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

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A Gram-positive, motile, moderately halophilic and rod-shaped bacterium, strain AHS-1^T, was isolated from a marine solar saltern in Korea, and its exact taxonomic position was investigated by use of a polyphasic study. Strain AHS-1^T grew optimally at pH 6.5–7.0 and 37–40 °C in the presence of 10–12% (w/v) NaCl. Phylogenetic analyses based on 16S rRNA gene sequences indicated that strain AHS-1^T belonged to the genus *Lentibacillus*. Strain AHS-1^T exhibited a 16S rRNA gene sequence similarity value of 97.0% to the type strain of *Lentibacillus salarius* and values of 95.0–96.7% to the type strains of other recognized *Lentibacillus* species. Strain AHS-1^T had cell-wall peptidoglycan based on *meso*-diaminopimelic acid. It contained MK-7 as the predominant menaquinone. The major fatty acids (>10% of total fatty acids) were anteiso-C_{15:0} and anteiso-C_{17:0}, and the major polar lipids were phosphatidylglycerol, diphosphatidylglycerol and two unidentified glycolipids. The DNA G+C content was 49.0 mol%. Strain AHS-1^T shared a mean level of DNA–DNA relatedness of 8.5% with *L. salarius* KCTC 3911^T. Strain AHS-1^T could also be distinguished from recognized *Lentibacillus* species based on several phenotypic properties. On the basis of phenotypic, chemotaxonomic, phylogenetic and genetic data, strain AHS-1^T is considered to represent a novel species of the genus *Lentibacillus*, for which the name *Lentibacillus salinarum* sp. nov. is proposed. The type strain is AHS-1^T (=KCTC 13162^T=CCUG 54822^T).

The genus *Lentibacillus* was created by Yoon *et al.* (2002) with the description of a single species, *Lentibacillus salicampi*. Subsequently, six further species of the genus, *Lentibacillus salarius* (Jeon *et al.*, 2005), *L. lacisalsi* (Lim *et al.*, 2005), *L. juripiscarius* (Namwong *et al.*, 2005), *L. halophilus* (Tanasupawat *et al.*, 2006), *L. kapialis* (Pakdeeto *et al.*, 2007) and *L. halodurans* (Yuan *et al.*, 2007), have been described. Here we report on the taxonomic characterization of a novel bacterial strain, AHS-1^T, which was isolated from a marine solar saltern in Korea.

Sediment samples collected from a marine solar saltern of the Yellow Sea, Byunsan, Korea, were used as the source for the isolation of bacterial strains. The sediment samples (each 0.5 mg) were inoculated in 100 ml modified S-G (MSG) medium (Sehgal & Gibbons, 1960) containing (per litre of distilled water): 100 g NaCl, 20 g MgSO₄·7H₂O,

2 g KCl, 3 g trisodium citrate, 10 g yeast extract and 7.5 g Casamino acids (pH 7.2). This medium was incubated at 30 °C on a horizontal shaker at 150 r.p.m. After incubation for 30 days, 0.1 ml of the suspension was transferred into 100 ml fresh medium and the medium was reincubated under the same conditions. Strain AHS-1^T was isolated from the enrichment culture by means of dilution plating on MSG medium solidified with 1.5% (w/v) agar. *L. salarius* KCTC 3911^T, which was used as a reference strain for DNA–DNA hybridization experiments, was obtained from the Korean Collection for Type Cultures (KCTC), Taejeon, Korea. The morphological, cultural, physiological and biochemical characteristics of strain AHS-1^T were investigated by using routine cultivation at 37 °C on MSG medium (pH 7.2). Cell morphology was examined by light microscopy (Nikon E600) 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, cells were negatively stained with 1% (w/v) phosphotungstic acid and the grids were examined after being air-dried. The Gram reaction was determined by using the bioMérieux

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain AHS-1^T is EF601571.

