Antonie van Leeuwenhoek (2016) 109:721–728
DOI 10.1007/s10482-016-0674-9

ORIGINAL PAPER

Paenibacillus salinicaeni sp. nov., isolated from saline silt sample

Xiang Guo · Shan Zhou · Yan-Wei Wang · Hui-Min Wang · De-Long Kong · Jie Zhu · Wei-Wei Dong · Ming-Xiong He · Bing-Qiang Zhao · Guo-Quan Hu · Zhi-Yong Ruan

Received: 26 January 2016 / Accepted: 29 February 2016 / Published online: 15 March 2016

Abstract A novel facultatively anaerobic bacterium, designated strain LAM0A28^T, was isolated from a saline silt sample collected from the Chinese Sea of Death located in Suining city, Sichuan province, China. Cells of strain LAM0A28^T were observed to be Gram-stain positive, motile, endospore-forming and straight-rod shaped. Strain LAM0A28^T was found to be able to grow at 15–45 °C (optimum: 30–35 °C), pH 5.0–10.0 (optimum: 7.5) and 0–5 % NaCl (w/v) (optimum: 0.5 %). The 16S rRNA gene sequence similarity analysis showed that strain LAM0A28^T is closely related to *Paenibacillus jilunlii* DSM 23019^T (97.5 %) and *Paenibacillus graminis* DSM 15220^T (97.2 %). The DNA–DNA hybridization values between the isolate and *P. jilunlii* DSM 23019^T, *P. graminis* DSM 15220^T were 30.2 ± 1.6 % and 44.7 ± 2.1 %, respectively. The DNA G+C content was found to be 51.2 mol% as determined by the T_m method. The major cellular fatty acids were identified as anteiso-$C_{15:0}$, $C_{16:0}$, iso-$C_{16:0}$ and $C_{14:0}$. The major isoprenoid quinone was identified as MK-7. The cell wall peptidoglycan was found to contain *meso*-diaminopimelic acid. The major polar lipids were found to be diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, two aminophospholipids and six unidentified lipids. Based on the phylogenetic, phenotypic and chemotaxonomic characteristics, strain LAM0A28^T is concluded to represent a novel species within the genus *Paenibacillus*, for which the name *Paenibacillus salinicaeni* sp. nov. is proposed. The type strain is LAM0A28^T (=ACCC 00741^T = JCM 30850^T).

Keywords *Paenibacillus salinicaeni* sp. nov. · Polyphasic taxonomy · Nitrogen-fixing · Chinese Sea of Death · Saline silt

Electronic supplementary material The online version of this article (doi:10.1007/s10482-016-0674-9) contains supplementary material, which is available to authorized users.

X. Guo · Y.-W. Wang · M.-X. He · G.-Q. Hu (✉)
Key Laboratory of Development and Application of Rural Renewable Energy (Ministry of Agriculture), Biogas Institute of Ministry of Agriculture, Chengdu 610041, People's Republic of China
e-mail: huguoquan1@hotmail.com

X. Guo · S. Zhou · H.-M. Wang · D.-L. Kong · J. Zhu · W.-W. Dong · B.-Q. Zhao · Z.-Y. Ruan (✉)
Key Laboratory of Microbial Resources (Ministry of Agriculture, China), Institute of Agricultural Resources and Regional Planning, CAAS, Beijing 100081, People's Republic of China
e-mail: ruanzhiyong@caas.cn

Introduction

The genus *Paenibacillus* was proposed by Ash et al. (1993) on the basis of analysis of the 16S rRNA gene sequences of group 3 bacilli, with *Paenibacillus polymyxa* as the type species. At the time of writing, the genus *Paenibacillus* comprises more than 150

recognised species (http://www.bacterio.net/p/paenibacillus.html),some (flagged→) at LPSN are reclassified. Members of the genus *Paenibacillus* are either Gram-stain positive or negative, facultatively anaerobic or strictly aerobic, non-pigmented, rod-shaped, motile and produce ellipsoidal spores (Ash et al. 1993; Osman et al. 2006; Priest 2009). The DNA G+C content ranges from 39 to 59 mol% (Yao et al. 2014). The predominant menaquinone is MK-7 and the main cellular fatty acid is anteiso-$C_{15:0}$ (Ludwig et al. 2009).

