Marinobacter salicampi sp. nov., isolated from a marine solar saltern in Korea

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A Gram-negative, motile, rod-shaped, *Marinobacter*-like bacterial strain, ISL-40^T, was isolated from a marine solar saltern of the Yellow Sea in Korea. The taxonomic position of the novel strain was investigated using a polyphasic approach. Strain ISL-40^T grew optimally at pH 7.0–8.0 and at 30 °C. It contained Q-9 as the predominant ubiquinone. The major fatty acids were C_{16:0}, C_{16:1 ω 7c} and/or iso-C_{15:0} 2-OH and 10-methyl C_{16:0}. The DNA G + C content was 58.1 mol%. A phylogenetic analysis based on 16S rRNA gene sequences showed that strain ISL-40^T belongs to the genus *Marinobacter*. Strain ISL-40^T exhibited 16S rRNA gene sequence similarity values of 93.5–96.4 % to the type strains of recognized *Marinobacter* species. The differential phenotypic properties and phylogenetic distinctiveness of strain ISL-40^T revealed that it is separate from recognized *Marinobacter* species. On the basis of phenotypic, phylogenetic and genetic data, therefore, strain ISL-40^T represents a novel species of the genus *Marinobacter*, for which the name *Marinobacter salicampi* sp. nov. is proposed. The type strain is ISL-40^T (=KCTC 12972^T=CCUG 54357^T).

The genus *Marinobacter*, which was described by Gauthier *et al.* (1992), currently comprises 16 species with validly published names including the recently described species *Marinobacter algicola* (Green *et al.*, 2006), *Marinobacter vinifirmus* (Liebgott *et al.*, 2006), *Marinobacter koreensis* (Kim *et al.*, 2006), *Marinobacter gudaonensis* (Gu *et al.*, 2007) and *Marinobacter salsuginis* (Antunes *et al.*, 2007). Phylogenetic analyses based on 16S rRNA gene sequences have shown that the genus *Marinobacter* falls within the class *Gammaproteobacteria* (Anzai *et al.*, 2000; Gu *et al.*, 2007). Here, we report the taxonomic characterization of a *Marinobacter*-like strain, ISL-40^T, isolated from a marine solar saltern.

Sediment collected from a marine solar saltern of the Yellow Sea at Byunsan, Korea, was used as the source for the isolation of bacterial strains. Strain ISL-40^T was isolated by means of the standard 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-40^T were investigated using routine cultivation at 30 °C on MA supplemented with 6 % (w/v) NaCl. Cell morphology was examined by using light microscopy (E600; Nikon) and transmission electron microscopy (TEM). Flagellation was determined by using a TEM (CM-20; Philips) with cells

from exponentially growing cultures. To visualize flagella, 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 supplemented with 6 % (w/v) NaCl and on MA supplemented with 6 % (w/v) NaCl supplemented with potassium nitrate (0.1 %, w/v), both of which had been prepared anaerobically using nitrogen. 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 marine broth 2216 (MB; Difco) or trypticase soy broth (Difco). Growth at various temperatures (4–45 °C) was measured on MA supplemented with 6 % (w/v) NaCl. 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 supplemented with 6 % (w/v) NaCl, 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 supplemented with 6 % (w/v) NaCl was used for the preparation of 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).

Abbreviation: TEM, transmission electron microscope/microscopy.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain ISL-40^T is EF486354.

Susceptibility to antibiotics was investigated on MA supplemented with 6% (w/v) NaCl using discs containing the following concentrations of antibiotic: 100 U polymyxin B; 50 µg streptomycin; 20 U penicillin G; 100 µg chloramphenicol; 10 µg ampicillin; 30 µg cephalothin; 30 µg gentamicin; 5 µg novobiocin; 30 µg tetracycline; 30 µg kanamycin; 15 µg lincomycin; 15 µg oleandomycin; 30 µg neomycin and 100 µg carbenicillin. Acid production from carbohydrates was tested as described by Leifson (1963). Utilization of various substrates for growth was determined as described 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 supplemented with 6% (w/v) NaCl at 30 °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 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-40^T was harvested from agar plates after cultivation for 7 days at 30 °C on MA supplemented with 6% (w/v) NaCl. The fatty acids were extracted and the fatty acid methyl esters prepared according to the standard protocol of the MIDI/Hewlett Packard Microbial Identification System (Sasser, 1990). The DNA G + C content was determined 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.

