Lentibacillus persicus sp. nov., a moderately halophilic species isolated from a saline lake

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A Gram-staining-positive, moderately halophilic bacterium, designated strain Amb31^T, was isolated from water of the hypersaline lake Aran-Bidgol in Iran and characterized taxonomically using a polyphasic approach. Cells were rods, motile and able to produce ellipsoidal endospores at a central position in swollen sporangia. Strain Amb31^T was facultatively anaerobic and catalase- and oxidase-positive. The strain grew in a complex medium supplemented with 3–25 % (w/v) NaCl (optimum 7.5–10 %). Optimal growth was at 30–35 °C and pH 7.5. Phylogenetic analysis based on 16S rRNA gene sequence comparisons showed that strain Amb31^T belonged to the genus *Lentibacillus*; it exhibited 16S rRNA gene sequence similarity values of 96.8 and 96.4 % to *Lentibacillus salicampi* SF-20^T and *Lentibacillus salinarum* AHS-1^T, respectively, and values of 95.9–94.7 % to the type strains of other recognized species of *Lentibacillus*. The cell-wall peptidoglycan of strain Amb31^T was based on meso-diaminopimelic acid and MK-7 was the respiratory isoprenoid quinone. The major fatty acids were anteiso-C_{15:0} (44.7 %), iso-C_{16:0} (21.4 %) and anteiso-C_{17:0} (15.9 %) and the polar lipid pattern consisted of phosphatidylglycerol, diphosphatidylglycerol, five phospholipids and a glycolipid. The DNA G + C content was 44.1 mol%. All these features confirmed the placement of strain Amb31^T within the genus *Lentibacillus* and the strain could be clearly differentiated from strains of the other species of *Lentibacillus* on the basis of several phenotypic, genotypic and chemotaxonomic features. DNA–DNA relatedness with the type strain of the most closely related strain, *L. salicampi* DSM 16425^T, was 28 %. Therefore, strain Amb31^T represents a novel species of the genus *Lentibacillus*, for which the name *Lentibacillus persicus* sp. nov. is proposed. The type strain is Amb31^T (=CCM 7683^T =CECT 7524^T =DSM 22530^T =LMG 25304^T).

The genus *Lentibacillus* was described by Yoon *et al.* (2002) to accommodate a single species, *Lentibacillus salicampi*, isolated from a salt field of the Yellow Sea in Korea. Namwong *et al.* (2005) described *Lentibacillus juripiscarius*, isolated from sauce produced in Thailand by the fermentation of fish. Jeon *et al.* (2005) described a third species within this genus, *Lentibacillus salarii*, isolated from saline sediment of a salt lake in China, and emended the description of the genus. This genus includes Gram-variable rods that are catalase-positive, oxidase-variable and urease-negative. The cell-wall peptidoglycan contains meso-diaminopimelic acid, and the predominant mena-

quinone is MK-7. The major polar lipids are diphosphatidylglycerol and phosphatidylglycerol and the major fatty acids are anteiso-C_{15:0} and iso-C_{16:0}. The G + C content of the DNA of this genus is in the range 42–44 mol% (Jeon *et al.*, 2005). At the time of writing, the genus *Lentibacillus* includes six additional species: *Lentibacillus lacisalsi* from a salt lake in China (Lim *et al.*, 2005), *Lentibacillus halophilus* from a fish-sauce fermentation (Tanasupawat *et al.*, 2006) and *Lentibacillus kapialis* from fermented shrimp paste (Pakdeeto *et al.*, 2007) produced in Thailand, *Lentibacillus halodurans* from a sediment sample of a salt lake in China (Yuan *et al.*, 2007), *Lentibacillus salinarum* from a marine solar saltern of the Yellow Sea, Korea (Lee *et al.*, 2008a), and *Lentibacillus salis* from soil of a salt lake in China (Lee *et al.*, 2008b). All these species are considered moderately halophilic (Ventosa *et al.*, 1998) and grow optimally at 4–15 % NaCl, except *L. halophilus*, which is an extreme halophile with optimal

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain Amb31^T (=CS-2009^T) is FN376846.

Fatty acid compositions of strain Amb31^T and type strains of the genus *Lentibacillus* are available as supplementary material with the online version of this paper.

growth at 20–26 % NaCl (Tanasupawat *et al.*, 2006). Here we report on the taxonomic characterization of a novel bacterial strain, Amb31^T, which was isolated from a hypersaline lake in Iran by using a polyphasic approach and represents a novel *Lentibacillus* species.