Some *Paenibacillus* species have shown the considerable potential applications in agricultural production because they can fix nitrogen, produce various enzymes (Choi et al. 2008; Li et al. 2014) and some antimicrobial substances (Jin et al. 2011; Xie et al. 2012). During the course of screening of anaerobic petroleum-degrading bacteria from saline silt samples collected from the Chinese Sea of Death located in Suining city, Sichuan province, China, a *Paenibacillus*-like strain, designated strain LAM0A28^T was isolated. By using a polyphasic taxonomic approach, we conclude here that strain LAM0A28^T represents a novel species of the genus *Paenibacillus*, for which the name *Paenibacillus salinicaeni* sp. nov. is proposed.

Materials and methods

Isolation and culture of bacterial strains

Strain LAM0A28^T was isolated from a sea silt sample collected from the Chinese Sea of Death located in Suining city, Sichuan province, China. An inorganic salt medium (ISM) was used for this study and consisted of the following materials (per liter): 1 g of NH_4NO_3, 0.2 g of $MgSO_4{\cdot}7H_2O$, 0.03 g of $CaCl_2{\cdot}2H_2O$, 1 g of K_2HPO_4, 1 g of KH_2PO_4, 0.25 g L-Cysteine HCl, 1 mg resazurin, pH adjusted to 7.5 with 1 N NaOH. The medium was sterilised by autoclaving at 121 °C for 20 min. The enrichment and isolation medium was ISM supplemented with 1 % (v/v) petroleum or hexadecane. The anaerobic technique of Hungate (Hungate, 1969; Bryant 1972; Miller and Wolin 1974) was used throughout the enrichment and isolation. One of the isolates obtained, designated strain LAM0A28^T, was purified at least twice before being preserved in 25 % (v/v) glycerol at -80 °C for further study.

Biomass for chemotaxonomic and molecular studies was obtained from TSB medium (BD/Difco 211825, Sparks, MD, USA) at 30 °C for 2 days. The proposed minimal standards for the description of aerobic, endospore-forming bacteria (Logan et al. 2009) were followed. The reference type strains *Paenibacillus jilunlii* DSM 23019^T and *Paenibacillus graminis* DSM 15220^T were obtained from the German Collection of Microorganisms and Cell Cultures (DSMZ; Germany). All strains were cultured under the same conditions for comparative analyses.

Morphological, physiological and biochemical characteristics

Cell morphology of an exponentially growing culture of strain LAM0A28^T were examined using light microscope (Nikon 80i, Tokyo, Japan) and transmission electron microscope (Hitachi 7500, Tokyo, Japan) (Chen et al. 2015; Han et al. 2015). Gram-staining reaction was carried out according to the method described by Smibert and Krieg (1994). Cell motility was examined in semi-solid TSB medium (0.4 % agar added). The pH, temperature and NaCl ranges for growth were determined in LD medium (per litre water: 10 g tryptone, 5 g yeast extract, 2.5 g NaCl, pH 7.0). Growth characteristics of strain LAM0A28^T were determined at various temperatures (4, 10, 15, 20, 25, 30, 35, 40, 45 and 50 °C) and pH values (4.0-11.0, at 0.5 unit intervals) in LD medium for 5 days. Tolerance of NaCl was tested at different salt concentrations [0-3 % (w/v) at 0.5 unit intervals and 3–8 % (w/v) at 1 unit intervals]. The medium was adjusted to the desired pH by using sterile solutions of citric acid/Na_2HPO_4 (pH 4.0–5.0), Na_2HPO_4/NaH_2PO_4 buffer (pH 6.0–8.0), $NaHCO_3/Na_2CO_3$ buffer (pH 9.0–10.0) or Na_2HPO_4/NaOH buffer (pH 11.0) (Ruan et al. 2014). Catalase activity was detected by placing drops of 3 % (v/v) H_2O_2 onto plate grown cultures and observing the production of oxygen bubbles. Oxidase activity was determined by using 1 % (w/v) tetramethyl-*p*-phenylenediamine. Examinations of antibiotic susceptibility were performed on TSA medium by using susceptibility discs with various antibiotics and the inhibition zones were judged according to the manufacturer's instruction. The following biochemical characteristics were studied using the methods described by Smibert and Krieg (1994): starch hydrolysis, gelatin liquefaction, Tween 20, Tween

60 and Tween 80 digestion, methyl red tests. More biochemical properties were determined by using the Biolog GP2 MicroPlates (Biolog, Hayward, CA, USA) and API 20NE, API ZYM, API 50CH test systems (bioMérieux, ĹEtoile, France) according to the manufacturers' instructions. The nitrogenase activity of strain LAM0A28^T was tested with the acetylene reduction assay method (Berge et al. 2002).

