Lentibacillus halodurans sp. nov., a moderately halophilic bacterium isolated from a salt lake in Xin-Jiang, China

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A Gram-positive, rod-shaped, spore-forming and moderately halophilic bacterium (strain 8-1^T) was isolated from a sediment sample of a neutral salt lake in Xin-Jiang, China. The strain grew optimally at 30 °C, pH 7.0–7.5 and 8–12 % (w/v) NaCl. The major cellular fatty acids were anteiso-C_{15:0}, iso-C_{15:0} and anteiso-C_{17:0}. The predominant isoprenoid quinone was MK-7 and the cell-wall peptidoglycan contained meso-diaminopimelic acid. Phosphatidylglycerol, diphosphatidylglycerol and two unidentified glycolipids were found to be the major polar lipid components. The genomic DNA G + C content was 43.4 mol%. Phylogenetic analysis based on the 16S rRNA gene sequence revealed that strain 8-1^T was closely related to *Lentibacillus salarius* KCTC 3911^T (98.0 % sequence similarity) and other recognized species within the genus *Lentibacillus* (94.5–95.9 %). The level of DNA–DNA relatedness between strain 8-1^T and *L. salarius* KCTC 3911^T was 40 %. Based on the phenotypic and genotypic data presented, strain 8-1^T is considered to represent a novel species of the genus *Lentibacillus*, for which the name *Lentibacillus halodurans* sp. nov. is proposed. The type strain is 8-1^T (= CGMCC 1.3702^T = DSM 18342^T).

Moderately halophilic bacteria (MHB), i.e. those that grow optimally in media containing 3–15 % (w/v) NaCl (Kushner, 1978), are distributed globally from oceanic to diverse terrestrial saline environments, including salterns, saline soils, salted foods and soda lakes. Numerous MHB are assigned taxonomically to the genera *Halobacillus* (Spring *et al.*, 1996), *Virgibacillus* (Heyndrickx *et al.*, 1998), *Gracilibacillus* (Wainø *et al.*, 1999), *Filobacillus* (Schlesner *et al.*, 2001), *Jeotgalibacillus* (Yoon *et al.*, 2001), *Lentibacillus* (Yoon *et al.*, 2002), *Tenuibacillus* (Ren & Zhou, 2005a) and *Salinibacillus* (Ren & Zhou, 2005b), many of which were originally affiliated to the genus *Bacillus*.

In recent years, several Gram-positive MHB belonging to the family *Bacillaceae* have been isolated from the salt lakes in Xin-Jiang, China (Jeon *et al.*, 2005; Ren & Zhou, 2005a, b). Here we present phenotypic and genotypic data from a moderately halophilic strain, designated 8-1^T, and show that this represents a novel species of the genus *Lentibacillus*.

The sampling site (Ai-Ding Lake), sample collection and treatment and the isolation of MHB were as described by Ren & Zhou (2005a). Strain 8-1^T was grown routinely on

HM medium (Ventosa *et al.*, 1982) with the addition of 10 % (w/v) NaCl. Cell morphology was observed by using an AOM Optical 1-20 light microscope and a transmission electron microscope (Hitachi S-570) as described by Zhu *et al.* (2003). Gram staining was performed as described by Gerhardt *et al.* (1981). Motility was determined in wet mounts by using phase-contrast microscopy (AOM Optical 1-20) and by culture in soft-agar medium. The presence of flagella was demonstrated by staining (Kodaka *et al.*, 1982) and transmission electron microscopy. On HM medium with 10 % (w/v) NaCl, strain 8-1^T formed creamy, circular colonies that were slightly convex at the centre. Cells of strain 8-1^T were rod-shaped, measuring approximately 0.5 × 1.5–2.5 µm. Cells were non-motile and no flagella were observed. In contrast, Jeon *et al.* (2005) reported that cells of *Lentibacillus salarius* KCTC 3911^T were motile by means of two flagella on the sides of the cells.

General physiological and biochemical tests were performed as described by Smibert & Krieg (1981). The NaCl, temperature and pH ranges for growth of the new isolate were determined as described by Ren & Zhou (2005a). Utilization of carbon and energy sources was investigated by using basal medium to which appropriate substrates (0.5 %, w/v) had been added (Xin *et al.*, 2001). Twenty-six different saccharides, polysaccharides and sugar alcohols were each used as sole carbon sources. Hydrolysis of starch, casein,

Abbreviation: MHB, moderately halophilic bacteria.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain 8-1^T is AY321433.

gelatin and Tweens 20, 40, 60 and 80 was determined on a modified HM plate (Ventosa *et al.*, 1982) by replacing the saccharide in the medium with the appropriate substrate. Susceptibility to antibiotics was determined on agar-medium plates by using absorbent paper discs impregnated with antibiotics. Strain 8-1^T showed moderate halophily, growing in the presence of 5–30% (w/v) NaCl. It grew optimally on media with 8–12% (w/v) NaCl. Strain 8-1^T grew in the pH range 6.0–9.0 (optimum pH 7.0–7.5) and a temperature range of 22–45 °C (optimum 30 °C). Other biochemical properties are detailed in Table 1 and in the species description below.

