Salimicrobium flavidum sp. nov., isolated from a marine solar saltern

Jung-Hoon Yoon, So-Jung Kang, Ki-Hoon Oh and Tae-Kwang Oh

Correspondence

Jung-Hoon Yoon
jhyoon@kribb.re.kr

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

A Gram-variable-staining, motile and coccoid-, ovoid- or rod-shaped bacterium, strain ISL-25^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-25^T grew optimally at pH 7.0–8.0 and 30–37 °C. Strain ISL-25^T contained *meso*-diaminopimelic acid as the cell-wall peptidoglycan, MK-7 as the predominant menaquinone and anteiso-C_{15:0}, anteiso-C_{17:0} and iso-C_{16:0} as the major fatty acids. The DNA G + C content was 49.3 mol%. Phylogenetic analyses based on 16S rRNA gene sequences showed that strain ISL-25^T belongs to the genus *Salimicrobium*. The similarity values between the 16S rRNA gene sequence of strain ISL-25^T and those of the type strains of the three currently recognized *Salimicrobium* species were 97.6–98.3 %. Mean DNA–DNA relatedness values between strain ISL-25^T and the type strains of the genus *Salimicrobium* were 9–15 %. Differential phenotypic properties of strain ISL-25^T, together with the phylogenetic and genetic distinctiveness, revealed that this strain could be differentiated from other *Salimicrobium* species. Therefore, strain ISL-25^T represents a novel species within the genus *Salimicrobium*, for which the name *Salimicrobium flavidum* sp. nov. is proposed. The type strain is ISL-25^T (=KCTC 13260^T=CCUG 56755^T).

The genus *Salimicrobium* was proposed by Yoon *et al.* (2007) by the reclassification of *Marinococcus albus* and *Bacillus halophilus* as *Salimicrobium album* and *Salimicrobium halophilum*, respectively, and the description of a novel species, *Salimicrobium luteum*. In this study, we report the taxonomic characterization of a *Salimicrobium*-like bacterial strain that was isolated from a marine solar saltern in Korea.

Strain ISL-25^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 (8 % NaCl MA). The type strains of the three *Salimicrobium* species were used as reference strains for DNA–DNA hybridization experiments and phenotypic characterization. *S. album* LMG 17430^T was obtained from the Laboratorium voor Microbiologie Universiteit Gent and *S. halophilum* KCTC 3566^T was obtained from the Korean Collection for Type Cultures. *S. luteum* BY-5^T was obtained from a previous study (Yoon *et al.*, 2007). The physiological and biochemical characteristics of strain ISL-25^T were investigated by using routine cultivation at 37 °C on 8 % NaCl MA. The morphology of strain ISL-25^T was observed after cultivation on 8 % NaCl MA, MH agar (Rodriguez-Valera *et al.*, 1980) and MH agar with 15 % (w/v) salt (Ventosa *et al.*, 1982) at 37 °C. Morphological, physiological, cultural and

biochemical properties were determined as described by Yoon *et al.* (2007). Growth in the absence of NaCl was investigated using trypticase soy broth (Difco), prepared according to the manufacturer's instructions except that NaCl was excluded, both with and without 0.45 % (w/v) MgCl₂·6H₂O and 0.06 % (w/v) KCl. Growth at various NaCl concentrations (0.5 % and 1.0–28.0 %, w/v, at increments of 1.0 %) was investigated in marine broth 2216 (MB; Difco) or trypticase soy broth supplemented with 0.45 % MgCl₂·6H₂O. Growth at various temperatures (4, 10, 20, 25, 28, 30, 35, 40 and 45 °C) was measured on 8 % NaCl MA. Acid production from carbohydrates was tested as described by Leifson (1963) with the modification that 8 % NaCl was added to the media.

Cell biomass for DNA extraction and the analysis of the cell-wall composition and isoprenoid quinones was obtained from cultures grown at 37 °C in MB supplemented with 8 % NaCl. For cellular fatty acid analysis, cell mass of strain ISL-25^T was harvested from 8 % NaCl MA plates after cultivation for 3 days at 37 °C. Chromosomal DNA was isolated and purified according to the method described by Yoon *et al.* (1996), with the exception 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, 5'-GAGTTTGATCCTGGCTCAG-3' and 5'-AGAAAGGAGGTGATCCAGCC-3', as described previously (Yoon *et al.*, 1998). Sequencing and phylogenetic

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain ISL-25^T is FJ357160.

analysis were performed as described by Yoon *et al.* (2003). Chemotaxonomic properties were examined as described by Yoon *et al.* (2007). DNA–DNA hybridization experiments were performed as described by Yoon *et al.* (2007), based on the method of Ezaki *et al.* (1989).