Strain Amb31^T was isolated from water of the hypersaline lake Aran-Bidgol, the largest playa in the centre of Iran (35.7047° N 51.3962° E), which is situated 1000 km from the coast and at an altitude of 800 m. This playa was formed by the deposition of halite sediments in different geological periods. The lake is triangular and has an area of about 650 km². The predominant salts in this lake are NaCl, Na₂SO₄, MgCl₂ and MgSO₄ and there are traces of carbonate ions. The salinity reaches saturation. The pH of the water is neutral (about pH 7.0–7.5). Strain Amb31^T was isolated by diluting a water sample in sterile 10 % (w/v) salt solution, plating on 10 % HM medium, which contained (l⁻¹) 81 g NaCl, 7 g MgCl₂, 9.6 g MgSO₄, 0.36 g CaCl₂, 2 g KCl, 0.06 g NaHCO₃, 0.026 g NaBr, 3.5 g proteose-peptone, 10 g yeast extract and 1 g glucose (pH 7.5; Ventosa *et al.*, 1982), and incubating aerobically at 35 °C. The strain was purified three times by subsequent plating on the same medium and maintained on 10 % HM agar and at –80 °C in HM broth supplemented with 30 % (v/v) glycerol. *L. salicampi* DSM 16425^T was used as a reference strain and was cultured under the same conditions as strain Amb31^T.

Cell morphology was examined by light microscopy (model CX 31; Olympus) using cells from exponentially growing cultures. Gram staining was performed by the Burke method (Murray *et al.*, 1994) and the result was confirmed by the KOH test (Baron & Finegold, 1990). The presence of endospores was investigated by using the Schaeffer–Fulton staining method (Murray *et al.*, 1994). Motility was analysed by the wet-mount method (Murray *et al.*, 1994). To determine the optimal temperature and pH for growth of the strain, broth cultures were incubated at 10–50 °C at intervals of 5 °C and at pH 5–11 at intervals of 0.5 pH units (using sodium acetate/acetic acid, Tris/HCl or glycine/sodium hydroxide to adjust the medium to pH <6, 6–9 and >9, respectively). Growth at 0, 2.5, 5, 7.5, 10, 15, 20, 25 and 30 % (w/v) NaCl was tested on HM medium at pH 7.5. Growth was monitored by OD₆₀₀ using a spectroscopic method (model UV-160 A; Shimadzu). Catalase, oxidase and urease activities, nitrate reduction, aesculin hydrolysis, indole production and methyl red and Voges–Proskauer tests were examined as recommended by Smibert & Krieg (1994). Hydrolysis of Tweens 20, 40, 60 and 80 was examined as described by Harrigan & McCance (1976). Determination of acid production from carbohydrates, as well as utilization of carbon and nitrogen sources, was performed as recommended by Ventosa *et al.* (1982). Antibiotic susceptibility tests were performed on Mueller–Hinton agar plus 10 % (w/v) sea salts (Ventosa *et al.*, 1982) seeded with a bacterial suspension containing 1.5 × 10⁶ c.f.u. ml⁻¹ using discs impregnated with various antibiotics (HiMedia). The

plates were incubated at 35 °C for 48 h and inhibition zone was interpreted according the manufacturer's manual. Other physiological and biochemical tests were performed as described previously (Mata *et al.*, 2002; Quesada *et al.*, 1984; Ventosa *et al.*, 1982).

Strain Amb31^T was a Gram-staining-positive, motile rod and produced ellipsoidal endospores at a central position in swollen sporangia. When grown for 2 days at 35 °C on 10 % HM medium, colonies were circular, entire, convex, smooth and cream coloured, with a diameter of 1.5–2.0 mm. Strain Amb31^T was moderately halophilic, growing with 3–25 % (w/v) NaCl (optimally with 7.5–10 % NaCl). No growth was observed in the absence of NaCl. The isolate grew between pH 6.0 and 9.5 (optimally at pH 7.5) and at 15–50 °C (optimally at 30–35 °C). Other phenotypic features are included in Table 1 and the species description.