Phylogenetic and genomic related analyses

Extraction and purification of genomic DNA from strain LAM0A28^T was performed as described by Mandel and Marmur (1968). The 16S rRNA gene was amplified by PCR using prokaryotic 16S rRNA universal primers 27F and 1492R (Weisburg et al. 1991). The *nifH* gene was amplified using PolF and PolR (Poly et al. 2001). Sequence similarity and multiple sequence alignment were analysed using the EzTaxon-e service (Kim et al. 2012), NCBI BLAST program (http://www.ncbi.nlm.nih.gov) and CLUSTAL W (Thompson et al. 1994). Phylogenetic trees were constructed using MEGA 6 software (Tamura et al. 2013). Tree topology was evaluated by neighbor-joining method (Saitou and Nei 1987), maximum likelihood (Felsenstein 1981) and maximum-parsimony method (Fitch 1971) with 1000 replications bootstrap analysis.

The genomic DNA G+C content was determined by the thermal denaturation method (Marmur and Doty 1962) using a Beckman DU 800 spectrophotometer (Beckman Coulter, Brea, CA, USA). *Escherichia coli* K-12 was used as the reference strain. The DNA–DNA reassociation values were detected by measuring the renaturation rates of the denatured DNAs as described by De Ley et al. (1970) and Huss et al. (1983). The experiments were carried out in quintuplicate.

Chemotaxonomic characterisation

Chemotaxonomic analyses were executed on the strain LAM0A28^T and the reference strains under the same conditions. All strains were incubated in TSB medium. Cells were harvested in the late exponential phase of growth at 30 °C. The cellular fatty acid analyses were performed on strain LAM0A28^T, *P. jilunlii* DSM 23019^T and *P. graminis* DSM 15220^T as described by Sakamoto et al. (2002). Identification and quantification of the cellular acids were performed using the Sherlock Microbial Identification System with the standard MIS Library Generation Software (Version 6.0 and Date 4, Microbial ID Inc., Newark, DE, USA). The respiratory quinones were analysed from strain LAM0A28^T by using reversed-phase HPLC as described previously (Komagata and Suzuki 1987). The polar lipids of strain LAM0A28^T were extracted and separated on silica gel plate (10 × 10 cm, Merck 5554) (Kates 1986) and further analyzed with the method described by Minnikin et al. (1984) and Xu et al. (2011). Molybdatophosphoric acid was used to reveal total polar lipids. Aminolipids were determined using ninhydrin reagent and phospholipids were identified by Zinzadze reagent. The results were analysed as described by Fang et al. (2012). The cell wall peptidoglycan structure of strain LAM0A28^T was analysed by TLC (Komagata and Suzuki 1987) with the methods described by Schleifer (1985). The sugar profile of strain LAM0A28^T was analysed according to the method described by Lechevalier and Lechevalier (1980).

Results and discussion

Morphological, physiological and biochemical characteristics

Cells of LAM0A28^T were observed to be motile, straight-rod shaped with a cell size of 0.6–1.0 μm in width and 2.2–12.6 μm in length (Fig. 1). The isolate was found to be Gram-stain positive, facultatively aerobic and spore-forming (Fig. S1). Colonies of strain LAM0A28^T cultivated on TSA for 72 h at 30 °C were observed to be milky, circular, convex, semi-translucent and smooth. Growth was observed at 15–45 °C (optimum 30–35 °C), pH 5.0–10.0 (optimum pH 7.5) and 0–5 % (w/v) NaCl (optimum 0.5 %). Growth was not inhibited by 0.001 % (w/v) lysozyme. Strain LAM0A28^T was found to be catalase positive and oxidase negative. Cells were found to be positive for the methyl red test. Strain LAM0A28^T was found to be resistant to (μg per disc unless otherwise indicated) carbenicillin (100), chloramphenicol (30), kanamycin (30), streptomycin (10) and tetracycline (30), but sensitive to ampicillin (10), gentamicin (10), neomycin (30) and polymixin B (300 U). In API ZYM tests, strain LAM0A28^T was found to be positive for alkaline phosphatase, leucine arylamidase, trypsin, α-chymotrypsin, acid phosphatase, naphthol-AS-BI-

