Antonie van Leeuwenhoek (2015) 108:659–666
DOI 10.1007/s10482-015-0521-4

ORIGINAL PAPER

Paenibacillus populi sp. nov., a novel bacterium isolated from the rhizosphere of *Populus alba*

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Received: 25 March 2015 / Accepted: 27 June 2015 / Published online: 2 July 2015

Abstract A novel aerobic bacterium, designated strain LAM0705^T, was isolated from the rhizosphere of *Populus alba* in the Peking University Third Hospital. Cells of strain LAM0705^T were observed to be Gram-stain positive, motile, spore-forming and rod-shaped. The optimal temperature and pH for growth were found to be 30 °C and pH 7.5, respectively. Strain LAM0705^T was found to be able to grow in the presence 0–5 % NaCl (w/v) (optimum 1.0 %). The major fatty acids of strain LAM0705^T were identified as anteiso-$C_{15:0}$, $C_{16:0}$ and iso-$C_{16:0}$. The dominant polar lipids were found to consist of diphosphatidylglycerol, phosphatidylethanolamine and phosphatidylglycerol. The cell wall peptidoglycan of strain LAM0705^T was found to contain *meso*-diaminopimelic acid. The predominant menaquinone was identified as MK-7. The G+C content of genomic DNA was found to be 48 mol% when determined by the T_m method. The 16S rRNA gene sequence similarity analysis indicated that strain LAM0705^T is closely related to *Paenibacillus agaridevorans* DSM 1355^T and *Paenibacillus thailandensis* KCTC 13043^T with 97.8 and 96.1 % sequence similarity, respectively. The DNA–DNA hybridization value between strain LAM0705^T and *P. agaridevorans* DSM 1355^T was 47 ± 0.8 %. On the basis of its phenotypic, phylogenetic and chemotaxonomic characteristics, strain LAM0705^T is concluded to represent a novel species of the genus *Paenibacillus*, for which the name *Paenibacillus populi* sp. nov. is proposed. The type strain is LAM0705^T (=ACCC 06427^T = JCM 19843^T).

Keywords *Paenibacillus populi* sp. nov. · Polyphasic taxonomy · 16S rRNA gene · Rhizosphere · *Populus alba*

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Electronic supplementary material The online version of this article (doi:10.1007/s10482-015-0521-4) contains supplementary material, which is available to authorized users.

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Introduction

The genus *Paenibacillus* belongs to the family *Paenibacillaceae* of the phylum *Firmicutes*, and was proposed by Ash et al. (1993, 1994) by reclassification of eleven species of the genus *Bacillus*. In 1996, the genus *Paenibacillus* was reassessed on the basis of polyphasic taxonomic results (Heyndrickx et al.

1996). Generally, members of the genus *Paenibacillus* are aerobic or facultatively anaerobic, endospore-forming and Gram-stain positive, although some strains are Gram-negative. Their genomic DNA G+C content ranges from 39–54 mol% and anteiso-$C_{15:0}$ is the predominant fatty acid (Shida et al. 1997; Montes et al. 2004; Khianngam et al. 2009; Priest 2009; Behrendt et al. 2010; Kämpfer et al. 2012; Wu et al. 2013). At the time of writing, the genus comprises more than 150 recognised species with validly published names (http://www.bacterio.net/paenibacillus.html). Since the description of the genus, members of the genus *Paenibacillus* have been found widely distributed throughout the biosphere such as volcanic soil (Uetanabaro et al. 2003), soil (Khianngam et al. 2009), sediment (Park et al. 2011) and sputum (Kim et al. 2010).

While studying the bacterial diversity of the rhizosphere of *Populus alba* in the Third Hospital of Peking University, a *Paenibacillus*-like strain, designated LAM0705^T, was obtained. By using a polyphasic taxonomic approach, we conclude that strain LAM0705^T represents a novel species of the genus *Paenibacillus*, for which the name *Paenibacillus populi* sp. nov. is proposed.

Materials and methods

Bacterial strains and culture condition

Strain LAM0705^T was isolated from the rhizosphere of *P. alba* in the Third Hospital of Peking University. TSA medium (BD/BBL 236950, Sparks, MD, USA) was used for the isolation. Soil samples were diluted with sterilised water and spread onto TSA medium plates and incubated under aerobic condition at 30 °C for 2 days. One of the isolates obtained, designated strain LAM0705^T, which was purified at least twice before preservation in 25 % (v/v) glycerol at −80 °C, was selected for further study.