Genomic DNA from strain Amb31^T was prepared using the method described by Marmur (1961). The 16S rRNA gene was amplified by PCR with the forward primer 16F27 [5'-AGAGTTTGATC(AC)TGGCTCAG-3'] and the reverse primer 16R1488 (5'-CGGTTACCTTGTTAGGACTTCA-CC-3'). Direct sequence determination of the PCR-amplified DNA was carried out using an automated DNA sequencer model ABI 3130XL (Applied Biosystems). 16S rRNA gene sequence analysis was performed with the ARB software package (Ludwig *et al.*, 2004). Published sequences from closely related bacteria were aligned with that from strain Amb31^T and the alignment was confirmed and checked against both primary and secondary structures of the 16S rRNA molecule using the alignment tool of the ARB software package. Phylogenetic trees were constructed using neighbour-joining (Saitou & Nei, 1987), maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Fitch, 1971) algorithms in the ARB software.

An almost-complete 16S rRNA gene sequence (1480 bp) of strain Amb31^T was obtained and used for initial BLAST searches in GenBank and for phylogenetic analysis. Identification of phylogenetic neighbours and calculation of pairwise 16S rRNA gene sequence similarity were achieved using the EzTaxon server (<http://www.eztaxon.org>; Chun *et al.*, 2007). Phylogenetic analysis based on the neighbour-joining algorithm revealed that strain Amb31^T was included in the cluster of strains of the genus *Lentibacillus* (Fig. 1). The most closely related strains were *L. salicampi* SF-20^T (96.8 % 16S rRNA gene sequence similarity), *L. salinarum* AHS-1^T (96.4 %) and *L. halodurans* 8-1^T (95.9 %); similarities with the other *Lentibacillus* strains were 95.6–94.7 %. Other closely related strains were the type strains of *Virgibacillus kekensis* (95.3 % sequence similarity), *Virgibacillus olivae* E₃₀8^T (95.1 %) and *Virgibacillus marismortui* 123^T (95.0 %). Maximum-likelihood and maximum-parsimony methods resulted in highly similar tree topologies (not shown) and confirmed the phylogenetic cluster formed by strain Amb31^T and strains of the genus *Lentibacillus*.

Table 1. Characteristics that distinguish strain Amb31^T from strains of the genus *Lentibacillus*

Strains: 1, *Lentibacillus persicus* sp. nov. Amb31^T; 2, *L. salicampi* JCM 11462^T (unless indicated, data from Yoon *et al.*, 2002); 3, *L. salinarum* KCTC 13162^T (Lee *et al.*, 2008a); 4, *L. halodurans* DSM 18342^T (Yuan *et al.*, 2007); 5, *L. juripiscarius* JCM 12147^T (Namwong *et al.*, 2005); 6, *L. kapialis* JCM 12580^T (Pakdeeto *et al.*, 2007); 7, *L. salarius* KCTC 3911^T (Jeon *et al.*, 2005); 8, *L. salis* KCTC 3936^T (Lee *et al.*, 2008b); 9, *L. lacisalsi* KCTC 3915^T (Lim *et al.*, 2005); 10, *L. halophilus* JCM 12149^T (Tanasupawat *et al.*, 2006). +, Positive; –, negative; ND, no data available.

Characteristic	1	2	3	4	5	6	7	8	9	10
Cell width (µm)	0.9	0.4–0.7	0.7–1.2	0.5	0.4–0.5	0.2–0.4	0.2–0.3	0.4–0.6	0.4–0.6	0.4–0.6
Cell length (µm)	1.5–4.0	2.0–4.0	2.0–4.0	1.5–2.5	1.5–6.0	0.8–2.5	1.5–3.0	0.8–2.5	1.2–3.0	1.0–3.0
Endospore position*	C, S	T	ND	T	T	T	T	SS	T	T
Endospore shape†	E	SP–O	O	SP–O	O	SP	SP–O	SP	SP	SP
Pigmentation‡	CR	CR	CR–Y	WH	WH–CR	R	CR	LY	CR	WH–CR
Motility	+	+	+	–	–	–	+	+	+	+
NaCl concentration for growth (% w/v)										
Range	3–25	2–23	3–24	5–30	3–30	5–30	1–20	5–15	5–25	12–30
Optimum	7.5–10.0	4–8	10–12	8–12	10	15	12–14	10	12–15	20–26
pH for growth										
Range	6.0–9.5	5.0–8.5§	6–9.5	6–9	5–9	5–9	6–8.5	7–9.2	7–9.5	6–8
Optimum	7.5	6–8	6.5–7	7–7.5	7	7	7–7.5	8	8	7–7.5
Temperature for growth (°C)										
Range	15–50	15–40	15–45	22–45	10–45	15–45	15–50	20–45	15–40	15–42
Optimum	30–35	30	37–40	30	37	37	30–35	37	30–32	30–37
Anaerobic growth	+	–	+	–	+	–	–	–	–	–
Nitrate reduction	+	+	+	–	+	+	+	+	+	–
Hydrolysis of:										
Aesculin	–	–	+	–	–	–	+	–	–	–
Casein	+	+	–	–	+	–	–	–	–	–
Tween 80	–	+	–	–	+	–	–	–	–	–
Urea	–	–	–	–	–	+	–	–	–	–
Acid production from:										
D-Fructose	+	–	–	+	+	+	+	+	+	–
D-Glucose	+	–	+	+	+	+	+	+	–	–
Lactose	–	–	–	–	–	–	+	–	–	–
Maltose	–	–	–	–	–	–	+	–	–	–
D-Mannitol	–	–	–	–	–	+	+	+	–	–
D-Mannose	–	–	–	+	–	+	+	–	–	–
D-Ribose	+	–	+	–	+	+	+	–	+	–
Trehalose	+	–	–	–	–	–	+	+	–	–
D-Xylose	–	–	–	–	+	–	+	+	+	–
DNA G + C content (mol%)	44.1	44	49	43.4	43	41.6	43	46.2	44	42.1–43.1¶