The morphological, cultural, physiological and biochemical characteristics of strain ISL-40^T are given in the species description (see below) or shown in Table 1. The almost-complete 16S rRNA gene sequence of strain ISL-40^T determined in this study comprised 1496 nt, representing approximately 96% of the *Escherichia coli* 16S rRNA gene sequence. 16S rRNA gene sequence analysis revealed that strain ISL-40^T was phylogenetically most closely related to the genus *Marinobacter*. In the phylogenetic tree

Table 1. Differential phenotypic characteristics of strain ISL-40^T and two phylogenetically related *Marinobacter* species

Taxa: 1, ISL-40^T; 2, *M. bryozorum* KMM 3840^T (data from Romanenko *et al.*, 2005); 3, *M. gudaonensis* SL014B61A^T (Gu *et al.*, 2007). +, Positive; –, negative; w, weakly positive; ND, not determined. All of the strains are positive for motility, catalase, oxidase, nitrate reduction to nitrite and the utilization of acetate and pyruvate. All of the strains are negative for hydrolysis of gelatin, starch and urea, nitrite reduction to N₂ and the utilization of L-arabinose, D-mannose, formate and L-glutamate.

Characteristic	1	2	3
Cell size (µm)	0.4–0.8 × 1.0–7.0	0.4–0.5 × 1.0–1.3	0.3–0.5 × 1.2–1.8
Growth without NaCl	–	–	+
Growth with 18% NaCl	–	–	+
Temperature range for growth (°C)	4–39	4–42	10–45
Hydrolysis of Tween 80	+	–	+
Utilization of:			
D-Cellobiose	–	–	w
Citrate	–	–	+
D-Fructose	–	–	+
D-Galactose	–	–	w
D-Glucose	–	–	+
L-Malate	+	–	ND
Maltose	–	–	+
Succinate	+	–	+
Sucrose	–	+	+
Trehalose	–	–	+
D-Xylose	–	+	+
Susceptibility to:			
Gentamicin	–	ND	+
Kanamycin	–	ND	+
Tetracycline	–	ND	+
DNA G + C content (mol%)	58.1	59.6	57.9

constructed using the neighbour-joining algorithm, strain ISL-40^T fell within the clade comprising species of the genus *Marinobacter* (Fig. 1). Strain ISL-40^T exhibited 16S rRNA gene sequence similarity values of 96.3 and 96.4 % to *Marinobacter bryozoorum* and *M. gudaonensis*, respectively, and values of 93.5–95.9 % with the type strains of the other species of the genus *Marinobacter*. The gene sequence similarity values with respect to other species used in the phylogenetic analysis were less than 90.4 %.

The chemotaxonomic properties of ISL-40^T confirm the result of the phylogenetic analysis, namely that the novel strain is affiliated with the genus *Marinobacter*. The predominant isoprenoid quinone detected in strain ISL-40^T was Q-9, at a peak area ratio of approximately 96 %, as is the case for other species of the genus *Marinobacter* (Yoon *et al.*, 2003; Gu *et al.*, 2007). The fatty acids of strain ISL-40^T consisted of the following (each constituting >0.5 % of total fatty acids): straight-chain fatty acids C_{16:0} (18.4 %), C_{12:0} (6.7 %), C_{18:0} (5.2 %), C_{17:0} (3.3 %) and C_{16:0} alcohol (0.6 %); summed feature 3 (C_{16:1}ω7c and/or iso-C_{15:0} 2-OH, 18.3 %); 10-methyl fatty acids C_{16:0} (16.9 %), C_{17:0} (2.0 %) and C_{18:0} (1.2 %); saturated fatty acids C_{18:1}ω9c (7.7 %), C_{18:1}ω7c (5.5 %), C_{17:1}ω8c (1.8 %), C_{16:1}ω9c (1.2 %) and C_{18:1}ω5c (0.8 %); hydroxyl fatty acids C_{12:0} 3-OH (6.1 %) and C_{11:0} 3-OH (0.6 %); branched fatty acid iso-C_{17:0} (1.2 %) and summed feature 2 (iso-C_{16:1}, C_{14:0} 3-OH and/or an unknown fatty acid with an equivalent chain-length of 10.928, 0.7 %). This fatty acid profile was similar to those of other species of the genus *Marinobacter*, although there were differences in the proportions of some fatty acids, probably because of differences in cultivation conditions and extraction procedures (Green *et al.*, 2006; Kim *et al.*, 2006; Gu *et al.*, 2007). The DNA G+C content of strain ISL-40^T was 58.1 mol%.