Biomass for chemotaxonomic and molecular studies was obtained by cultivation in shaking flasks with TSB medium (BD/ Difco 211825, Sparks, MD, USA) at 35 °C for 2 days. The recommended Minimal Standards for describing new taxa of aerobic, endospore-forming bacteria as described by Logan et al. (2009) were followed. The reference type strains *Paenibacillus agaridevorans* DSM 1355^T and *Paenibacillus thailandensis* KCTC 13043^T were obtained from the German Collection of Microorganisms and Cell Cultures (DSMZ; Germany) and Korean Collection for Type Cultures (KCTC; Korean), respectively. Reference type strains were cultured under the same conditions as strain LAM0705^T for comparative analyses.

Morphological, physiological and biochemical characteristics

Morphological characteristics of an exponentially growing culture of strain LAM0705^T were examined by using a light microscope (Nikon 80i, Tokyo, Japan), transmission electron microscope and scanning electron microscope (Hitachi 7500, Tokyo, Japan). Motility was tested in TSB medium with 0.4 % agar. Growth at temperatures of 10, 15, 20, 30, 37, 45, 50, 55 and 60 °C; and pH ranges of 4, 5, 6, 7, 8 and 9 were investigated in TSB medium. NaCl concentrations of 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12 % (w/v) were produced in TSB medium prepared according to the formula of TSB but varying the addition of NaCl. The medium was adjusted to the desired pH by using sterile solutions of citric acid/Na_2HPO_4 (pH 4.0–5.0), MES (pH 5.5–6.0), PIPES (pH 6.5–7.0), Tricine (pH 7.5–8.5) or CAPSO (pH 9.0–9.5) added at a final concentration of 30 mM (Ruan et al. 2014). All tests were conducted independently in duplicate. Growth under aerobic, trace-oxygen, and anaerobic conditions were examined at 35 °C up to 7 days. Gram staining was carried out by using the procedure described by Buck (1982). Sporulation was conducted with TSA medium supplemented with 5 mg L^{-1} $MnSO_4$ (Logan et al. 2009). Catalase activity was determined based on bubble production in 3 % (v/v) H_2O_2. Oxidase activity was determined by using 1 % (w/v) tetramethyl-*p*-phenylenediamine. Hydrolysis of casein and starch were carried out on skimmed milk agar and starch agar, respectively. Nitrate reduction was conducted as described by Smibert and Krieg (1994). A variety of tests to determine the biochemical characteristics were also performed by using the Biolog GP2 MicroPlates (Biolog, Hayward, CA, USA), API 20E, API ZYM and API 50CH test systems (bioMérieux, ĹEtoile, France) according to the manufacturers' instructions.

Chemotaxonomic characterisation

The major fatty acid analyses were performed on strain LAM0705^T, *P. agaridevorans* DSM1355^T and *P. thailandensis* KCTC 13043^T. The three strains were incubated on TSA medium at 35 °C for 36 h. The fatty acid methyl esters were obtained from the cells collected from the plates. The Sherlock Microbial Identification System with the standard MIS Library Generation Software (Microbial ID Inc., Newark, DE, USA) was used for the identification and quantification of the fatty acid methyl esters as well as the numerical analysis of the fatty acid profiles according to the manufacturer's instruction. The isomer type of the diamino acid in the cell wall peptidoglycan was determined as described by Komagata and Suzuki (1987). The respiratory quinones of strain LAM0705^T were prepared and analysed according to the method described by Minnikin et al. (1984) and Tindall (1990) by using high performance liquid chromatography. The polar lipids were extracted and separated on silica gel plates (10 × 10 cm, Merck 5554) and further analysed by using 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 data were interpreted as described by Fang et al. (2012).

Molecular studies

Genomic DNA for PCR amplification of strain LAM0705^T was extracted and purified using a TIANamp Bacter DNA Kit (Tiangen Biotech CO., Ltd) according to the manufacturer's instruction. The 16S rRNA gene was amplified by PCR with the universal bacterial primers 27F and 1492R (Weisburg et al. 1991). Purified PCR products of approximately 1.5 kb were cloned into pGEM-T vector and sequenced by the Majorbio Company (Beijing, China). The EzTaxon-e service (Kim et al. 2012) was used to analyse the sequence similarities. Phylogenetic trees were built via the neighbour-joining method (Saitou and Nei 1987) and maximum parsimony method (Fitch 1971) with the MEGA6 program package (Tamura et al. 2013). Evolutionary distances were calculated according to the algorithm of the Kimura's two-parameter model (Kimura 1980) for the neighbour-joining method.

