



Paenibacillus mellifer sp. nov., isolated from gut of the honey bee *Apis mellifera*

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Received: 22 July 2022 / Revised: 3 August 2022 / Accepted: 8 August 2022 / Published online: 16 August 2022
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Abstract

An isolate, designated MBLB2552^T, was isolated from the gut of the honey bees (*Apis mellifera* L.) and identified as a member of the genus *Paenibacillus* based on the sequences of the 16S rRNA gene sequences. The most closely related species to strain MBLB2552^T were *Paenibacillus timonensis* 2301032^T, *Paenibacillus barengoltzii* NBRC 101215^T, and *Paenibacillus macerans* IAM 12467^T, with similarity values of 98.1, 97.21 and 97.0%, respectively, based on 16S rRNA gene sequences. The genome size and G + C content of MBLB2552^T were 5.2 Mb and 52.4%, respectively. The Ortho average nucleotide identity (OrthoANI) and in silico DNA–DNA hybridization (*is*DDH) values between strain MBLB2552^T and the type strains of the closest species were below the species delineation threshold. Comparative genomic analysis showed that most core POGs of strain MBLB2552^T and other related taxa were related to translation, ribosomal structure and biogenesis (J) and carbohydrate metabolism in the COG category and KEGG pathways, respectively. Strain MBLB2552^T was Gram stain-positive, spore-forming, rod-shaped, facultative anaerobic, motile, and grew at 20–45 °C in 0–2% (w/v) NaCl at pH 6.0–9.0. The major polar lipids identified were diphosphatidylglycerol, phosphatidylglycerol, phosphatidylcholine, phosphatidylserine, unidentified polar lipids, and an unidentified glycolipid. We propose that strain MBLB2552^T represents the type strain of the genus *Paenibacillus* and its name *Paenibacillus mellifer* sp. nov. is proposed. The type of strain was MBLB2552^T (=JCM 35371^T = KCTC 43386^T).

Keywords *Paenibacillus mellifer* · Honey bee · Polyphasic taxonomy · Isolation

Introduction

Honey bees (*Apis mellifera* L.) are the most important insects, both economically and ecologically, to humans, as they are critical pollinating insects globally and honey producers (Batra 1995; Dedej and Delaplane 2003). Therefore, the health of honey bees has become a significant concern in many countries (Goulson et al. 2015). Culture-dependent gut microbial communities in *A. mellifera* (Gilliam et al. 1997; Inglis et al. 1998; Saccà and Lodesani 2020) are complex and not fully understood or even known (Elzeini et al. 2021). Microflora found in the gut are members of: yeasts, Gram-positive bacteria, Gram-negative or gram variable bacteria (Mohr and Tebbe 2006; Elzeini et al. 2021).

The genus *Paenibacillus* was proposed in 1993 after Ash et al. (1993) conducted a broad comparative analysis of the 16S rRNA gene sequences of the genus *Bacillus*. The genus *Paenibacillus* has increased to 277 validly named species and 5 subspecies of *P. polymyxa* as the type species (<http://www.bacterio.net/paenibacillus.html>). Members of

Communicated by Erko Stackebrandt.

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this genus can be widely found in various ecological environments and harbor strains of industrial and agricultural importance relevant to humans, animals, and plants (Grady et al. 2016).

During the isolation and characterization of the gut bacterial community in *Apis mellifera*, 81 isolates were obtained. Among them, strain MBLB2552^T belonging to the genus *Paenibacillus* was identified as a candidate novel strains by comparative 16S rRNA gene sequence analysis. In this study, we report a polyphasic taxonomic description of strain MBLB2552^T isolated from the honey bee gut.

Materials and methods

Isolation of the bacterial strain and culture condition

Healthy nurse honey bees (*A. mellifera* L.) were collected from Incheon National University, Incheon, Republic of Korea in the summer (June 2021) for bacterial isolation. The bee surface was sterilized with 100% ethanol and dissected the whole intestinal tract (esophagus to rectum). The whole intestinal tract of the bees was suspended and diluted in PBS. The suspensions were spread onto tryptic soy agar (TSA, Becton, Dickinson and Company, USA) medium and then incubated at 30 °C for 1 week. Cycloheximide at a concentration of 0.01% (v/v) was added to the media to prevent fungi growth. Colonies with distinct morphological characteristics based on color, shape, size, and rough or smooth surfaces were selected and subsequently re-streaked at least three times on the same type of fresh medium to obtain pure colonies.