*C, Central; S, swollen; SS, swollen sporangia; T, terminal.

†E, Ellipsoidal; O, oval; SP, spherical.

‡CR, Cream; LY, light yellow; R, red; WH, white; Y, yellow.

§Data for *L. salicampi* DSM 16425^T obtained in this study.

||Positive results were obtained for *L. salicampi* DSM 16425^T in this study.

¶Range of values for 13 strains including the type strain.

The G + C content of the genomic DNA was determined from the midpoint value (T_m) of the thermal denaturation profile (Marmur & Doty, 1962) by using the equation of Owen & Hill (1979). Cell biomass for analysis of the cell-wall peptidoglycan, isoprenoid quinones and polar lipids was obtained by cultivation on 10 % HM agar at 35 °C. The cell-wall peptidoglycan type, respiratory lipoquinone and polar lipids of strain Amb31^T were analysed by the Identification

Service and Dr B. J. Tindall at DSMZ, Braunschweig, Germany. For the fatty acid determination, cells were grown on marine agar (Difco) with 10 % NaCl (pH 7.2) at 37 °C for 48 h. The whole-cell fatty acid composition was determined by gas chromatography with the Microbial Identification System (MIDI) by the Identification, Characterization and Molecular Typing Service at the BCCM/LMG Bacteria Collection, Gent, Belgium.

Fig. 1. Neighbour-joining phylogenetic tree, based on the 16S rRNA gene sequence comparison, showing the relationship of strain Amb31^T with related strains of the genera *Lentibacillus*, *Virgibacillus*, *Halobacillus*, *Gracilibacillus*, *Paraliobacillus*, *Filobacillus* and *Tenuibacillus*. Bootstrap values (≥ 70%) based on 1000 replications are shown as percentages at branch nodes. *Brevibacillus brevis* JCM 2503^T was used as an outgroup. Bar, 0.01 substitutions per nucleotide position.

The G+C content of the DNA of strain Amb31^T was 44.1 mol%. This value is within the range described for *Lentibacillus* species (Table 1). The polar lipids detected were phosphatidylglycerol, diphosphatidylglycerol, five phospholipids and an unidentified glycolipid. Strain Amb31^T contained MK-7 as the unique menaquinone and the peptidoglycan cell-wall type A1 γ , with meso-diaminopimelic acid as the diagnostic diamino acid. These features are defined characteristics for the genus *Lentibacillus* (Jeon *et al.*, 2005). The cellular fatty acid profile of strain Amb31^T is shown in Supplementary Table S1 (available in IJSEM Online). The predominant fatty acids are anteiso-C_{15:0} (44.7%), iso-C_{16:0} (21.4%) and anteiso-C_{17:0} (15.9%), which are the major fatty acids detected in *Lentibacillus* species (Jeon *et al.*, 2005).