Cells of strain ISL-25^T were Gram-variable-staining and non-spore-forming. On 8% NaCl MA, cells were short rods or rods (0.4–0.7 µm × 0.8–3.5 µm), whereas they were

larger cocci, ovals or rods on MH agar (0.8–1.3 µm × 0.8–2.9 µm) and on MH agar with 15% salt (0.8–1.3 µm × 0.9–9.0 µm). Morphological, cultural, physiological and biochemical characteristics of strain ISL-25^T are given in the species description and in Table 1. The almost-complete 16S rRNA gene sequence of strain ISL-25^T that was determined for this study comprised 1524 nucleotides, which covered approximately 96% of the *Escherichia coli* 16S rRNA gene sequence. In the phylogenetic tree based on

Table 1. Differential phenotypic characteristics of strain ISL-25^T and the type strains of *Salimicrobium* species

Taxa: 1, ISL-25^T (*Salimicrobium flavidum* sp. nov.); 2, *S. album* (data from Hao *et al.*, 1984; Li *et al.*, 2005); 3, *S. halophilum* (Ventosa *et al.*, 1989; Arahal *et al.*, 1999); 4, *S. luteum* (Yoon *et al.*, 2007). All are positive for catalase, oxidase, NaCl requirement for growth, susceptibility to penicillin G, chloramphenicol, ampicillin, cephalothin, novobiocin, carbenicillin, lincomycin and oleandomycin, and activities of esterase (C4) and esterase lipase (C8). All are negative for anaerobic growth, hydrolysis of casein and starch, acid production from L-arabinose, D-galactose and lactose, susceptibility to polymyxin B and tetracycline, and activities of lipase (C14), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, β-glucuronidase, α-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase. +, Positive; –, negative; w, weakly positive; ND, not determined.

Characteristic	1	2	3	4
Cell morphology	Cocci, ovals or rods	Cocci	Rods	Cocci
Cell size (µm)	0.4–1.3 × 0.8–9.0*	1.0–1.2	0.5–1.0 × 2.5–9.0	0.7–2.4*
Motility	+	+	+	–
Colony colour	Pale yellow	White	Non-pigmented	Yellow
Nitrate reduction	–	+	–	–
Optimal NaCl concentration for growth (%)	10	5–15	15	10
Growth at:				
10 °C	+	ND	–	+
45 °C	–	ND	+	–
Hydrolysis of:				
Aesculin	–	–	+	–
Gelatin	–	–	–	+
Tween 80	+	–	+	–
Acid production from:				
D-Fructose	+	–	–	+
D-Glucose	+	–	+	+
Maltose	–	–	–	+
Sucrose	+	–	+	+
Trehalose	+	–	+	+
D-Xylose	–	–	+	–
D-Mannitol	+	–	–	+
Susceptibility to antibiotics (µg per disc)†				
Streptomycin (50)	w	+	–	–
Gentamicin (30)	–	+	–	–
Kanamycin (30)	+	+	–	+
Neomycin (30)	–	+	–	–
APY ZYM†				
Alkaline phosphatase	+	+	–	+
Acid phosphatase	–	+	–	–
Naphthol-AS-BI-phosphohydrolase	–	w	–	+
β-Galactosidase	w	–	–	+
β-Glucosidase	–	+	–	–
DNA G + C content (mol%)	49.3	44.9‡	51.5‡	47.9

*Differences may be observed with different cultivation conditions.

†Data from this study.

‡Determined by *T_m* method.

the neighbour-joining algorithm, strain ISL-25^T fell within the clade comprising *Salimicrobium* species (Fig. 1). Strain ISL-25^T exhibited 16S rRNA gene sequence similarity values of 98.3, 98.2 and 97.6 % with *S. luteum* BY-5^T, *S. halophilum* DSM 4771^T and *S. album* DSM 20748^T, respectively, 94.5–95.3 % with the type strains of *Halobacillus* species and less than 92.7 % with the other strains used in the phylogenetic analysis.

Strain ISL-25^T had meso-diaminopimelic acid as the diagnostic diamino acid in the cell-wall peptidoglycan. The predominant menaquinone detected in strain ISL-25^T was MK-7 (with a peak area ratio of approximately 93 %). The cellular fatty acid profile of strain ISL-25^T is shown in Table 2, together with those of the *Salimicrobium* strains analysed previously (Yoon *et al.*, 2007). Strain ISL-25^T contained large amounts of branched fatty acids; the major components (>10 % of total fatty acids) were anteiso-C_{15:0}, anteiso-C_{17:0} and iso-C_{16:0}. This fatty acid profile of strain ISL-25^T was similar to those of the three recognized *Salimicrobium* species, although there were differences in the proportions of some fatty acids (Table 2). The DNA G + C content of strain ISL-25^T was 49.3 mol%. The results obtained from the chemotaxonomic analyses support those from the phylogenetic analysis and suggest that strain ISL-25^T belongs to the genus *Salimicrobium*.

Strain ISL-25^T exhibited mean DNA–DNA relatedness values of 15, 12 and 9 % to *S. halophilum* KCTC 3566^T, *S. luteum* BY-5^T and *S. album* LMG 17430^T, respectively. Strain ISL-25^T was distinguishable from *Salimicrobium*

Table 2. Cellular fatty acid compositions (%) of *Salimicrobium flavidum* sp. nov. ISL-25^T and *Salimicrobium* type strains after growth on MA with 8 % NaCl at 37 °C

Strains: 1, ISL-25^T (*Salimicrobium flavidum* sp. nov.); 2, *S. album* LMG 17430^T; 3, *S. halophilum* KCTC 3566^T; 4, *S. luteum* BY-5^T. Data for reference strains were obtained from Yoon *et al.* (2007). Fatty acids representing <0.5 % in all strains are not shown. –, Not detected/not reported.