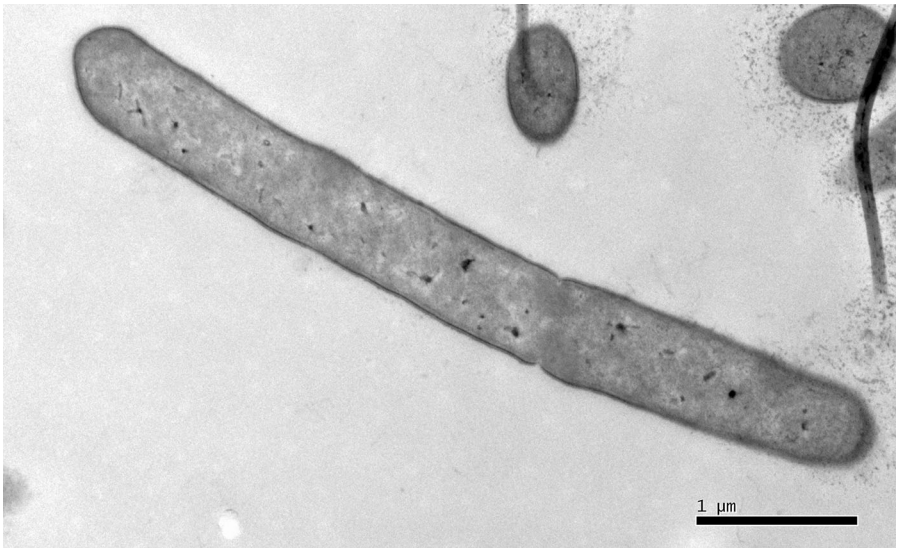

Fig. 1 Transmission electron micrograph of cells of strain LAM0A28^T. *Bar*, 1 µm

phosphohydrolase and α-glucosidase; and weakly positive for esterase (C4), esterase lipase (C8), valine arylamidase and β-glucosidase. In the API 20NE system, the hydrolysis of esculin and 4′-nitrophenyl-β-D-galactopyranoside, and the assimilation of D-glucose, L-arabinose, D-mannose, mannitol, *N*-acetyl-glucosamine, D-maltose and potassium gluconate were found to be positive. Nitrate was reduced to nitrite. With API 50CH, acid was found to be produced from glycerol, L-arabinose, D-ribose, D-xylose, D-galactose, D-glucose, D-fructose, D-mannose, inositol, D-mannitol, amygdalin, arbutin, aesculin, salicin, D-cellobiose, D-maltose, D-lactose, D-melibiose, D-sucrose, D-trehalose, D-raffinose, starch, glycogen, D-gentiobiose, D-turanose, D-fucose and potassium gluconate. The differences in the physiological and biochemical characteristics between strain LAM0A28^T and its relatives are shown in Table 1. The detailed results obtained from the API system are shown in Table S1. The result of nitrogenase activity assay (Table 2) indicated that strain LAM0A28^T exhibits a relatively high nitrogenase activity compared to the reference strains.

Molecular analyses

Phylogenetic analysis based on the nearly complete 16S rRNA gene sequence of strain LAM0A28^T (1476 nt, GenBank accession number KM260652) indicated that the strain is a member of the genus *Paenibacillus* and closely related to strains *P. jilunlii* DSM 23019^T and *P. graminis* DSM 15220^T with 97.5 and 97.2 % sequence similarity, respectively (Fig. 2). Phylogenetic trees constructed based on the maximum-likelihood and maximum-parsimony algorithms also supported this conclusion (Fig. S2 and S3). Levels of 16S rRNA gene sequence similarity between strain LAM0A28^T and other recognised members of the genus *Paenibacillus* were below 97.0 %. The DNA–DNA hybridization values between strain LAM0A28^T and *P. jilunlii* DSM 23019^T and *P. graminis* DSM 15220^T were 30.2 ± 1.6 and 44.7 ± 2.1 %, respectively. The DNA G+C content of strain LAM0A28^T was determined to be 51.2 mol%, which is within the range of values reported for members of the genus *Paenibacillus*. A 365 bp segment of the *nifH* gene (GenBank accession number KU171013) was amplified from strain LAM0A28^T. The *nifH* gene sequence similarities between strain LAM0A28^T and *P. graminis* DSM 15220^T, *P. riograndensis* SBR5^T, *P. sonchi* X19-5^T, *P. jilunlii* DSM 23019^T are 97, 92, 91 and 91 %, respectively. Phylogenetic analysis based on *nifH* gene sequences revealed that strain LAM0A28^T clusters with species of the genus *Paenibacillus* (Fig. S4, S5 and S6).