DNA-DNA hybridizations were performed by using the thermal denaturation and renaturation method (De Ley et al. 1970) as modified by Huß et al. (1983), using a Beckman DU800 spectrophotometer. The genomic DNA G+C content was determined by the thermal denaturation method using a Beckman DU 800 spectrophotometer (Beckman Coulter, Brea, CA, USA). *Escherichia coli* K-12 was used as a reference strain.

Results and discussion

Morphological, physiological and biochemical characteristics

Cells of strain LAM0705^T were observed to be aerobic, motile, Gram-stain positive, endospore-forming, rod-shaped with a cell size of 0.5–1.2 μm in width and 0.75–3.0 μm in length (Fig. S1) and motile by peritrichous flagella (Fig. S2). Colonies were observed to be white, circular, opaque, convex, smooth and approximately 2–4 mm in diameter after incubation on TSA medium at 35 °C for 2 days. Spherical to ellipsoidal endospores were observed to be formed terminal in swollen sporangia (Fig. S3). The pH and temperature ranges for growth were found to be 6.0–8.0 (optimum 7.5) and 25–45 °C (optimum 30 °C), respectively. The strain was found to grow well in medium with 1 % (w/v) NaCl and tolerated up to 5 %. The physiological and biochemical characteristics of strain LAM0705^T that differentiate it from the reference strains are shown in Table 1. The detailed physiological and biochemical characteristics of the type strain LAM0705^T are given in the species description.

Chemotaxonomic characteristics

The major fatty acids of strain LAM0705^T were identified as anteiso-$C_{15:0}$ (46.9 %), $C_{16:0}$ (12.1 %) and iso-$C_{16:0}$ (10.9 %). The detailed fatty acid compositions of strain LAM0705^T, *P. agaridevorans* DSM 1355^T and *P. thailandensis* KCTC 13043^T are shown in Table 2. The cell wall peptidoglycan of strain LAM0705^T was found to contain *meso*-diaminopimelic acid as the diamino-acid. The predominant isoprenoid quinone was identified as menaquinone-7 (MK-7). The main polar lipids

Table 1 Differential phenotypic, physiological and genotypic characteristics of strain LAM0705^T and its relatives: all data were obtained from this study

Characteristics	LAM0705^T	DSM 1355^T	KCTC 13034^T
Temperature range (°C)	25–45	25–40	20–55
pH range	6.0–8.0	5.0–7.0	7.0–9.0
Tolerance to NaCl (%, w/v)	0–5	0–5	0–3
Nitrate reduction	−	+	−
Voges–Proskauer reaction	+	−	+
Gelatin hydrolysis	−	+	−
Urease	−	+	−
Acid production from			
Amygdalin	+	−	+
D-Arabinose	−	−	+
L-Arabinose	+	−	+
D-Arabitol	−	−	+
L-Arabitol	−	−	+
D-Cellobiose	+	−	+
Dulcitol	−	−	+
Erythritol	−	−	+
D-Fucose	−	−	+
D-Fructose	−	−	+
Glycerol	+	−	+
D-Galactose	+	−	+
Glycogen	−	−	+
D-Gentiobiose	−	−	+
Inulin	−	−	+
D-Lyxose	−	−	+
D-Mannose	−	−	+
D-Mannitol	−	−	+
D-Maltose	−	+	+
D-Melibiose	−	−	+
D-Melezitose	−	−	+
D-Ribose	−	−	+
L-Rhamnose	−	−	+
D-Raffinose	−	−	+
L-Sorbose	−	−	+
Sorbitol	−	−	+
D-Sucrose	−	+	−
D-Trehalose	−	+	+
D-Turanose	−	−	+
D-Tagatose	−	−	+
Xylose	−	−	+
DNA G+C content (mol%)[a]	48.0	50.5	53.2

+, Positive; −, negative

[a] DNA G+C content was determined by T_m method

were found to be diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol, one unidentified phospholipid and three unidentified lipids (Fig. S4).