DNA–DNA hybridization studies were performed by the competition procedure of the membrane method (Johnson, 1994) described in detail by Arahall *et al.* (2001a, b). The hybridization temperature was 47.9 °C, which is within the limit of validity for the filter method (De Ley & Tijtgaat, 1970), and the percentage of hybridization was calculated according to Johnson (1994). The experiments were carried out in triplicate. The percentage of DNA–DNA hybridization between strain Amb31^T and *L. salicampi* DSM 16425^T was 28%. This level of DNA–DNA relatedness is low enough to classify strain Amb31^T within a genotypically distinct novel species of *Lentibacillus* (Stackebrandt & Goebel, 1994; Stackebrandt *et al.*, 2002).

Overall, our results show clearly that strain Amb31^T is phylogenetically closely related to strains of the genus *Lentibacillus*; however, there are differences in some phenotypic and chemotaxonomic features (Table 1). Therefore, we propose that the strain represents a novel species of the genus *Lentibacillus*, for which the name *Lentibacillus persicus* sp. nov. is proposed.

Description of *Lentibacillus persicus* sp. nov.

Lentibacillus persicus [per' si.cus. L. masc. adj. *persicus* Persian, pertaining to Persia (Iran), from where the type strain was isolated].

Cells are Gram-staining-positive motile rods, 0.9 × 1.5–4.0 µm. Ellipsoidal endospores form at a central position in swollen sporangia. Colonies are cream coloured, circular, convex and smooth with entire margins and are 1.5–2.0 mm in diameter after 2 days of incubation at 35 °C on 10% HM medium. Facultatively anaerobic. Catalase- and oxidase-positive but urease-negative. Moderately halophilic. Requires 3–25% NaCl for growth (optimum 7.5–10% NaCl); no growth without NaCl. Grows at 15–50 °C (optimum 30–35 °C) and pH 6.0–9.5 (optimum pH 7.5). Reduces nitrate and nitrite. Hydrolyses casein, gelatin, Tweens 40 and 60 and DNA, but not starch, Tweens 20 or 80, aesculin, tyrosine, xanthine or hypoxanthine. Indole and H₂S are not produced. Methyl red and Voges–Proskauer tests are negative. Arginine dihydrolase and ornithine and lysine decarboxylases are not detected. Produces acid from D-fructose, D-glucose, D-ribose and

trehalose, but not from D-amylgdalin, D-arabinose, arbutin, cellobiose, L-citrulline, dulcitol, DL-methionine, D-galactose, glycerol, *myo*-inositol, inulin, lactose, maltose, D-mannitol, D-mannose, melezitose, melibiose, raffinose, sorbitol, sucrose, xylitol or D-xylose. The following compounds are used as sole carbon and energy sources: fructose, D-glucose, maltose, ribose, sucrose, trehalose, aesculin, melezitose, butanol, *myo*-inositol, xylitol, acetate, benzoate, ethanol, fumarate, glycerol and hippurate. The following compounds are not used as sole carbon and energy sources: starch, D-arabinose, cellobiose, D-fucose, D-galactose, lactose, D-mannose, melibiose, raffinose, D-xylose, salicin, dulcitol, D-mannitol, propanol, D-sorbitol, methanol, citrate, formate, propionate, succinate, valerate, malate and tartrate. The following compounds are used as sole carbon, nitrogen and energy sources: L-alanine, glutamine, L-lysine and L-threonine. The following compounds are not used as sole carbon, nitrogen and energy sources: L-arginine, aspartate, L-cysteine, phenylalanine, L-methionine, L-ornithine, L-serine, tryptophan, isoleucine and valine. Sensitive to (μ g per disc unless otherwise indicated) amikacin (30), amoxicillin (30) bacitracin (10 U), carbenicillin (100), cefoxitin (30), chloramphenicol (30), erythromycin (15), gentamicin (30), nitrofurantoin (300), novobiocin (5), rifampicin (5), streptomycin (10), tetracycline (30) and tobramycin (10). Resistant to kanamycin (30) and polymixin B (100 U). Peptidoglycan contains *meso*-diaminopimelic acid. The menaquinone is MK-7. Major fatty acids are anteiso-C_{15:0}, iso-C_{16:0} and anteiso-C_{17:0}. Phosphatidylglycerol, diphosphatidylglycerol, five phospholipids and an unidentified glycolipid are present as major polar lipids. The DNA G+C content of the type strain is 44.1 mol% (T_m).

The type strain, Amb31^T (=CCM 7683^T =CECT 7524^T =DSM 22530^T =LMG 25304^T), was isolated from the hypersaline lake Aran-Bidgol in Iran.

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