Chemotaxonomic characteristics

The major fatty acids of strain LAM0A28^T were identified as anteiso-$C_{15:0}$ (35.8 %), $C_{16:0}$ (17.9 %), iso-$C_{16:0}$ (14.0 %) and $C_{14:0}$ (10.9 %). The detailed fatty acid compositions of strain LAM0A28^T, *P.*

Table 1 Differential characteristics of strain LAM0A28^T and its close phylogenetic relatives

Characteristic	1	2	3
Cell size (μm)	0.6–1.0 × 2.2–12.6	1.0–1.5 × 2.5–5.0	0.5–1.0 × 3.0–6.0
Growth temperature Range (°C)	15–45	15–40	10–40
Growth in the presence of 5 % NaCl	+	−	−
Catalase	+	−	+
Hydrolysis of esculin	+	w	+
Antibiotic resistance (μg per disc)			
Carbenicillin (100)	+	−	+
Chloramphenicol (30)	+	−	+
Tetracycline (30)	+	−	+
Ampicillin (10)	−	−	+
Assimilation of (API 20NE)			
D-glucose	+	+	w
N-acetyl-glucosamine	+	−	+
Enzyme activities (API ZYM):			
Esterase	w	+	w
Esterase lipase	w	w	−
Cystine arylamidase	−	w	−
Naphthol-AS-BI-phosphohydrolase	+	w	w
α-Galactosidase	−	w	w
Acid production from (API 50CH)			
Methyl β-D-xylopyranoside	−	+	+
Inulin	−	+	−
D-Melezitose	−	w	+
D-Arabitol	w	−	−
Potassium gluconate	+	−	−
DNA G+Ccontent (mol %) (T_m)	51.2	52.9	52.1

All data are from this study

Strains 1 strain LAM0A28^T, *2 P. jilunlii* DSM 23019^T, *3 P. graminis* DSM 15220^T, + positive reaction, − negative reaction, *w* weakly positive

Table 2 Nitrogenase activity of strain LAM0A28^T compared with the reference type strains

Strains	Nitrogenase activity [nmol C_2H_2 (mg protein)$^{-1}$]
LAM0A28^T	579.6 ± 35.3
P. jilunlii DSM 23019^T	418.2 ± 52.6
P. graminis DSM 15220^T	201.1 ± 16.8

All data are from this study. The values are presented as the mean ± SD of triplicate reactions

jilunlii DSM 23019^T and *P. graminis* DSM 15220^T are shown in Table 3. The predominant menaquinone was identified as MK-7. The major polar lipids of strain LAM0A28^T were identified as diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, two aminophospholipids and six unidentified lipids (Fig. S7). The diamino acid of the cell wall peptidoglycan was determined to be *meso*-diaminopimelic acid. The sugar profile was found to contain xylose, ribose and traces of galactose (Fig. S8).

Taxonomic conclusion

Strain LAM0A28^T was observed to be Gram-positive, facultatively aerobic, motile, endospore-forming and rod-shaped. The predominant fatty acids and menaquinone were anteiso-$C_{15:0}$ and MK-7, respectively. The major

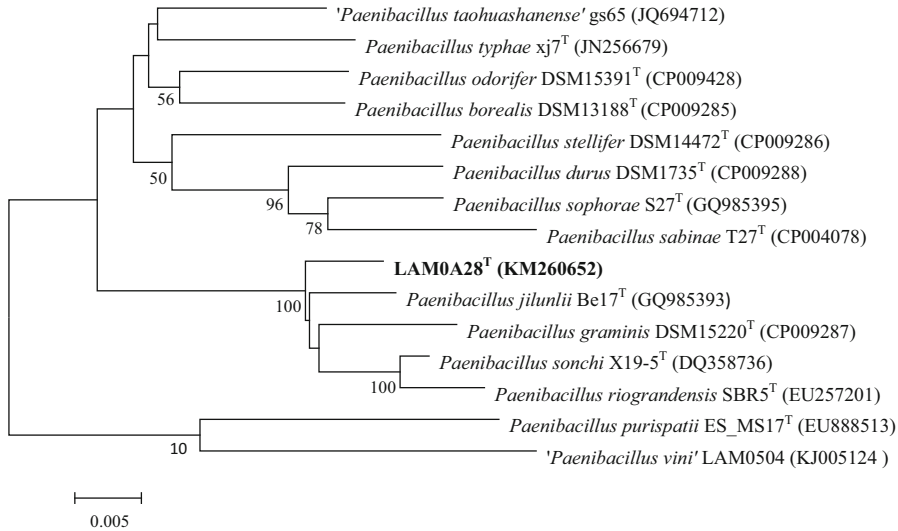

Fig. 2 Neighbor-joining phylogenetic tree based on a comparison of the 16S rRNA gene sequences of strain LAM0A28^T and its closest relatives. Genbank accession numbers are given in parentheses. *Bar*, 5 nucleotide changes per 1000 nucleotides. Only bootstrap values >50 % are shown at the branch points