Molecular characterisation

The 16S rRNA gene sequence (1460 nt; GenBank accession number KJ000069) was obtained from

Table 2 Fatty acid composition of strain LAM0705^T and its relatives

Major fatty acids	LAM0705^T	DSM 1355^T	KCTC 13043^T
Unbranched			
$C_{14:0}$	2.0	2.0	0.5
$C_{15:0}$	ND	ND	tr
$C_{16:0}$	**12.1**	**16.3**	5.6
$C_{16:1}\omega7c$ alcohol	0.7	1.7	ND
$C_{16:1}$ $\omega11c$	1.2	5.7	ND
$C_{17:0}$	0.6	ND	ND
$C_{18:0}$	0.8	0.9	tr
$C_{18:1}$ $\omega9c$	0.7	tr	ND
iso-Unbranched			
iso-$C_{14:0}$	2.1	4.2	1.3
iso-$C_{15:0}$	7.5	7.4	7.4
iso-$C_{16:0}$	**10.9**	**12.1**	**18.6**
iso-$C_{17:0}$	3.8	3.3	6.1
iso-$C_{17:1}\omega10c$	ND	2.1	ND
anteiso-Unbranched			
anteiso-$C_{14:0}$	tr	ND	ND
anteiso-$C_{15:0}$	**46.9**	**36.6**	**41.0**
anteiso-$C_{17:0}$	8.5	4.6	18.3
Summed features			
4	tr	2.3	ND
5	0.5	tr	tr

Major components in each strain are highlighted in bold

Data for the major fatty acids of LAM0705^T, DSM 1355^T, KCTC 13034^T are from this study

Summed feature 4 including iso—$C_{17:1}$ I and /or anteiso-B

Summed feature 5 including anteiso—$C_{18:0}$ and/or $C_{18:2}\omega6,9c$

tr Trace amount (less than 0.5 %), *ND* not detected

strain LAM0705^T. Phylogenetic analysis based on the 16S rRNA gene sequences indicated that strain LAM0705^T is a member of the genus *Paenibacillus* and closely related to *P. agaridevorans* DSM 1355^T and *P. thailandensis* KCTC 13043^T with sequence similarity of 97.8 and 96.1 %, respectively (Fig. 1). The topologies of phylogenetic trees built using maximum parsimony and maximum-likelihood method supported the finding that strain LAM0705^T formed a stable clade with these related species (Figs. S5, S6). The DNA–DNA hybridization value between strain LAM0705^T and *P. agaridevorans* DSM 1355^T was 47 ± 0.8 %. The genomic DNA G+C content of strain LAM0705^T was found to be 48 mol% as determined by the T_m method, which is in the range reported for the members of the genus *Paenibacillus*.

Taxonomic conclusion

Based on its characterisation as Gram-positive, rod-shaped, endospore-forming cells; positive for catalase, oxidase and β-galactosidase activities; the major fatty acid (anteiso-$C_{15:0}$, $C_{16:0}$ and iso-$C_{16:0}$); the predominant polar lipids (diphosphatidylglycerol, phosphatidylethanolamine and phosphatidylglycerol); the predominant menaquinone (MK-7); the cell wall peptidoglycan diamino acid (*meso*-diaminopimelic acid); the genomic DNA G+C content (48 mol%); and the phylogenetic analyses, all these data suggest that strain LAM0705^T belongs to the genus *Paenibacillus*. However, strain LAM0705^T showed notable differences in comparison to the type strains of the closely related species *P. agaridevorans* DSM 1355^T and *P. thailandensis* KCTC 13043^T with regard to morphological characteristics, growth ranges (temperature, pH and NaCl tolerance), nitrate reduction, Voges–Proskauer test, hydrolysis of gelatin and urease, and acid production from carbohydrates (Table 1). The closely related reference strain *P. agaridevorans* DSM 1355^T could grow under trace-oxygen conditions in TSB medium whereas strain LAM0705^T could not. The profiles of the major fatty acids of strain LAM0705^T, *P. agaridevorans* DSM 1355^T and *P. thailandensis* KCTC 13043^T were similar but differences were found in the abundance of