Fatty acid	1	2	3	4
Straight-chain				
C _{15:0}	1.8	–	0.9	1.7
C _{16:0}	1.0	2.4	1.6	2.4
C _{18:0}	–	4.9	–	0.9
C _{20:0}	–	4.0	–	–
Branched				
iso-C _{14:0}	3.4	–	1.5	2.3
iso-C _{15:0}	3.2	1.8	37.3	26.1
anteiso-C _{15:0}	49.8	47.0	24.7	28.7
iso-C _{16:0}	10.6	–	3.4	6.8
iso-C _{17:0}	1.4	2.1	14.4	11.8
anteiso-C _{17:0}	26.4	29.5	11.2	14.5
iso-C _{17:1} ω10c	–	–	1.4	–
anteiso-C _{19:0}	–	8.2	–	–
Unsaturated				
C _{16:1} ω7c alcohol	1.4	–	1.5	3.0
C _{16:1} ω11c	0.2	–	0.9	–
Hydroxy				
C _{16:0} 2-OH	–	–	–	0.8
Summed feature 4*	0.6	–	1.2	1.0

*Summed features represent two or three fatty acids that cannot be separated by the Microbial Identification System. Summed feature 4 contains iso-C_{17:1} I and/or anteiso-C_{17:1} B.

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the positions of *Salimicrobium flavidum* sp. nov. ISL-25^T, *Salimicrobium* species and some other related taxa. Bootstrap values (>50 %) based on 1000 replications 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. *Alicyclobacillus acidocaldarius* DSM 446^T was used as the outgroup. Bar, 0.01 substitutions per nucleotide position.

species by differences in several phenotypic characteristics, as listed in Table 1. The phylogenetic and genetic distinctiveness and differential phenotypic properties of strain ISL-25^T were sufficient to categorize it as a member of a species that is distinct from the recognized *Salimicrobium* species (Wayne *et al.*, 1987; Stackebrandt & Goebel, 1994). On the basis of the data presented, strain ISL-25^T represents a novel species of the genus *Salimicrobium*, for which the name *Salimicrobium flavidum* sp. nov. is proposed.

Description of *Salimicrobium flavidum* sp. nov.

Salimicrobium flavidum (fla'vi.dum. L. neut. adj. *flavidum* pale yellow).

Cells are Gram-variable-staining and coccoid-, ovoid- or rod-shaped (0.4–1.3 × 0.8–9.0 μm). Motile by means of peritrichous flagella. Colonies are circular, slightly convex, glistening, smooth and pale yellow, 1.5–2.0 mm in diameter after incubation for 3 days at 37 °C on MA with 8 % NaCl. Growth occurs at 4 and 40 °C but not at 45 °C

(optimum, 37 °C), at pH 6.0 but not at pH 5.5 (optimum, pH 7.0–8.0), and in the presence of 1.0–26.0 % NaCl (optimum, 10 % NaCl). Anaerobic growth does not occur on MA with 8 % NaCl with or without nitrate supplementation. Mg^{2+} ions are required for growth. Catalase- and oxidase-positive. Tweens 20, 40, 60 and 80 are hydrolysed, but aesculin, casein, gelatin, starch, hypoxanthine, xanthine and L-tyrosine are not. H_2S is not produced. Acid is produced from D-fructose, D-glucose, D-mannitol, D-mannose, melibiose, D-ribose, sucrose and trehalose, but not from L-arabinose, cellobiose, D-galactose, lactose, maltose, melezitose, raffinose, L-rhamnose, myo-inositol, D-sorbitol or D-xylose. Susceptible to the following antibiotics (μg per disc, unless indicated otherwise): ampicillin (10), carbenicillin (100), cephalothin (30), chloramphenicol (100), kanamycin (30), lincomycin (15), novobiocin (5), oleandomycin (15) and penicillin G (20 U) and weakly susceptible to streptomycin (50), but not to gentamicin (30), neomycin (30), polymyxin B (100 U) or tetracycline (30). In assays with the API ZYM system, alkaline phosphatase, esterase (C4) and esterase lipase (C8) are present and β -galactosidase is weakly present, but lipase (C14), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, α -chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α -galactosidase, β -glucuronidase, α - and β -glucosidase, N-acetyl- β -glucosaminidase, α -mannosidase and α -fucosidase are absent. The cell-wall peptidoglycan contains meso-diaminopimelic acid as the diamino acid. The predominant menaquinone is MK-7. Major fatty acids (>10 % of total fatty acids) are anteiso- $C_{15:0}$, anteiso- $C_{17:0}$ and iso- $C_{16:0}$. Other phenotypic characteristics are given in Table 1. The DNA G+C content of the type strain is 49.3 mol% (HPLC).

The type strain, ISL-25^T (=KCTC 13260^T=CCUG 56755^T), was isolated from a marine solar saltern of the Yellow Sea in Korea.

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