polar lipids were diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, two aminophospholipids and six unidentified lipids. In combination with the analysis of the 16S rRNA gene sequence, *nifH* gene sequence and DNA G+C content, it is concluded that strain LAM0A28^T is a member of the genus *Paenibacillus*. However, evident distinctions were exhibited between strain LAM0A28^T and the closely related strains *P. jilunlii* DSM 23019^T and *P. graminis* DSM 15220^T in morphological features, growth conditions, catalase activity, hydrolysis of esculin, antibiotic resistance, enzyme activities, and acid production from carbohydrates (Table 1). The predominant fatty acids in strain LAM0A28^T were the same as or similar to the reference strains in this study (Table 3), although $C_{16:1}\omega11c$ was found only in strain LAM0A28^T. Differences in the nitrogenase activity were also found between strain LAM0A28^T and the reference strains. The low DNA–DNA hybridization values (30.2 ± 1.6 and 44.7 ± 2.1 %) between the novel strain and its close relatives precludes genomic relatedness and supports the designation of strain LAM0A28^T as the type strain of a novel species within the genus *Paenibacillus* (Stackebrandt and Goebel 1994). Based on the phylogenetic, phenotypic, and chemotaxonomic characteristics, strain LAM0A28^T is considered to represent a novel species of the genus *Paenibacillus*, for which the name *Paenibacillus salinicaeni* sp. nov. is proposed.

Table 3 Cellular fatty acid contents of strain LAM0A28^T and the related type strains of the genus *Paenibacillus*

Fatty acids	1	2	3
$C_{14:0}$	**10.9**	10.0	8.9
$C_{16:0}$	**17.9**	**21.6**	**26.9**
$C_{18:0}$	tr	1.1	tr
$C_{16:1}\omega11c$	1.1	ND	ND
iso-$C_{14:0}$	8.9	6.5	7.8
iso-$C_{15:0}$	6.1	7.1	5.5
iso-$C_{16:0}$	**14.0**	**14.8**	**12.0**
iso-$C_{17:0}$	tr	1.5	1.2
Anteiso-$C_{15:0}$	**35.8**	**34.7**	**32.9**
Anteiso-$C_{17:0}$	1.5	2.0	1.5

All data were obtained from this study. All strains were cultivated with the same medium and growth conditions. Values were percentages of total fatty acids. tr, trace amount (<1.0 %). The data marked in bold represented the major fatty acids (>10 %) of all strains

Strains 1 strain LAM0A28^T, *2 P. jilunlii* DSM 23019^T, *3 P. graminis* DSM 15220^T

ND not detected

Description of *Paenibacillus salinicaeni* sp. nov

Paenibacillus salinicaeni (sa.li.ni.cae'ni. L. adj. *salinus* saline; L. neut. n. *caenum* mud, silt; N.L. gen. n. *salinicaeni* of saline silt).

Cells are Gram-stain positive, facultatively anaerobic, motile and straight-rod shaped. Ellipsoidal terminal spores are formed in swollen sporangia. Growth occurs with 0–5 % (w/v) NaCl (optimum 0.5 %), at pH 5.0–10.0 (optimum pH 7.5) and at 15–45 °C (optimum 30–35 °C). Catalase positive and oxidase negative. Methyl red reaction is positive. Growth is not inhibited by 0.001 % (w/v) lysozyme. Nitrate is reduced to nitrite. Starch, esculin and 4′-nitrophenyl-β-D-galactopyranoside are hydrolysed but gelatin, urea, Tween 20, Tween 60 and Tween 80 are not. The major fatty acids (>10 % of total) are anteiso-$C_{15:0}$, $C_{16:0}$, iso-$C_{16:0}$ and $C_{14:0}$. The cell wall peptidoglycan contains *meso*-diaminopimelic acid. The predominant menaquinone is MK-7. The major polar lipids are diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, two aminophospholipids and six unidentified lipids. The genomic DNA G+C content is 51.2 mol% as determined by the T_m method.

The type strain is LAM0A28^T (=ACCC 00741^T = JCM 30850^T), which is isolated from a saline silt sample collected from Chinese Sea of Death located in Suining city, Sichuan province, China. The 16S rRNA gene sequence of strain LAM0A28^T has been deposited in GenBank under accession number KM260652.

