Current Microbiology (2019) 76:995–1002
https://doi.org/10.1007/s00284-019-01710-y

Paenibacillus lutimineralis sp. nov., Isolated From Bentonite

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Received: 27 August 2018 / Accepted: 24 May 2019 / Published online: 8 June 2019

Abstract
Strain MBLB1234^T was isolated from bentonite samples collected at Guryong mining area located in Pohang, Republic of Korea and was taxonomically characterized by a polyphasic approach. This strain was a Gram-stain-negative, motile, endospore-forming, facultative anaerobic, catalase-positive, oxidase-negative, and rod-shaped bacterium. Strain MBLB1234^T was able to grow at 20−45 °C (optimum, 37 °C), pH 6.0−10.0 (optimum, 7.0–8.0), and 0−5.0% (w/v) NaCl (optimum, 0.5%). Genome size was 6,497,679 bp with a G + C content of 46.4 mol %. The genome was predicted to contain 5233 protein-coding genes, and 135 rRNA genes consisted of 10 5S rRNAs, 10 16S rRNAs, 10 23S rRNAs, and 105 tRNAs. Phylogenetic analysis based on the 16S rRNA gene sequences revealed that strain MBLB1234^T clustered with *Paenibacillus motobuensis* JCM 12774^T and *P. aceti* JCM 31170^T with 98.3–98.5% and 97.2–97.4% sequencing similarity, respectively. The major fatty acids of strain MBLB1234^T were anteiso-$C_{15:0}$ (35.7%), anteiso-$C_{17:0}$ (17.8%), iso-$C_{17:0}$ (14.5%), and $C_{16:0}$ (11.0%). The polar lipids were diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylmethylethanolamine, and one unidentified phospholipid, six unidentified aminophospholipids, and one unidentified lipid. The predominant isoprenoid quinone was menaquinone-7. DNA–DNA hybridization values between strain MBLB1234^T and *P. motobuensis* JCM 12774^T and *P. aceti* JCM 31170^T were 34 and 38%, respectively. Average nucleotide identity value between strains MBLB1234^T and *P. aceti* L14^T was 82.3%. Based on characteristics of genomic, phenotypic, chemotaxonomic, and phylogenetic analyses, strain MBLB1234^T represents a novel species of the genus *P.* , for which the name *P. lutimineralis* sp. nov. is proposed. The type strain is MBLB1234^T (= JCM 32684^T = KCTC 33978^T).

Introduction

The genus *Paenibacillus*, belonging to the family *Paenibacillaceae* in phylum *Firmicutes*, was first proposed by Ash et al. [1] by dividing the genus *Bacillus* sensu lato into five distinct groups based on comparative 16S rRNA gene sequence analysis according to which the genus *Paenibacillus* was established as group 3 [2]. Subsequently, an emended description of the genus *Paenibacillus* was provided by Shida et al. [3]. At the time of writing, the genus *Paenibacillus* comprises 238 species and four subspecies with validly published names (http://www.bacterio.net/paenibacillus.html), among which *Paenibacillus polymyxa* is the type species of the genus [4]. The members of the genus *Paenibacillus* are widely distributed in various habitats and mostly isolated from human-enriched soils, decomposing plant materials, other soil samples [5, 6].

Bentonite is a clay mineral mostly composed of smectite, predominant composition of which is montmorillonite [7]. Bentonite containing various elements, such as potassium, sodium, aluminum, calcium, have been investigated for the adsorption of heavy metals and molecular species in a variety of environments and industries and also identified as low-cost adsorbents [8–10]. In addition to studies on the physicochemical properties of bentonites, some studies on the antibacterial effect of bentonite against *Escherichia coli* and *Staphylococcus aureus* have been reported [11]. Therefore, it has been applied to the development of health-related

The GenBank/EMBL/DDBJ accession number for genome and the Digital Protologue Database Taxon Number for of strain MBLB1234^T are CP034346 and TA00598, respectively.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00284-019-01710-y) contains supplementary material, which is available to authorized users.