Strain ISL-40^T is distinguishable from two phylogenetically related recognized species of the genus *Marinobacter* by means of differences in several phenotypic characteristics, as shown in Table 1. The phylogenetic distinctiveness of ISL-40^T, together with the differential phenotypic properties, is sufficient to allocate the strain to a separate species from the recognized *Marinobacter* species (Stackebrandt & Goebel, 1994). Therefore, on the basis of the data presented, strain ISL-40^T should be placed within the genus *Marinobacter* as a novel species, for which the name *Marinobacter salicampi* sp. nov. is proposed.

Description of *Marinobacter salicampi* sp. nov.

Marinobacter salicampi (sa.li.cam'pi. L. n. *sal* salt; L. n. *campus* field; N.L. gen. n. *salicampi* of a salt field).

Cells are Gram-negative, aerobic, straight or curved rods (0.4–0.8 × 1.0–7.0 μm). Motile by means of a single flagellum. Colonies on MA supplemented with 6 % (w/v) NaCl are circular to slightly irregular, raised, smooth, yellowish white in colour and 0.8–1.2 mm in diameter after 7 days incubation at 30 °C. Optimal growth occurs at 30 °C. Optimal pH for growth is between 7.0 and 8.0; growth occurs at pH 5.5, but not at pH 5.0. Optimal growth occurs in the presence of approximately 8 % (w/v) NaCl. Growth occurs in the presence of 15 % (w/v) NaCl, but not in the absence of NaCl or in the presence of more than 16 % (w/v) NaCl. Tweens 20, 40 and 60 are hydrolysed, but aesculin, casein, L-tyrosine, hypoxanthine and xanthine are not. 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-mannitol, D-sorbitol and *myo*-inositol. Benzoate and salicin are not utilized. Susceptible to ampicillin, carbenicillin, cephalothin, chloramphenicol, penicillin G, polymyxin

Fig. 1. Neighbour-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the positions of strain ISL-40^T, species of the genus *Marinobacter* and some other related taxa. Bootstrap percentages (from 1000 replications) >50 % are shown at branch points. *E. coli* ATCC 11775^T was used as an outgroup. Bar, 0.01 substitutions per nucleotide position.

B and streptomycin, but not to lincomycin, novobiocin, neomycin or oleandomycin. In assays with the API ZYM system, alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, naphthol-AS-BI-phosphohydrolase and *N*-acetyl- β -glucosaminidase are present, but lipase (C14), valine arylamidase, cystine arylamidase, trypsin, α -chymotrypsin, acid phosphatase, α -galactosidase, β -galactosidase, β -glucuronidase, α -glucosidase, β -glucosidase, α -mannosidase and α -fucosidase are absent. The predominant ubiquinone is Q-9. The major fatty acids (each constituting >10% of total fatty acids) are C_{16:0}, summed feature 3 (C_{16:1} ω 7c and/or iso-C_{15:0} 2-OH) and 10-methyl C_{16:0}. The DNA G+C content is 58.1 mol% (determined by HPLC). Other phenotypic characteristics are given in Table 1.

The type strain, ISL-40^T (=KCTC 12972^T=CCUG 54357^T), was isolated from a marine solar saltern of the Yellow Sea at Byunsan, Korea.

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