A table detailing the cellular fatty acid compositions of strain AHS-1^T and related *Lentibacillus* species is available as supplementary material with the online version of this paper.

Gram stain kit according to the manufacturer's instructions. Growth under anaerobic conditions was determined after incubation in a Forma anaerobic chamber on MSG agar and on MSG agar supplemented with potassium nitrate (0.1 %, w/v), both of which had been prepared anaerobically under nitrogen. The pH range for growth was determined in MSG broth that was adjusted to various pH values (pH 4.5–10.5 at intervals of 0.5 pH units). Growth at various NaCl concentrations (0–30 % at intervals of 1 %) was investigated by using liquid media prepared according to the formula of the MSG medium except that NaCl was excluded. Growth at various temperatures (4–50 °C) was measured on MSG agar. Catalase and oxidase activities were determined as described by Cowan & Steel (1965). Hydrolysis of casein, starch, hypoxanthine, tyrosine and xanthine was tested on MSG agar by using the substrate concentrations described by Cowan & Steel (1965). Hydrolysis of aesculin, gelatin, Tween 80 and urea and reduction of nitrate were investigated as described by Lanyi (1987) with the modification that artificial seawater (Bruns *et al.*, 2001) supplemented with 8 % (w/v) NaCl was used for preparation of media. Acid production from carbohydrates was determined as reported by Leifson (1963) but using supplementation with 8 % (w/v) NaCl.

Cell biomass for DNA extraction and for the analyses of cell-wall, isoprenoid quinones and polar lipids was obtained from cultivation in MSG broth 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 RNA contamination. The 16S rRNA gene was amplified by PCR by using two universal primers as given by Yoon *et al.* (1998). Sequencing of the amplified 16S rRNA gene and phylogenetic analysis were performed as described by Yoon *et al.* (2002). The isomer type of the diamino acid in the cell-wall peptidoglycan was analysed via TLC according to the method described by Komagata & Suzuki (1987). Isoprenoid quinones were analysed according to Komagata & Suzuki (1987) by using reversed-phase HPLC. Polar lipids were extracted according to the procedures of Minnikin *et al.* (1984) and were identified by two-dimensional TLC followed by spraying with appropriate detection reagents (Minnikin *et al.*, 1984; Komagata & Suzuki, 1987). For cellular fatty acid analysis, cell mass of strain AHS-1^T was harvested from agar plates after cultivation for 2 days at 37 °C on marine agar 2216 (Difco) supplemented with 10 % (w/v) NaCl. 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 according to the method of Tamaoka & Komagata (1984) with the modification that DNA was hydrolysed by using nuclease P1 (Sigma) and the resultant nucleotides were analysed by reversed-phase HPLC. DNA–DNA hybridization experiments were performed fluorometrically according to the method of Ezaki *et al.* (1989). Hybridization was

performed with five replications for each sample. The highest and lowest values obtained in each sample were excluded, and the means of the remaining three values were quoted as DNA–DNA relatedness values.

Morphological, cultural, physiological and biochemical characteristics of strain AHS-1^T are detailed in Table 1 or are given in the species description below. Growth occurred at 15–45 °C (optimum, 37–40 °C), at pH 6.0–9.5 (optimum, pH 6.5–7.0) and in the presence of 3–24 % (w/v) NaCl (optimum, 10–12 % NaCl). After incubation for 4 days on MSG agar at 37 °C, colonies were circular, slightly convex, smooth, entire, cream-yellow in colour and 0.5–1.2 mm in diameter.

The almost-complete 16S rRNA gene sequence of strain AHS-1^T determined in this study comprised 1521 nt. In the phylogenetic tree based on the neighbour-joining algorithm, strain AHS-1^T fell within the radiation of the cluster comprising recognized *Lentibacillus* species (Fig. 1). Strain AHS-1^T showed highest 16S rRNA gene sequence similarity to the type strain of *L. salarius* (97.0 %), and showed values of 95.0–96.7 % to the type strains of other recognized *Lentibacillus* species. The mean level of DNA–DNA relatedness between strain AHS-1^T and *L. salarius* KCTC 3911^T was 8.5 %, when their DNAs were used individually as labelled DNA probes for cross-hybridization, indicating that these taxa represent different genomic species.