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products including functional foods, cosmetics, and pharmaceuticals [8]. Although there have been several studies on the characterizations of bentonite properties themselves and its biological effects, few studies have reported the microbial diversity in bentonite [12] and the isolation of novel microorganism present in bentonite [13]. Accordingly, a large number of microbial strains were isolated during a study targeting the culture-dependent microbial diversity in bentonite collected from Guryong mining area located in Pohang, Republic of Korea. Based on 16S rRNA gene sequence similarity, one of the isolated strains designated MBLB1234^T was found to be a member of the genus *Paenibacillus*. Further study of strain MBLB1234^T based on the polyphasic analyses also determined the taxonomic position as a representative of a novel species of the genus *Paenibacillus*.

Materials and Methods

Isolation and Culture Conditions

Four bentonite samples were collected at Guryong mining area located in Pohang, Gyeongsangbuk-do, Republic of Korea (36°00′55.1″N 129°32′02.9″E), pooled in autoclaved bag and stored at 4 °C before the isolation of bacterial strains. The chemical compositions of bentonite samples were analyzed using energy dispersive X-ray fluorescence spectrometer (Rigaku NEX CG) [14], resulting that major chemical components of bentonite samples were SiO_2 with 52.4–62.6 wt%, followed by Al_2O_3 with 13.4–16.4 wt%. The bentonite samples were thoroughly crushed and diluted in 0.85% (w/v) NaCl. The suspensions were spread on TSB agar (BD Difco) to isolate bacterial strains. The plates were then incubated at 30 °C for 2 weeks until colonies formed. These colonies were subsequently re-streaked at least three times on the same kind of fresh medium to obtain pure colonies. After 16S rRNA gene sequencing analysis of the isolates, one isolate designated strain MBLB1234^T exhibiting less than 99.0% similarity in 16S rRNA gene sequence was finally selected as the primary candidate for novel species and then stored at − 80 °C in 25% (w/v) glycerol stock solution until further analysis. Strain MBLB1234^T has been deposited at the JCM (Japan Collection of Microorganisms) and the KCTC (Korean Collection for Type Cultures).

Primary Identification

To monitor the taxonomic information, a HiYield™ Genomic DNA Mini Kit (RBC, Taiwan) was used to extract the genomic DNA of the isolates. The 16S rRNA gene was amplified by PCR using universal primers 27F and 1492R [15], after which the PCR products were sequenced at Macrogen Co., Ltd. and the 16S rRNA gene sequences were assembled using the SeqMan software (DNAStar) [16]. To align the 16S rRNA gene sequence of each isolate with related species, SILVA (http://www.arb-silva.de/aligner) was used [17].

Whole Genome Sequencing, Genome Annotation and Phylogenetic Tree Construction

Whole genome of strain MBLB1234^T was sequenced and assembled using the PacBio RS II sequencing system and Hierachical Genome Assembly Process (HGAP) (Pacific Biosciences). Genome annotation was performed using the Rapid Annotation using Subsystem Technology (RAST) server for gene prediction, RNAmmer 1.2 sever (http://www.cbs.dtu.dk/services/RNAmmer/) [18] for fining rRNA, tRNA scan-SE (http://lowelab.ucsc.edu/tRNAscan-SE/) [19] for scanning tRNA, eggNOG web (http://eggnogdb.embl.de/) [20] for comparing orthologous groups of proteins at different taxonomic levels and summarizing functional annotations, and the Bacterial Pan Genome Analysis pipeline (BPGA) (http://iicb.res.in/bpga/index.html) for analyzing the core, accessory, unique, and exclusively absent genes. The 16S rRNA gene sequence of strain MBLA1234^T was sorted from the whole genome using the RAST and RNAmmer 1.2 servers. To construct the phylogenetic tree, the 16S rRNA gene sequences of the strains belonging to the genus *Paenibacillus* were subsequently obtained from the EzBioCloud server (http://www.ezbiocloud.net/) [21], after which the evolutionary distances were calculated using the Kimura two-parameter model [22]. A phylogenetic tree was built with the MEGA7 program [23] using the maximum likelihood (ML) [24], maximum parsimony (MP) [25], and neighbor-joining (NJ) [26] methods (each employed 1000 replicates).