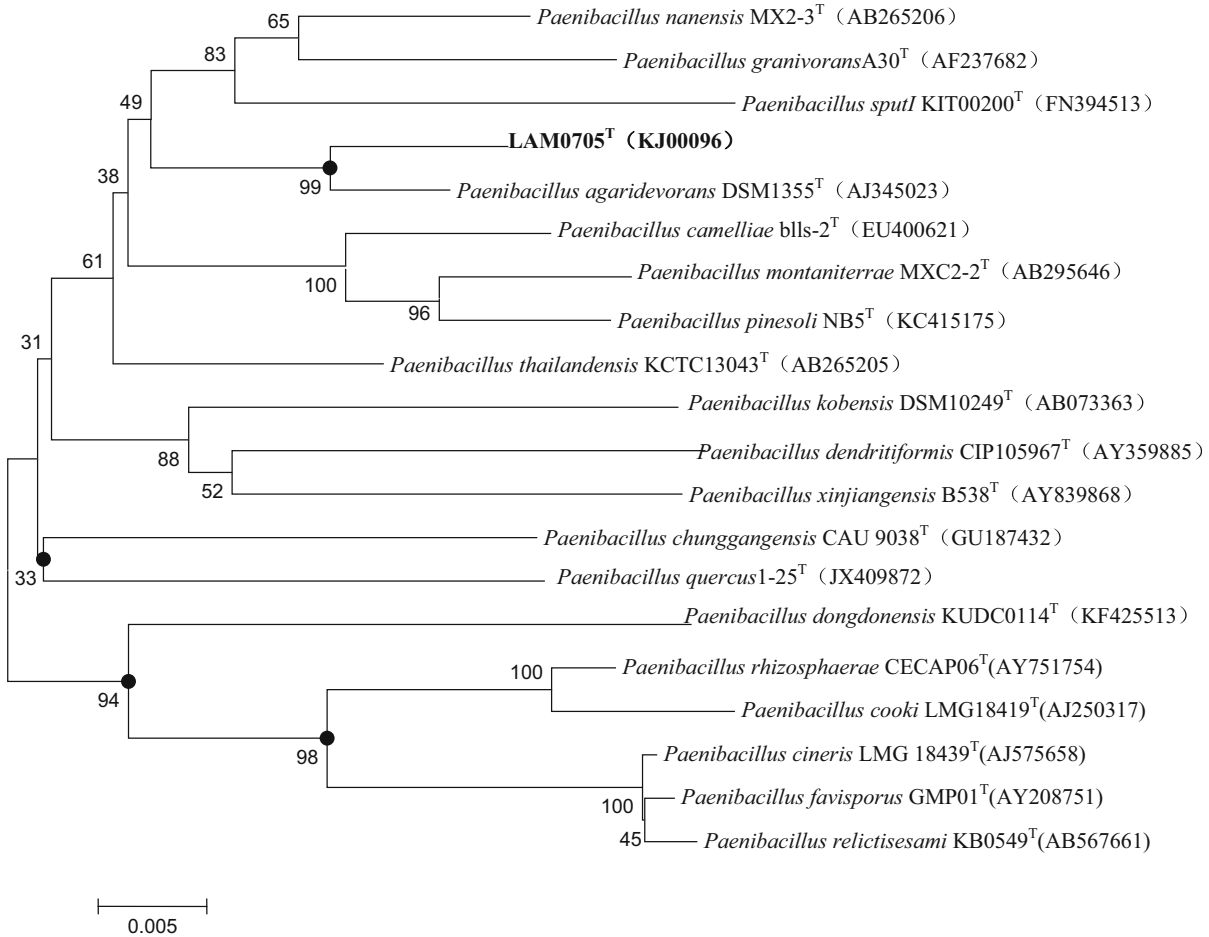

Fig. 1 Neighbour-joining phylogenetic tree based on a comparison of the 16S rRNA gene sequences of strain LAM0705^T and its closest relatives. Genbank accession numbers are given in parentheses. *Bar* 5 nucleotide changes per 1000 nucleotides. Branching nodes supported by the maximum-likelihood and maximum-parsimony algorithms are marked with *filled circles*