The results obtained from chemotaxonomic analyses support the phylogenetic results suggesting that strain AHS-1^T represents a member of the genus *Lentibacillus* (Yoon *et al.*, 2002; Jeon *et al.*, 2005; Tanasupawat *et al.*, 2006; Yuan *et al.*, 2007). The diagnostic diamino acid in the cell-wall peptidoglycan was *meso*-diaminopimelic acid. The predominant isoprenoid quinone detected in strain AHS-1^T was menaquinone-7 (MK-7) and the major polar lipids were diphosphatidylglycerol, phosphatidylglycerol and two unidentified glycolipids. Strain AHS-1^T contained large amounts of branched- and straight-chain fatty acids; the major components (>10 % of the total fatty acids) were anteiso-C_{15:0} (54.0 %) and anteiso-C_{17:0} (27.0 %) (see Supplementary Table S1 in IJSEM Online). The fatty acid profile of strain AHS-1^T was similar to those of recognized *Lentibacillus* species, although there were some differences in the proportions of some components, perhaps due to differences in the cultivation, extraction and analysis conditions used (see Supplementary Table S1 in IJSEM Online). The DNA G+C content of strain AHS-1^T was 49.0 mol%, a value higher than those of other *Lentibacillus* species. Strain AHS-1^T was distinguishable from recognized *Lentibacillus* species by differences in several phenotypic characteristics (Table 1). Accordingly, phylogenetic and genetic distinctiveness, together with differential phenotypic properties, was sufficient to allocate strain AHS-1^T in a species that is separate from recognized *Lentibacillus* species (Wayne *et al.*, 1987; Stackebrandt & Goebel, 1994). On the basis of the data presented, strain AHS-1^T represents a novel

Table 1. Differential phenotypic characteristics between strain AHS-1^T (*L. salinarum* sp. nov.) and recognized *Lentibacillus* species

Strains: 1, AHS-1^T; 2, *L. salicampi* KCCM 41560^T (data from Yoon *et al.*, 2002; Namwong *et al.*, 2005); 3, *L. juripiscarius* JCM 12147^T (Namwong *et al.*, 2005); 4, *L. salarius* KCTC 3911^T (Jeon *et al.*, 2005); 5, *L. lacisalsi* KCTC 3915^T (Lim *et al.*, 2005); 6, *L. halophilus* JCM 12149^T (Tanasupawat *et al.*, 2006); 7, *L. kapolis* JCM 12580^T (Pakdeeto *et al.*, 2007); 8, *L. halodurans* DSM 18342^T (Yuan *et al.*, 2007). +, Positive reaction; –, negative reaction; w, weakly positive reaction; NA, no data available. All are rod-shaped, Gram-positive and catalase-positive, and negative for hydrolysis of starch, acid production from melibiose, raffinose and rhamnose (no data for *L. halodurans* DSM 18342^T) and myo-inositol (no data for *L. halodurans* DSM 18342^T, *L. salarius* KCTC 3911^T and *L. lacisalsi* KCTC 3915^T).