Genomic DNA–DNA Relatedness

DNA–DNA hybridization (DDH) values of strain MBLB1234^T and two reference strains were determined by using photobiotin-labeled DNA probes and microdilution wells [27]. The highest and lowest values were omitted for each sample, and the means of the remaining three values were used as the DNA–DNA relatedness values. Genomic DNA–DNA relatedness between strain MBLB1234^T and closely related reference genome was computed using the Average Nucleotide Identity (ANI) calculator (ver. 0.93.1) supplied by EzBioCloud sever.

Reference Strains

Paenibacillus motobuensis JCM 12774^T and *Paenibacillus aceti* JCM 31170^T were selected as reference strains to

analyze the biochemical and chemotaxonomic characteristics of strain MBLA1234^T. These reference strains were purchased from JCM and routinely cultivated on TSB agar at 30 °C. We have also used the genomes of *P. aceti* L14 (MDDO01000000), *P. glucanolyticus* 5162 (CP015286), *P. pini* JCM 16418 (BAVZ00000000), *P. solani* FJAT-22460 (LIUT00000000), and *P. terrae* HPL-003 (CP003107) as reference strains for genomic analysis of strain MBLA1234^T.

Phenotype and Biochemical Characterization and In Silico Phenotypic Analysis

The cell morphology of strain MBLB1234^T was observed by light microscopy (model CX 23; Olympus) and transmission electron microscopy (JEM-101; JEOL). Gram staining was conducted using a BD Gram-stain kit according to the manufacturer's instructions. Anaerobic growth was evaluated using a GasPak™ EZ anaerobe gas-generating pouch system with indicator (BD) on TSA at 30 °C for 4 weeks. Growth range and optimal growth were determined with modified TSA. To determine the optimal temperature, strain MBLB1234^T was incubated on TSA at 4, 10, 15, 20, 25, 30, 35, 37, 40, 45, 50, and 55 °C for 4 weeks. To measure the NaCl tolerance, TSA as basal medium was modified by adjusting the NaCl in the medium to 0, 0.5% (w/v), and 1.0−10.0% (w/v) at intervals of 1.0%. The growth range in different pH was determined by cultivating on TSA at 37 °C after adjusting the medium pH using the following buffer systems: pH 5.0 and 6.0 with 10 mM 2-(*N*-morpholino) ethanesulfonic acid; pH 7.0–9.0 with 10 mM Bis–Tris propane; pH 10.0 and 11.0 with 10 mM 3-(cyclohexylamino)-1-propanesulfonic acid. Catalase activity was determined by testing bubble production in 3% (w/v) hydrogen peroxide, while oxidase activity was assessed using 1% (w/v) tetramethyl-*p*-phenylenediamine solution. Hydrolysis of starch, casein, Tweens 20, 40, and 80, gelatin, and L-tyrosine were evaluated as described by Benson [28], while H_2S production was tested according to Gerhardt et al. [29]. Utilization of various substrates as sole carbon and energy sources, and enzyme activities of strain MBLB1234^T were determined using the API 20NE, API 50CH, and API ZYM (bioMérieux) strips according to the manufacturer's instructions. Antibiotic susceptibility was tested by the disk diffusion plate method as described by Bauer et al. [30]. Disks were impregnated with the following antibiotics (μg ml^{-1} disk unless otherwise indicated): ampicillin (10), carbenicillin (100), cephalothin (30), ciprofloxacin (10), erythromycin (25), gentamicin (30), kanamycin (30), lincomycin (15), neomycin (30), norfloxacin (20), novobiocin (10), penicillin G (20 UI), polymyxin B (100 UI), streptomycin (50), and tetracycline (30). The strain was incubated at 30 °C for 2 weeks. In silico phenotypic traits' analyses were performed with the predicted genes from genome annotation using the RAST, eggNOG, and BPGA.