Identification, sequence similarity analysis and genomic analysis

After obtaining pure single colonies, genomic DNA from each colony was extracted using the HiYield™ Genomic DNA Mini Kit (RBC, Taiwan) according to the manufacturer's protocol. Universal bacterial primer pairs 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492R (5'-TACGGYTACCTTGTTACGACTT-3') were used to amplify the 16S rRNA gene for bacterial identification (Lane 1991). The PCR products of the amplified 16S rRNA genes were sequenced by Macrogen Co., Ltd and assembled as previously described by Roh et al. (2008) using the SeqMan program (DNA star). The obtained 16S rRNA gene sequence was analyzed using the BLAST program provided by GenBank (<https://www.ncbi.nlm.nih.gov/>) and EzBioCloud server (<http://www.ezbiocloud.net/>) (Yoon et al. 2017) for phylogenetic analysis. A phylogenetic tree was constructed using MEGA 7 (Kumar et al. 2016) after a gap deletion and

multiple alignments of data via the CLUSTAL W program (Thompson et al. 1994). Evolutionary distances were calculated by the Kimura two-parameter model (Kimura 1983). A phylogenetic tree was built using the neighbor-joining (NJ) (Saitou and Nei 1987), maximum-likelihood (ML) (Felsenstein 1981), and maximum-parsimony (MP) (Kluge and Farris 1969) algorithms (each employed 1000 replicates) with the MEGA7 program.

The genome of strain MBLB2552^T was sequenced and assembled using the Illumina SPAdes v. 3.10.1 next-generation sequencing platform and SOAP denovo v. 3.10.1 de novo assemblies. The genome sequence authenticity and the absence of contamination by strain MBLB2552^T were confirmed by the proposed minimal standards for using prokaryotes genome data (Chun et al. 2018). Authenticity was checked by comparing the 16S rRNA gene sequences obtained, one was the conventional Sanger sequencing method as previously mentioned, and the other was whole-genome sequencing. Contamination in the strain MBLB2552^T genome was evaluated using the ContEst16S (<https://www.ezbiocloud.net/tools/contest16s>) (Lee et al. 2017).

Genome annotation, phylogenetic analysis

Clusters of Orthologous Groups (COG) category analysis was conducted using EggNOG v5.0 for homologous gene predicted search (Tatusov et al. 2000; Huerta-Cepas et al. 2015). To perform comparative genomic analysis, the NCBI genome database (<http://www.ncbi.nlm.nih.gov/genome/>) was referred to obtain closely related species of the genus *Paenibacillus* with strain MBLB2552^T. The OrthoANI and *is*DDH values among the strain MBLB2552^T and other species of the genus *Paenibacillus* were measured using OAT software version 0.93.1 (Lee et al. 2015) and Genome-to-Genome Distance Calculator program (GGDC 2.1; <http://ggdc.dsmz.de/distcalc2.php>) (Meier-Kolthoff et al. 2013) with the recommended formula 2.2 based on DNA–DNA hybridization, respectively.

Comparative genomic analysis

Pan-genome analysis was performed using the Bacterial Pan Genome Analysis (BPGA) software (Chaudhari et al. 2016). The genomes of strain MBLB2552^T and related genus *Paenibacillus* strains were categorized as core (conserved for all strains), accessory (more than two species shared but not core), and unique (only strain-specific) genes. Pan-genome function and pathway analyses were assigned based on the COG database (<https://www.ncbi.nlm.nih.gov/research/cog/>) and the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (<https://www.genome.jp/kegg/>) (Kanehisa and Goto 2000), respectively. COG and KEGG pan-genome

orthologous groups (POGs) were clustered using the USE-ARCH algorithm with a 50% sequence identity cut-off value. For sequence alignment of the concatenated core POG sequences, the MAFFT v. 7.427 algorithm was used (Kato et al. 2002) and a phylogenetic tree based on core POG sequences was constructed using the UPGMA method with 1000 bootstrap replications (Sneath and Sokal 1973). To construct a balanced minimum evolution tree with branch support via FASTME 2.1.4 including SPR postprocessing, intergenomic distances were calculated by Type Strain Genome Server (TYGS) (<http://tygs.dsmz.de/>) (Lefort et al. 2015; Meier-Kolthoff and Göker 2019).