Acknowledgments We would like to thank Professor Aharon Oren from The Hebrew University of Jerusalem for assistance with Latin in deriving the specific epithet for the strain LAM0A28. This work was supported by Foundation of the Key Laboratory of Development and Application of Rural Renewable Energy (MOA, China) (No. 201502), National Nonprofit Institute Research Grant of CAAS (No. 2014-30), National Key Technology R&D Program of China (No. 2013BAD05B04F02) and National Infrastructure of Microbial Resources.

References

Ash C, Priest FG, Collins MD (1993) Molecular identification of rRNA group 3 bacilli (Ash, Farrow, Wallbanks and Collins) using a PCR probe test. Proposal for the creation of a new genus *Paenibacillus*. Antonie Van Leeuwenhoek 64:253–260

Berge O, Guinebretiere MH, Achouak W, Normand P, Heulin T (2002) *Paenibacillus graminis* sp nov and *Paenibacillus odorifer* sp nov., isolated from plant roots, soil and food. Int J Syst Evol Microbiol 52:607–616

Bryant MP (1972) Commentary on the Hungate technique for culture of anaerobic bacteria. Am J Clin Nutr 25:1324–1328

Chen XR, Shao CB, Wang YW, He MX, Ma KD, Wang HM, Kong DL, Guo X, Zhou YQ, Ruan ZY (2015) *Paenibacillus vini* sp. nov., isolated from alcohol fermentation pit mud in Sichuan Province, China. Anton Leeuw Int J G 107:1429–1436

Choi JH, Im WT, Yoo JS, Lee SM, Moon DS, Kim HJ, Rhee SK, Roh DH (2008) *Paenibacillus donghaensis* sp nov., a xylan-degrading and nitrogen-fixing bacterium isolated from East Sea sediment. J Microbiol Biotechn 18(2):189–193

De Ley J, Cattoir H, Reynaerts A (1970) The quantitative measurement of DNA hybridization from renaturation rates. Eur J Biochem 12:133–142

Fang MX, Zhang WW, Zhang YZ, Tan HQ, Zhang XQ, Wu M, Zhu XF (2012) *Brassicibacter mesophilus* gen. nov., sp. nov., a strictly anaerobic bacterium isolated from food industry wastewater. Int J Syst Evol Microbiol 62:3018–3023

Felsenstein J (1981) Evolutionary trees from DNA sequences: a maximum likelihood approach. J Mol Evol 17:368–376

Fitch WM (1971) Toward defining the course of evolution: minimum change for a specific tree topology. Syst Zool 20:406–416

Han TY, Tong XM, Wang YW, Wang HM, Chen XR, Kong DL, Guo X, Ruan ZY (2015) *Paenibacillus populi* sp. nov., a novel bacterium isolated from the rhizosphere of *Populus alba*. Anton Leeuw Int J G 108:659–666

Hungate RE (1969) A roll tube method for cultivation of strict anaerobes. In: Norris JR, Ribbons RW (eds) Methodsin microbiology, vol 3B. Academic Press, London, pp 117–132

Huss VAR, Festl H, Schleifer KH (1983) Studies on the spectrophotometric determination of DNA hybridization from renaturation rates. Syst Appl Microbiol 4:184–192

Jin HJ, Zhou YG, Liu HC, Chen SF (2011) *Paenibacillus jilunlii* sp nov., a nitrogen-fixing species isolated from the rhizosphere of Begonia semperflorens. Int J Syst Evol Micr 61:1350–1355

Kates M (1986) Techniques of lipidology, 2nd edn. Elsevier, Amsterdam

Kim OS, Cho YJ, Lee K, Yoon SH, Kim M, Na H, Park SC, Jeon YS, Lee JH, Yi H, Won S, Chun J (2012) Introducing EzTaxon-e: a prokaryotic 16S rRNA Gene sequence database with phylotypes that represent uncultured species. Int J Syst Evol Microbiol 62:716–721

Komagata K, Suzuki K (1987) Lipid and cell-wall analysis in bacterial systematics. Methods Microbiol 19:161–207

Lechevalier MP, Lechevalier HA (1980) The chemotaxonomy of actinomycetes. In: Dietz A, Thayer DW, Fairfax VA (eds) Actinomycete taxonomy, society for industrial microbiology. SIM Special Publication, Utrecht, pp 227–291

Li YF, Calley JN, Ebert PJ, Helmes EB (2014) *Paenibacillus lentus* sp nov., a beta-mannanolytic bacterium isolated

from mixed soil samples in a selective enrichment using guar gum as the sole carbon source. Int J Syst Evol Microbiol 64:1166–1172