Chemotaxonomic Characterization

Cellular fatty acid profiles of strains MBLA1234^T, *P. motobuensis* JCM 12774^T, and *P. aceti* JCM 31170^T were analyzed according to the methods described by Miller [31] using an Agilent 6890 gas chromatography system and a crosslinked methyl siloxane column (HP-1; A30 m × 0.320 mm × 0.25 μm). Cells of strain MBLB1234^T and related taxa were prepared by cultivation on TSA at 37 °C for 2 days, and cell pellets were saponified, methylated, and extracted to analyze the fatty acid profiles using the Sherlock MIS Software version 6.2 based on the TSBA6 database [32]. Polar lipids of strain MBLB1234^T and the reference strain *Paenibacillus motobuensis* JCM 12774^T were extracted according to the protocols of Minnikin et al. [33]. Two-dimensional thin-layer chromatography of polar lipids was analyzed on silica gel 60 F254 (10 × 10 cm; Merck) by spraying with proper reagents [33, 34]. To investigate the isoprenoid quinone, strain MBLB1234^T and related taxa were cultivated on TSA at 37 °C for 2 days. Freeze-dried cells were used to extract the isoprenoid quinone according to the method described by Collins and Jones [35] and identified using an HPLC system (YL9100; Younglin).

Results and Discussion

Genotypic Characteristics and Phylogenetic and Genomic Analyses

From the primary identification of the 16S rRNA gene sequence, one isolate designated strain MBLB1234^T was selected as the primary candidate for novel species within the genus *Paenibacillus*. After sequencing the whole genome of strain MBLB1234^T, the genome sequence was deposited in GenBank/EMBL/DDBJ under accession number CP034346. The genome size of strain MBLB1234^T was 6,497,679 bp. Base on the RAST, RNAmmer 1.2, and tRNA scan-SE sever, 135 RNAs were predicted with distribution of 10 5S rRNAs, 10 16S rRNAs, 10 23S rRNAs, and 105 tRNA. The genomic DNA G + C content was 46.4 mol % which is similar to values previously reported for the genus *Paenibacillus* (39–59 mol%) [6]. According to the eggNOG, 5233 protein-coding genes were predicted (Supplementary Table S1). Of them, functional unknown genes occupied the most as 24.31%. The next most predicted genes were related with carbohydrate transport metabolism (9.52%) and transcription (9.50%). From the BPGA, numbers of core, accessory, unique, and exclusively absent genes were expected to 1140, 2833, 1239, and 13, respectively

(Supplementary Table S2). Length of the predicted 10 16S rRNA gene sequences was 1555 bp, and similarities of between them were in the range of 99.4 to 99.9% (Fig. 1). Strain MBLB1234^T shared the highest sequence similarity with *P. motobuensis* JCM 12774^T (98.3–98.5%), followed by *P. aceti* JCM 31170^T (97.2–97.4%). The pairwise sequence similarities to another valid published species of the genus *Paenibacillus* including *Paenibacillus chibensis* JCM 9905^T and *Paenibacillus anaericanus* MH21^T were less than 96.1%. Strain MBLB1234^T clustered with *P. aceti* L14^T and *P. motobuensis* MC 10^T with a high bootstrap value in the phylogenetic tree analysis using the ML, MP, and NJ algorithms, while it formed a separate lineage remote from *P. polymyxa* ATCC 842^T as type species of the genus *Paenibacillus* (Fig. 1). The DDH values of strain MBLB1234^T with *P. motobuensis* JCM 12774^T and *P. aceti* JCM 31170^T were 34% and 38%, respectively. According to current prokaryotic systematics defining DDH values of < 70% as indicative of a distinct species [36], the determined DDH values indicated that the strain MBLB1234^T could be considered a new species within the genus *Paenibacillus*. Since the genome of *P. motobuensis* JCM 12774^T was not available, other related genomes including *P. aceti* L14, *P. glucanolyticus* 5162, *P. pini* JCM 16418, *P. solani* FJAT-22460, and *P. terrae* HPL-003, were obtained from the GenBank and then employed to analyze genomic DNA–DNA relatedness. ANI value between strain MBLB1234^T and *P. aceti* L14 was 82.29%, while ANI values with other reference strains ranged from 69.24 to 70.02% (data not shown). The ANI value was therefore far below the generally accepted rule in delineation of prokaryotic novel species [37]. Thus, strain MBLB1234^T was confirmed to represent a novel species in the genus *Paenibacillus*.

