *Marinimicrobium locisalis* sp. nov.

Marinimicrobium locisalis (lo.ci.sa'lis. L. n. *locus* place, locality; L. gen. n. *salis* of salt; N.L. gen. n. *locisalis* from a place of salt).

Cells are Gram-negative and non-spore-forming rods, 0.2–0.4 × 0.7–3.5 μm. Motile by means of a single polar flagellum. Colonies on MA are circular to irregular, raised to slightly convex, glistening, smooth, yellow-green and 1.0–1.5 mm in diameter after incubation at 30 °C for 5 days. Growth occurs at 4 and 40 °C, but not at 45 °C, and at pH 6.0, but not at pH 5.5 (optimum pH 7.0–8.0). Growth occurs in the presence of 18% (w/v) NaCl, but not

Table 2. Cellular fatty acid compositions (%) of *Marinimicrobium* species

Strains: 1, ISL-43^T (*M. locisalis* sp. nov.); 2, ISL-43^T; 3, *M. koreense* KCTC 12356^T; 4, *M. agarilyticum* KCTC 12357^T. Data from this study after incubation at 30 °C (column 1) or 35 °C (columns 2–4). Fatty acids <0.5% of the total in all strains are omitted. ECL, Equivalent chain-length; –, not detected.

Fatty acid	1	2	3	4
Straight-chain				
C _{9:0}	0.1	0.2	0.7	0.6
C _{10:0}	4.7	5.2	4.3	10.2
C _{11:0}	0.8	1.0	1.6	1.9
C _{12:0}	4.7	5.7	2.0	1.5
C _{14:0}	1.8	2.0	2.5	1.2
C _{15:0}	3.4	3.9	4.9	3.8
C _{16:0}	20.8	22.0	21.9	22.9
C _{17:0}	3.2	3.8	4.0	6.5
C _{18:0}	2.2	2.4	2.0	2.8
Unsaturated				
C _{17:1} ω8c	0.4	0.7	0.5	0.7
C _{18:1} ω7c	11.7	10.1	2.2	2.2
Hydroxy				
C _{10:0} 3-OH	8.4	9.8	6.2	8.6
C _{11:0} 3-OH	0.4	0.6	0.7	0.8
Cyclo				
C _{17:0}	0.5	1.0	3.0	1.4
C _{19:0} ω8c	18.7	17.1	27.7	17.9
Summed feature 3*	16.5	13.5	11.5	11.7
Summed feature 7*	–	–	0.7	0.2
Unknown fatty acid ECL 11.799	0.9	0.6	2.1	3.8

*Summed features represent groups of two or three fatty acids that could not be separated by GLC with the MIDI system. Summed feature 3 contains C_{16:1}ω7c and/or iso-C_{15:0} 2-OH. Summed feature 7 contains C_{19:1}ω6c, cyclo C_{19:0}ω10c and/or unknown fatty acid (ECL 18.846).

without NaCl or with more than 19% (w/v) NaCl. Optimum growth occurs in the presence of approximately 2% (w/v) NaCl. Anaerobic growth does not occur on MA or on MA supplemented with nitrate. H₂S is not produced. Tweens 40 and 60 are hydrolysed, but hypoxanthine is not. L-Arabinose, cellobiose, D-glucose, maltose, D-xylose and salicin are utilized, but D-fructose, D-galactose, D-mannose, trehalose, sucrose, acetate, citrate, succinate, benzoate, L-malate, pyruvate, formate and L-glutamate are not. Acid is produced from cellobiose and D-galactose, but not from melezitose, L-rhamnose or D-sorbitol. Susceptible to ampicillin, carbenicillin, cephalothin, chloramphenicol, gentamicin, neomycin, polymyxin B, oleandomycin and streptomycin, but not to kanamycin, lincomycin, novobiocin, penicillin G or tetracycline. The predominant ubiquinone is Q-8. The major fatty acids (>10% of total fatty acids) are C_{16:0}, C_{19:0}ω8c, C_{16:1}ω7c and/or iso-C_{15:0} 2-OH, and C_{18:1}ω7c. Other phenotypic characteristics are

given in Table 1. The DNA G + C content of the type strain is 58.4 mol%.

The type strain, ISL-43^T (=KCTC 22484^T=CCUG 56757^T), was isolated from a marine solar saltern of the Yellow Sea, Korea.

Emended description of the genus *Marinimicrobium* Lim et al. 2006

The description of the genus *Marinimicrobium* is as given by Lim et al. (2006) with the following amendments. Cells are short rods or rods. Catalase is negative or positive.

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