anteiso-$C_{15:0}$; the amount in strain LAM0705^T (46.9 %) was higher than in *P. agaridevorans* DSM 1355^T (36.6 %) and *P. thailandensis* KCTC 13043^T (41.0 %) (Table 2). Differences in the polar lipid profile also existed when compared to the reference strains (Fig. S4). Strain LAM0705^T shared low 16S rRNA gene sequence similarities to the type strains *P. agaridevorans* DSM 1355^T and *P. thailandensis* KCTC 13043^T (97.8 and 96.1 %, respectively). DNA–DNA hybridization data (47 ± 0.8 %) clearly differentiated strain LAM0705^T from strain *P. agaridevorans* DSM 1355^T. The low (<70 %) DNA–DNA relatedness value between the novel strain and its close relative precludes genomic relatedness and supports the designation of strain LAM0705^T as the representative of a novel species within the genus *Paenibacillus* (Stackebrandt and Goebel 1994). Based on the phenotypic, phylogenetic and chemotaxonomic characterisation, strain LAM0705^T is considered to represent a novel species of the genus *Paenibacillus* for which the name *Paenibacillus populi* sp. nov. is proposed.

Description of *Paenibacillus populi* sp. nov.

Paenibacillus populi (po′pu.li. L. gen. n. *populi* of a poplar, pertaining to *Populus*, the Latin name for the poplar, from the rhizosphere of which the type strain was isolated).

Cells are aerobic, Gram-stain positive, spore-forming and rod-shaped with a cell size of 0.5–1.2 μm in width and 0.75–3.0 μm in length. Cells are motile by means of peritrichous flagella. The pH and temperature ranges for growth are 6.0–8.0 (optimum 7.0) and 25–45 °C (optimum 30 °C), respectively. Grows well in TSB medium with 1 % NaCl (w/v) and tolerates up to 5 % NaCl. Acids are produced from glycerol, L-arabinose, D-galactose, D-glucose, inositol, amygdalin, esculin, salicin and D-cellobiose. Positive for the following enzymatic reactions: alkaline phosphatase, esterase (C4), esterase lipase (C8), naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase and α-glucosidase; weakly positive reaction for acid phosphatase; and negative reactions for lipase (C14), leucine arylamidase, valine arylamidase, cystine arylamidase, α-mannosidase, β-glucuronidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, trypsin and α-fucosidase. Positive reactions for Voges–Proskauer reaction, OPNG test, assimilation of D-glucose, inositol, amygdaloside, D-mannitol and L-rhamnose; negative for gelatin hydrolysis, urease, indole production, arginine dihydrolase, ornithine, citrate utilization, H_2S production, assimilation of L-arabinose, amygdaloside, melibiose, D-sucrose and sorbitol. Carbon sources utilised are dextrin, Tween 40, amygdalin, inositol, maltose, maltotriose, D-mannose, β-methyl-D-glucoside, palatinose, D-ribose, D-trehalose, turanose, D-xylose, acetic acid, D-cellobiose, D-galactose, α-D-glucose, succinic acid, mono-methyl ester, L-alaninamide, D-alanine, L-alanine, L-glutamic acid, L-pyroglutamic acid, glycerol, adenosine, 2′-deoxy adenosine, thymidine, uridine, thymidine-5′-monophosphate. Does not utilise α-cyclodextrin, glycogen, inulin, mannan, tween 80, *N*-acetyl-β-D-mannosamine, L-arabinose, D-fructose, gentiobiose, lactulose, D-melibiose, α-methyl-D-galactoside, D-psicose-β-hydroxybutyric acid, D-psicose, L-rhamnose, D-sorbitol, xylitol, D-malic acid, pyruvic acid, succinic acid, L-serine and putrescine. The cell-wall peptidoglycan contains *meso*-diaminopimelic acid. The major fatty acids are anteiso-$C_{15:0}$, $C_{16:0}$ and iso-$C_{16:0}$. The major polar lipids are diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol, one unidentified phospholipid and three unidentified lipids. The major isoprenoid quinone is MK-7. The genomic DNA G+C content of the type strain is 48 mol% as determined by the T_m method.

The type strain is LAM0705^T (=ACCC 06427^T = JCM 19843^T), which was isolated from the rhizosphere of *Populus alba* in the Third Hospital of Peking University. The GenBank accession number of the 16S rRNA gene sequence of the type strain is KJ000069.

Acknowledgments This work was supported by National Nonprofit Institute Research Grant of CAAS (No. 2014-30), National Key Technology R&D Program of China (No. 2013BAD05B04F02 and 2011BAD11B05), Foundation of the Key Laboratory of Development and Application of Rural Renewable Energy (MOA, China) (No. 2013002), Science Foundation of Modern Farming Group (No. MF20100518), and Science Foundation of Dajing Group.

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