Phenotype and Biochemical Characteristics and In Silico Phenotypic Analysis

Strain MBLB1234^T optimally grew at 37 °C and pH 7.0–8.0 and with 0.5% (w/v) NaCl, and was sensitive to ampicillin, carbenicillin, cephalothin, ciprofloxacin, gentamicin, kanamycin, lincomycin, neomycin, norfloxacin, novobiocin, polymyxin B, streptomycin, and tetracycline, but resistant to erythromycin, and penicillin G. Colonies of strain MBLB1234^T were observed to be circular, flat, and opaque

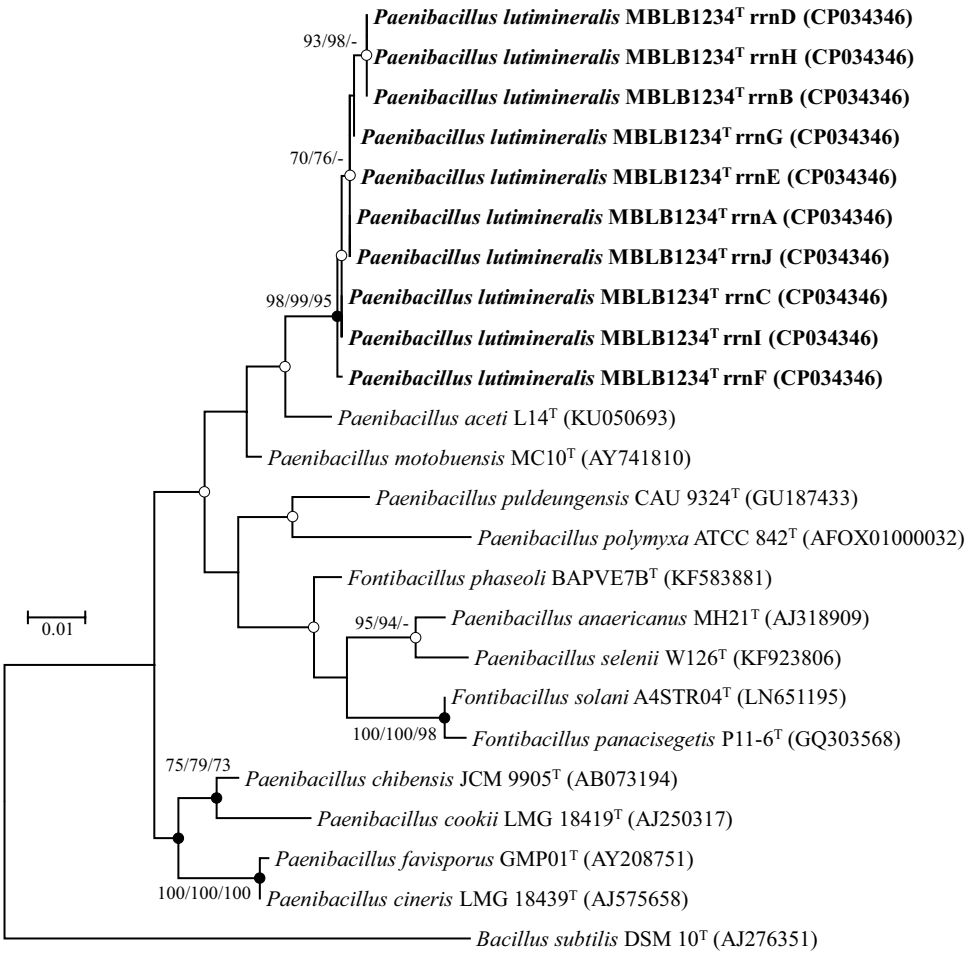

Fig. 1 Maximum likelihood (ML) phylogenetic tree, based on the 16S rRNA gene sequences, showing the position and relationship between strain MBLB1234^T and related taxa of the genus *Paenibacillus*. Numbers at nodes indicate bootstrap values (> 70%) calculated based on the ML/maximum-parsimony (MP)/neighbor-joining (NJ) algorithms for the branch point based on 1000 replications. Closed circles indicate that the corresponding nodes are also recovered by the MP and NJ. Open circles indicate that the corresponding nodes are also recovered by the MP or NJ. *Bacillus subtilis* DSM 10^T served as an outgroup. Bar, 0.01 substitutions per nucleotide position

white on TSA plates. The cells were Gram-stain-negative, motile by flagella, facultative anaerobic, endospore-forming, and rod-shaped (0.5−0.7 μm in width by 1.5−2.4 μm in length). An ellipsoidal endospore in swollen sporangia formed in the terminal region of the cell (Supplementary Fig. S1). The strain MBLB1234^T and reference strains were positive for H_2S production, but negative for indole production. While strain MBLB1234^T was found to be negative for nitrate reduction and Voges-Proskauer test, *P. motobuensis* JCM 12774^T was positive for these tests. Starch, casein, and L-tyrosine were hydrolyzed, while Tweens 20, 