Cell mass used for extraction of DNA (Sambrook *et al.*, 1989), determination of the cellular fatty acid profile (MIDI Sherlock microbial identification system; Microbial ID) and peptidoglycan composition (Schleifer & Kandler, 1972) and analysis of polar lipids (Minnikin *et al.*, 1984) and isoprenoid quinones (Collins *et al.*, 1977; Groth *et al.*, 1996) was obtained by cultivating strain 8-1^T at 30 °C and pH 7.2 on HM medium with 10% (w/v) NaCl. The genomic DNA G + C content was determined by the thermal denaturation method according to Marmur & Doty (1962). The major isoprenoid quinone of strain 8-1^T was MK-7. Strain 8-1^T

contained *meso*-diaminopimelic acid (*meso*-DAP) as a diagnostic diamino acid in the cell-wall peptidoglycan; by contrast, Jeon *et al.* (2005) reported that *L. salarius* KCTC 3911^T did not contain any diamino acids in its cell-wall peptidoglycan. The major polar lipids of strain 8-1^T were phosphatidylglycerol, diphosphatidylglycerol and two unidentified glycolipids. Cellular fatty acid analysis of strain 8-1^T revealed that the main components were anteiso-C_{15:0} (42.41%), iso-C_{15:0} (20.73%), anteiso-C_{17:0} (17.17%), iso-C_{16:0} (7.73%), iso-C_{17:0} (5.3%), iso-C_{14:0} (3.1%) and C_{16:0} (2.16%), contrasting with several closely related species of the genus *Lentibacillus*. The genomic DNA G + C content of strain 8-1^T was 43.4 mol%, a value similar to those reported for *L. salarius* KCTC 3911^T (43 mol%) and *Lentibacillus salicampi* KCCM 41560^T (44 mol%). Characteristics that are useful in the differentiation of strain 8-1^T from several closely related taxa are shown in Table 1.

The 16S rRNA gene of strain 8-1^T was amplified by PCR with universal primers as described by Duckworth *et al.* (1996). The almost-complete nucleotide sequence (1466 bp) was determined by direct sequencing and compared with available 16S rRNA gene sequences in the GenBank database by using the BLAST program. Multiple

Table 1. Differential characteristics of strain 8-1^T and other species of the genus *Lentibacillus*

Strains: 1, strain 8-1^T; 2, *L. salarius* KCTC 3911^T (data from Jeon *et al.*, 2005); 3, *L. salicampi* KCCM 41560^T (Yoon *et al.*, 2002); 4, *L. juri-piscarius* JCM 12147^T (Namwong *et al.*, 2005); 5, *L. lacisalsi* KCTC 3915^T (Lim *et al.*, 2005); 6, *L. halophilus* JCM 12149^T (Tanasupawat *et al.*, 2006). +, Positive; –, negative; W, weak; ND, no data.

Characteristic	1	2	3	4	5	6
Spore shape/position*	S/O, T	S/O, T	S/O, T	O, T	S, T	S, T
Motility	–	+	+	–	+	+
Optimal growth temperature (°C)	30	30–35	30	37	30–32	30–37
Optimal growth NaCl concentration (%)	8–12	12–14	4–8	10	12–15	20–26
NaCl range for growth (% w/v)	5–30	1–20	2–23	3–30	5–25	12–30
Nitrate reduction	–	+	ND	+	+	–
Oxidase	+	–	+	+	+	+
Acid produced from:						
D-Glucose	+	+	–	+	–	–
D-Fructose	+	+	–	+	+	–
D-Mannose	+	+	–	–	–	–
D-Xylose	–	+	–	+	W	–
Lactose	–	+	–	–	–	–
Maltose	–	+	–	–	–	–
Glycerol	–	+	ND	+	–	ND
Hydrolysis of:						
Aesculin	–	+	–	ND	–	–
Tween 80	–	–	+	+	–	–
Casein	–	–	+	+	–	–
Cell-wall diamino acid type	<i>meso</i> -DAP	–	<i>meso</i> -DAP	<i>meso</i> -DAP	<i>meso</i> -DAP	<i>meso</i> -DAP
Polar lipids†	PG, DPG, GL	PG, DPG	PG, DPG	PG, DPG, GL	PG, DPG	PG, DPG, GL
DNA G + C content (mol%)	43.4	43.0	44.0	43	44	42

*O, Oval; S, spherical; T, terminal.