Logan NA, Berge O, Bishop AH, Busse HL, Vos PD, Fritze D, Heyndrickx M, Kämper P, Rabinovitch L, Salkinoja-Salonen MS, Seldin L, Ventosa A (2009) Proposed minimal standards for describing new taxa of aerobic, endospore- forming bacteria. Int J Syst Evol Microbiol 59:2114–2121

Ludwig W, Schleifer KH, Whitman WB (2009) Family IV. *Paenibacillaceae* fam. nov. In: De Vos P, Garrity GM, Jones D, Krieg NR, Ludwig W, Rainey F, Schleifer KH, Whitman WB (eds) Bergey's manual of systematic bacteriology, vol 3, 2nd edn. Springer, New York, p 269

Mandel M, Marmur J (1968) Use of ultraviolet absorbance-temperature profile for determining the guanine plus cytosine content of DNA. Methods Enzymol 12:195–206

Marmur J, Doty P (1962) Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature. J Mol Biol 5:109–118

Miller TL, Wolin MJ (1974) A serum bottle modification of the Hungate technique for cultivating obligate anaerobes. Appl Microbiol 27:985–987

Minnikin DE, Odonnell AG, Goodfellow M, Alderson G, Athalye M, Schaal A, Parlett JH (1984) An integrated procedure for the extraction of bacterial isoprenoid quinones and polar lipids. J Microbiol Methods 2:233–241

Osman S, Satomi M, Venkateswaran K (2006) *Paenibacillus pasadenensis* sp. nov. and *Paenibacillus barengoltzii* sp. nov., isolated from a spacecraft assembly facility. Int J Syst Evol Microbiol 56:1509–1514

Poly F, Monrozier LJ, Bally R (2001) Improvement in the RFLP procedure for studying the diversity of *nifH* genes in communities of nitrogen fixers in soil. Res Microbiol 152:95–103

Priest FG (1994) Genus I. *Paenibacillus* Ash, Priest and Collins 1994, 852VP. In: De Vos P, Garrity GM, Jones D, Krieg NR, Ludwig W, Rainey FA, Schleifer K-H, Whitman WB (eds) Bergey's manual of systematic bacteriology. Springer, New York, pp 269–295

Ruan Z, Wang Y, Song J, Jiang S, Wang H, Li Y, Zhao B, Jiang R, Zhao B (2014) *Kurthia huakuii* sp. nov., isolated from biogas slurry, and emended description of the genus *Kurthia*. Int J Syst Evol Microbiol 64:518–521

Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4:406–425

Sakamoto M, Suzuki M, Umeda M, Ishikawa I, Benno Y (2002) Reclassification of bacteroides forsythus (Tanner et al. 1986) as *Tannerella forsythensis* corrig., gen. nov., comb.Nov. Int J Syst Evol Microbiol 52:841–884

Schleifer KH (1985) Analysis of the chemical composition and primary structure of murein. Methods Microbiol 18:123–156

Smibert RM, Krieg NR (1994) Phenotypic characterization. In: Gerhardt P, Murray RGE, Wood WA, Krieg NR (eds) Methods for general and molecular bacteriology. American Soc Microbiol, Washington, DC, pp 607–654

Stackebrandt E, Goebel BM (1994) Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. Int J Syst Evol Microbiol 44:846–849

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: molecular evolutionary genetics analysis version 6.0. Mol Biol Evol 30:2725–2729

Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res 22:4673–4680

Weisburg WG, Barns SM, Pelletier DA, Lane DJ (1991) 16S ribosomal DNA amplification for phylogenetic study. J Bacteriol 173:697–703

Xie JB, Zhang LH, Zhou YG, Liu HC, Chen SF (2012) *Paenibacillus taohuashanense* sp nov., a nitrogen-fixing species isolated from rhizosphere soil of the root of Caragana kansuensis Pojark. Anton Leeuw Int J Gen 102(4):735–741

Xu XW, Huo YY, Wang CS, Oren A, Cui HL, Vedler E, Wu M (2011) *Pelagibacterium halotolerans* gen. nov., sp. nov. and *Pelagibacterium luteolum* sp. nov., novel members of the family *Hyphomicrobiaceae*. Int J Syst Evol Microbiol 61:1817–1822

Yao R, Wang R, Wang D, Su J, Zheng SX, Wang G (2014) *Paenibacillus selenitireducens* sp. nov., a selenite-reducing bacterium isolated from a selenium mineral soil. Int J Syst Evol Microbiol 64:805–811