40, and 80 were not hydrolyzed in all strains. Strain MBLB1234^T and reference strains also shared numerous similarities including being positive for activities of esterase (C4) and esterase lipase (C8) and negative for lipase (C14) activity. However, some characteristics were found to discriminate strain MBLB1234^T from the reference strains. The detailed physiological and biochemical characteristics of strain MBLB1234^T are presented in Supplementary Table S3, and the species description and are compared to those of closely related *Paenibacillus* species in Table 1.

Through In silico phenotypic tests, glycerol dehydrogenase (EC 1.1.16), L-arabinose isomerase (EC 5.3.1.4), ribose ABC transporter system (TC 3.A.1.2.1), xylose isomerase (EC 5.3.1.5), xyloside transporter XynT, galactose/methyl galactoside ABC transporter (EC 3.6.3.17), glucose-6-phosphate 1-dehydrogenase (EC 1.1.1.49), *N*-acetylglucosamine-6-phosphate deacetylase (EC 3.5.1.25), cellobiose phosphotransferase system, maltose/maltodextrin ABC transporter, galactose-1-phosphate uridylyltrasnferase (EC 2.7.7.10), sucrose 6-phosphate hydrolase (EC 3.2.1.26), and trehalose phosphorylase (EC 2.4.1.64) were identified from genome of strain MBLB1234^T. Glycerol, L-arabinose, D-ribose, D-xylose, methyl-*β*-D-xyloside, D-galactose, D-glucose, *N*-acetylglucosamine, D-cellobiose, D-maltose, D-lactose, sucrose, and trehalose were utilized as a sole carbon source. Although 4-diphosphocytidly-2-C-metyl-D-erythritol kinase (EC 2.7.1.48), mannose-6-phosphate isomerase (EC 5.3.1.8), *α*-glucoside transporter system, glycogen phosphorylase, and 6-phosphogluconate dehydrogenase were detected from the genome of strain MBLB1234^T, erythritol, D-mannose, methyl-α-D-glucoside, glycogen,and gluconate were not used as a sole carbon source.