†DPG, Diphosphatidylglycerol; GL, unknown glycolipid(s); PG, phosphatidylglycerol.

alignment with closely related members was performed by using the CLUSTAL W program (Thompson *et al.*, 1994). Ambiguous and unalignable bases were manually omitted and the phylogenetic tree was then constructed by using the neighbour-joining, minimum-evolution and maximum-parsimony methods in the MEGA program (version 3.1; Kumar *et al.*, 2004). The stability of relationships was assessed by bootstrap analysis (1000 replications). The phylogenetic tree (Fig. 1) based on the neighbour-joining method indicated that strain 8-1^T formed a coherent cluster with recognized species of the genus *Lentibacillus*. Strain 8-1^T was related most closely to *L. salarius* KCTC 3911^T (98.0% 16S rRNA gene sequence similarity) and showed levels of similarity of 94.5–95.9% to other recognized species of the genus *Lentibacillus*. Similar tree topologies were obtained with the maximum-likelihood and maximum-parsimony algorithms (data not shown). DNA–DNA hybridization was performed by the spectrophotometric renaturation rate method (Huß *et al.*, 1983; De Ley *et al.*, 1970) to determine the level of relatedness between strain 8-1^T and *L. salarius* KCTC 3911^T, its closest relative as determined from 16S rRNA gene sequence analysis. The level of DNA–DNA relatedness between strain 8-1^T and *L. salarius* KCTC 3911^T was 40% (mean of three independent experiments, which did not differ by more than 6%). In combination with 16S rRNA gene sequence data, this indicates that strain 8-1^T is not genotypically related to any recognized species of the genus *Lentibacillus* (Stackebrandt & Goebel, 1994).

On the basis of the data presented, strain 8-1^T is considered to represent a novel species of the genus *Lentibacillus*, for which the name *Lentibacillus halodurans* sp. nov. is proposed.

Description of *Lentibacillus halodurans* sp. nov.

Lentibacillus halodurans (ha.lo.du'rans. Gr. n. *hals* salt; L. pres. part. *durans* enduring; N.L. part. adj. *halodurans* salt-enduring).

Cells are Gram-positive, rod-shaped, $0.5 \times 1.5\text{--}2.5$ µm, non-motile and strictly aerobic. Spherical or oval endospores form at the cell-terminal position in swollen sporangia. Colonies are white, slightly convex and 4–5 mm in diameter with regular margins after 2 days cultivation on HM medium with 10% (w/v) NaCl. Oxidase- and catalase-positive but urease-negative. Growth occurs at a temperature range of 22–45 °C (optimum 30 °C) and NaCl concentration of between 5 and 30% (w/v) (optimum 8–12%). No growth occurs without NaCl. The pH range for growth is 6.0–9.0 (optimum pH 7.0–7.5). Nitrate is not reduced to nitrite. D-Glucose, D-mannose, D-fructose, cellobiose, L-sorbose, inulin, D-raffinose, D-galactose, salicin, lactose, sucrose, aesculin, maltose, mannitol, melibiose, D-sorbose, trehalose, dulcitol, glycerol, inositol, erythritol, melezitose, arabinose, rhamnose and xylose are used as carbon sources. Acid is produced from D-mannose, D-fructose and D-glucose. Gelatin and Tween 40 are hydrolysed, but casein, starch, aesculin, and Tweens 20 and 80 are not. Cells are resistant to norfloxacin (10 µg), neomycin (30 µg), bacitracin (0.04 U), tetracycline (30 µg), streptomycin (10 µg), chloramphenicol (30 µg), kanamycin (30 µg) and penicillin G (10 U), but susceptible to novobiocin (30 µg), rifampicin (5 µg), erythromycin (15 µg), ciprofloxacin (5 µg), vancomycin (30 µg) and ampicillin (10 µg). The predominant menaquinone is MK-7. The diagnostic diamino acid in the cell-wall peptidoglycan is *meso*-DAP, and major polar lipids are phosphatidylglycerol, diphosphatidylglycerol and two unidentified glycolipids. The major fatty acids are anteiso-C_{15:0}, iso-C_{15:0}, anteiso-C_{17:0} and iso-C_{16:0}. The genomic DNA G + C content is 43.4 mol% (*T_m*).

The type strain, 8-1^T (=CGMCC 1.3702^T = DSM 18342^T), was isolated from a sediment sample of a neutral salt lake in Xin-Jiang, China.

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Fig. 1. Phylogenetic tree showing the relationships of strain 8-1^T and other related species, based on 16S rRNA gene sequences. The tree was constructed with the neighbour-joining method; bootstrap values >50% based on 1000 replications are given at branch points. Bar, 0.01 substitutions per nucleotide position.

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