Characteristic	1	2	3	4	5	6	7	8
Spore shape*	O	S/O	O	S/O	S	S	S	S/O
Motility	+	+	–	+	+	+	–	–
Maximum temperature for growth (°C)	45	40	45	50	40	42	45	45
NaCl range for growth (%)	3–24	3–25†	3–30	1–20	5–25	12–30	5–30	5–30
Optimal NaCl concentration (%)	10–12	4–8	10	12–14	12–15	20–26	15	8–12
Oxidase	+	+	+	–	+	+	+	+
Urease	–	–	–	–	–	–	+	–
Nitrate reduction	+	+	+	+	+	–	+	–
Hydrolysis of:								
Aesculin	+	–	–	+	–	–	–	–
Casein	–	+	+	–	–	–	–	–
Tween 80	–	+	+	–	–	–	–	–
Acid production from:								
L-Arabinose	–	–	–	+	+	–	–	NA
Cellobiose	–	w†	–	NA	NA	–	–	NA
D-Glucose	+	+	+	+	–	–	+	+
D-Galactose	w	w†	–	NA	NA	–	+	NA
D-Fructose	–	+	+	+	+	–	+	+
Lactose	–	–	–	+	–	–	–	–
Maltose	–	–	–	+	–	–	–	–
D-Mannose	–	w†	–	+	–	–	w	+
D-Ribose	+	+	+	+	+	–	+	NA
Salicin	–	w†	–	–	–	–	–	NA
Sucrose	–	–	w	NA	NA	–	w	NA
Trehalose	–	–	–	w	–	–	–	NA
D-Xylose	–	w†	+	+	w	–	–	–
D-Mannitol	–	–	–	w	–	–	+	NA
Sorbitol	–	–	–	NA	NA	–	+	NA
Polar lipids‡	PG, DPG, GL	PG, DPG	PG, DPG, GL	PG, DPG	PG, DPG	PG, DPG, GL	PG, DPG, GL	PG, DPG, GL
DNA G + C content (mol%)	49.0	44	43	43	44	42.4	41.6	43.4

*O, Oval; S, spherical.

†Data from Namwong *et al.* (2005).

‡DPG, Diphosphatidylglycerol; GL, unidentified glycolipid; PG, phosphatidylglycerol.

species of the genus *Lentibacillus*, for which the name *Lentibacillus salinarum* sp. nov. is proposed.

Description of *Lentibacillus salinarum* sp. nov.

Lentibacillus salinarum (sa.lin.a'rum. L. gen. pl. n. salinarum of salt-works).

Cells are Gram-positive, facultatively anaerobic, rod-shaped and 0.7–1.2 × 2.0–4.0 µm in size. Motile by means of peritrichous flagella. Colonies on MSG agar are circular, slightly convex, smooth, entire, cream-yellow in colour and

0.5–1.2 mm in diameter after incubation for 4 days at 37 °C. Optimal growth temperature is 37–40 °C; growth occurs at 15 and 45 °C, but not at 10 or 46 °C. Optimal pH for growth is 6.5–7.0; growth occurs at pH 6.0, but not at pH 5.5. Optimal growth occurs in the presence of approximately 10–12 % (w/v) NaCl; growth occurs in the presence of 24 % (w/v) NaCl, but not in the absence of NaCl or in the presence of >25 % (w/v) NaCl. Growth occurs under anaerobic conditions on MSG agar and on MSG agar supplemented with potassium nitrate. Catalase- and oxidase-positive. Urease-negative. Hypoxanthine,

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the positions of strain AHS-1^T, recognized *Lentibacillus* species and some other related taxa. Bootstrap values (expressed as percentages of 1000 replications) of >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. *Alicyclobacillus acidocaldarius* DSM 446^T was used as an outgroup. Bar, 0.01 substitutions per nucleotide position.

xanthine and tyrosine are not hydrolysed. Positive for nitrate reduction. The cell-wall peptidoglycan contains *meso*-diaminopimelic acid as the diamino acid. The predominant menaquinone is MK-7. Major fatty acids (>10% of the total) are anteiso-C_{15:0} and anteiso-C_{17:0}. Phosphatidylglycerol, diphosphatidylglycerol and two unidentified glycolipids are present as major polar lipids. The DNA G+C content of the type strain is 49.0 mol%.

The type strain, AHS-1^T (=KCTC 13162^T=CCUG 54822^T), was isolated from a marine solar saltern of the Yellow Sea, Byunsan, Korea.

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