Chemotaxonomic Characteristics

The respiratory quinone detected in strain MBLB1234^T was menaquinone-7 (MK-7), which was in accordance with the genus *Paenibacillus* [6]. The predominant fatty acids of strain MBLB1234^T (> 10% of the total fatty acids) were anteiso-$C_{15:0}$ (35.7%), anteiso-$C_{17:0}$ (17.8%), iso-$C_{17:0}$ (14.5%), and $C_{16:0}$ (11.0%). The anteiso-$C_{15:0}$ and anteiso-$C_{17:0}$ as major polar lipids were similar with two reference strains: *P. motobuensis* JCM 12774^T and *P. aceti* JCM 31170^T. However, a few differences between MBLB1234^T and the reference strains were shown, with more $C_{16:0}$ and less iso-$C_{15:0}$ in strain MBLB1234^T (Table 2). The polar lipids of strain MBLB1234^T were identified as diphosphatidylglycerol (DPG), phosphatidylglycerol (PG), phosphatidylethanolamine (PE), phosphatidylmethylethanolamine (PME), one unidentified phospholipid (PL), six unidentified aminophospholipids (APL1-APL6), and one unidentified lipid (L). The reference strain *P. motobuensis* JCM 12774^T showed a similar polar lipid profile to type strain MBLB1234^T, but two additional unidentified aminophospholipids were detected for the strain *P. motobuensis* JCM 12774^T (Supplementary Fig. S2). According to the available polar lipid data, DPG, PG, and PE are known to be the major polar lipids of the genus *Paenibacillus* [38, 39]. In summary, strain MBLB1234^T possessed chemotaxonomic characteristics typical of the genus *Paenibacillus,* and furthermore, some distinctions were also detected between MBLB1234^T and the related type strains.

All data generated by phenotypic, phylogenetic, chemotaxonomic, and genomic analyses suggest that the strain MBLB1234^T isolated from bentonite samples is considered to represent a novel species within the genus *Paenibacillus* for which the name *P. lutimineralis* sp. nov. is proposed.

Description of *Paenibacillus lutimineralis* sp. nov.

Paenibacillus lutimineralis (lu.ti.minera'lis. lŭtum L. n. lutum clay; minerális. L. gen. adj, mineralis of the mineral; N.L. gen. n. *lutimineralis*, of a clay mineral, the source from which the type strain was isolated).

Cells are Gram-stain-negative, facultative anaerobic, motile by flagella, spore-forming, and rod-shaped (0.5−0.7 × 1.5−2.4 μm). Colonies on TSA are nonpigmented, circular, convex, bright, and cream-colored with a diameter of 1.0−1.5 mm. Cell growth can be observed in the presence of 0−5.0% (w/v) NaCl (optimum, 0.5%) at 20−45 °C (optimum, 37 °C) and pH 6.0−10.0 (optimum, 7.0–8.0). Cells are positive for catalase test, H_2S production, and methyl red test, but negative for oxidase, indole production, and Voges−Proskauer test. Reduction of nitrate to nitrite is negative. Cells can hydrolyze casein, L-tyrosine, and starch, but not Tweens 20, 40, and 80, and gelatin does not occur. In API 20NE test, *β*-galactosidase utilization of glucose, mannose, mannitol, *N*-acetyl-glucosamine, maltose, and gluconate is positive. In API ZYM test, results show activities of alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, naphthol-AS-BI-phosphohydrolase, *α*-galactosidase, *β*-galactosidase, *α*-glucosidase, and *β*-glucosidase. In API 50CH assays, results for glycerol, L-arabinose, D-ribose, D-xylose, methyl-β-D-xyloside, D-galactose, D-glucose, D-fructose, *N*-acetylglucosamine,

Table 1 Differential characteristics of strain MBLB1234^T and closely related *Paenibacillus* species

Characteristics	1	2	3
Growth range of temperature (°C)	20–45	20–55	15–50
Growth range of NaCl (%, w/v)	0–5	0–5	0–8
Growth range of pH	6.0–10.0	6.0–8.0	5.0–10
Catalase	+	+	−
Oxidase	−	+	−
Nitrate reduction	−	+	−
Voges–Proskauer test	−	+	−
Enzyme activities of:			
Protease (gelatin)	−	−	+
Valine arylamidase	−	−	+
Cystine arylamidase	−	−	+
Trypsin	−	−	+
α-Chymotrypsin	−	+	+
Acid production from:			
L-Arabinose	+	+	−
D-Ribose	+	−	+
Methyl-β-D-xyloside	+	−	+
D-Mannose	−	−	+
L-Rhamnose	−	+	+
Methyl-β-D-mannoside	−	+	−
D-Raffinose	+	+	−
Glycogen	−	−	+
Xylitol	−	−	+
Utilization of:			
Arabinose	+	+	−
Mannose	−	−	+
Mannitol	+	−	+
Polar lipids	DPG, PG, PE, PME, PL, APL1-APL6, L	DPG, PG, PE, PME, APL1-APL8, L	DPG, PG, PE, PL[b]
Respiratory quinone	MK-7	MK-7	MK-7
gDNA G+C content (mol %)	46.4	47.0[a]	49.9[b]

Strains: 1, strain MBLB1234^T; 2, *Paenibacillus motobuensis* JCM 12774^T; 3, *Paenibacillus aceti* JCM 31170^T

All data are obtained in this study, unless otherwise noted

All strains were Gram-stain-negative. All strains were positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase; hydrolysis of starch, casein, and L-tyrosine; H_2S production; and methyl red reaction. All strains produced acid from glycerol, D-ribose, D-xylose, methyl-β-D-xyloside, D-galactose, D-glucose, D-fructose, *N*-acetylglucosamine, amygdalin, arbutin, esculin, salicin, D-cellobiose, D-maltose, D-lactose, D-melibiose, sucrose, D-trehalose, starch, gentiobiose, and D-turanose. Negative traits of strain MBLB1234^T are represented in Supplementary Table S3

+ positive, − negative

[a]Data from Lida et al. [39]

[b]Data from Li et al. [38]

amygdalin, arbutin, esculin, salicin, D-cellobiose, D-maltose, D-lactose, D-melibiose, sucrose, D-trehalose, D-raffinose, starch, gentiobiose, and D-turanose are positive. The predominant fatty acids are anteiso-$C_{15:0}$, anteiso-$C_{17:0}$, iso-$C_{17:0}$, and $C_{16:0}$. The major respiratory quinone is MK-7. The major polar lipids of strain MBLB1234^T are diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylmethylethanolamine, one unidentified phospholipid, six unidentified aminophospholipids, and one unidentified lipid. The DNA G+C content of the type strain is 46.4 mol%.

The type strain, MBLB1234^T (=JCM 32684^T=KCTC 33978^T), was isolated from bentonite samples collected at Guryong mining area located in Pohang, Republic of Korea.

Table 2 Cellular fatty acid contents (%) of strain MBLB1234^T and closely related *Paenibacillus* species

Fatty acids	1	2	3
Saturated			
$C_{12:0}$	Tr	Tr	Tr
$C_{14:0}$	1.8	1.5	1.1
$C_{16:0}$	11.0	5.8	2.9
Branched-chain fatty acid			
$C_{14:0}$ iso	1.3	1.4	2.1
$C_{15:0}$ iso	8.0	14.9	10.7
$C_{16:0}$ iso	7.5	9.7	16.6
$C_{17:0}$ iso	14.5	14.5	6.0
$C_{13:0}$ anteiso	Tr	ND	ND
$C_{15:0}$ anteiso	35.7	37.8	45.6
$C_{17:0}$ anteiso	17.8	13.1	13.3

Strains 1 MBLB1234^T; 2 *Paenibacillus motobuensis* JCM 12774^T; 3 *Paenibacillus aceti* JCM 31170^T. All data obtained from this study. Strains were grown at 37 °C for 2 days on TSA. Values are percentages of total fatty acids, and only fatty acids representing more than 1% for at least one of the strains are shown Tr, trace amount (< 1%) and ND, not detected

Acknowledgements This research was supported by the Basic Research Project (18-3214) of the Korea Institute of Geoscience and Mineral Resources (KIGAM) funded by the Ministry of Science and ICT. This research was also supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (NRF-2016R1D1A1B03931582), and the World Institute of Kimchi (KE1902-2) funded by the Ministry of Science, ICT & Future Planning.

Compliance with Ethical Standards

Conflict of interest The authors declare that there are no conflicts of interest.

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