Phenotype and biochemical characteristics

The morphology of strain MBLB2552^T was observed by light microscopy (model CX 23; Olympus) and transmission electron microscopy (JEM-101; JEOL). Gram staining was assessed with a BD Gram stain kit according to the manufacturer's protocols. Anaerobic growth was performed using a GasPak™ EZ anaerobe gas generating pouch system with an indicator (BD) on TSA at 30 °C for 3 weeks. To determine the condition for temperature growth, strain MBLB2552^T was incubated in TSB at 4, 10, 15, 20, 25, 30, 35, 37, 40, 45, 50 and 55 °C for 1 week. To measure the growth range of different NaCl concentration, TSB as the basal medium was modified that NaCl in the medium was changed to 0, 0.5% (w/v) and 1.0–5.0% (w/v) at intervals of 0.5%. The growth range in different pH was tested in TSB 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, which was incubated at 30 °C. The growth of each medium was measured based on the optical density every 24 h at 600 nm using a UV-1280 spectrophotometer (Shimadzu, Japan). Catalase and oxidase activity were checked by transferring fresh colonies from TSA to a glass slide and adding 3% H₂O₂ and 1% tetramethyl-*p*-phenylenediamine solution, respectively. Hydrolysis of starch, casein, Tweens 20 and 80, and gelatin was performed as described by Benson (2002). H₂S production was detected by cultivation in TSB broth containing sodium thiosulfate (5 g l⁻¹) with lead acetate paper. Other biochemical characterization of strain MBLB2552^T was experimented using the API 20NE and API 50CHE (bioMérieux, France) strips according to the manufacturer protocols. The susceptibility of the strain MBLB2552^T was checked by the disc diffusion plate method as described by Bauer et al. (1996). Discs were contained with the following antibiotics (µg ml⁻¹ disc unless indicated): ampicillin (10), cephalothin (30), ciprofloxacin (10), erythromycin (25), gentamicin (30), kanamycin (30), lincomycin (15), neomycin (30), norfloxacin (20), novobiocin (10), penicillin G

(20 IU), streptomycin (50), and tetracycline (30). The strain was incubated at 30 °C for one week.

Chemotaxonomic characteristics

Cells of strain MBLB2552^T for chemotaxonomic characterization were prepared by cultivation in TSB at 30 °C for 2 days. Cellular fatty acid and polar lipid profiles of strain MBLB2552^T were analyzed according to the methods described by Miller (1982) and Minnikin et al. (1984). Fatty acid profiles were obtained using the Sherlock MIS Software version 6.2 based on the TSBA6 database (Sasser 1990). Polar lipids of strain MBLB2552^T were analyzed by two-dimensional TLC on silica gel 60 F254 (10×10 cm; Merck) by spraying with the following staining reagents: sulfuric acid–ethanol (1:2, v/v) for total polar lipids, zinzade reagent (5% of ethanolic molybdatophosphoric acid), and ninhydrin for aminolipids (Minnikin et al. 1984; Komagata and Suzuki 1987). Freeze-dry cells of the strain MBLB2552^T were used to investigate isoprenoid quinone according to Collins and Jones method (1981) and identified using an HPLC system (YL9100; Younglin). The diamino acid type in the cell wall analysis was determined according to the protocols reported by Stanek and Roberts (1974).

Results and discussion

Whole-genome sequencing and phylogenetic analysis

The full-length 16S rRNA gene sequence (1483 bp) was obtained by conventional Sanger sequencing, as described above for the phylogenetic analysis which revealed that the isolated strain MBLB2552^T belongs to the genus *Paenibacillus*. Similarities in the 16S rRNA gene sequences between strains MBLB2552^T and *P. timonensis* 2301032^T, *P. barengoltzii* NBRC 101215^T, *P. macerans* IAM 12467^T, *P. phoenicis* 3PO2SA^T, *P. faecis* CIP 101062^T were 98.17, 97.21, 97.0, 96.96, and 96.96%, respectively. Similarities with another valid published species of the genus *Paenibacillus* were less than 96.8%. In the phylogenetic tree, strain MBLB2552^T was grouped with *P. timonensis* 2301032^T and the topologies of the phylogenetic trees built using NJ and MP algorithms supported the finding that strain MBLB2552^T formed a stable cluster with *P. timonensis* 2301032^T (Fig. 1). The genome sequences of *P. timonensis* 2301032^T (GCA009725205), *P. barengoltzii* NBRC 101215^T (GCA004000745), *P. macerans* IAM 12467^T (GCA004000965), *P. faecis* CIP 101062^T (GCA008084145), *P. physcomitrellae* XB^T (GCA014640575), and *P. polymyxa* G02^T (GCA900454525) were chosen for a more detailed comparison of genomic properties.

Fig. 1 Maximum-likelihood (ML) phylogenetic tree, based on the 16S rRNA gene sequences, showing the position and relationship between strain MBLB2552^T and related taxa of the genus *Paenibacillus*. Numbers at nodes indicate bootstrap values (> 70%) calculated based on the ML/ neighbor-joining (NJ) / maximum-parsimony (MP) algorithms for the branch point based on 1000 replications. Closed circles indicate that the corresponding nodes are also recovered by the NJ and MP. Open circles indicate that the corresponding nodes are also recovered by the NJ or MP. *Bacillus subtilis* DSM10^T served as an outgroup. Bar, 0.01 substitutions per nucleotide position

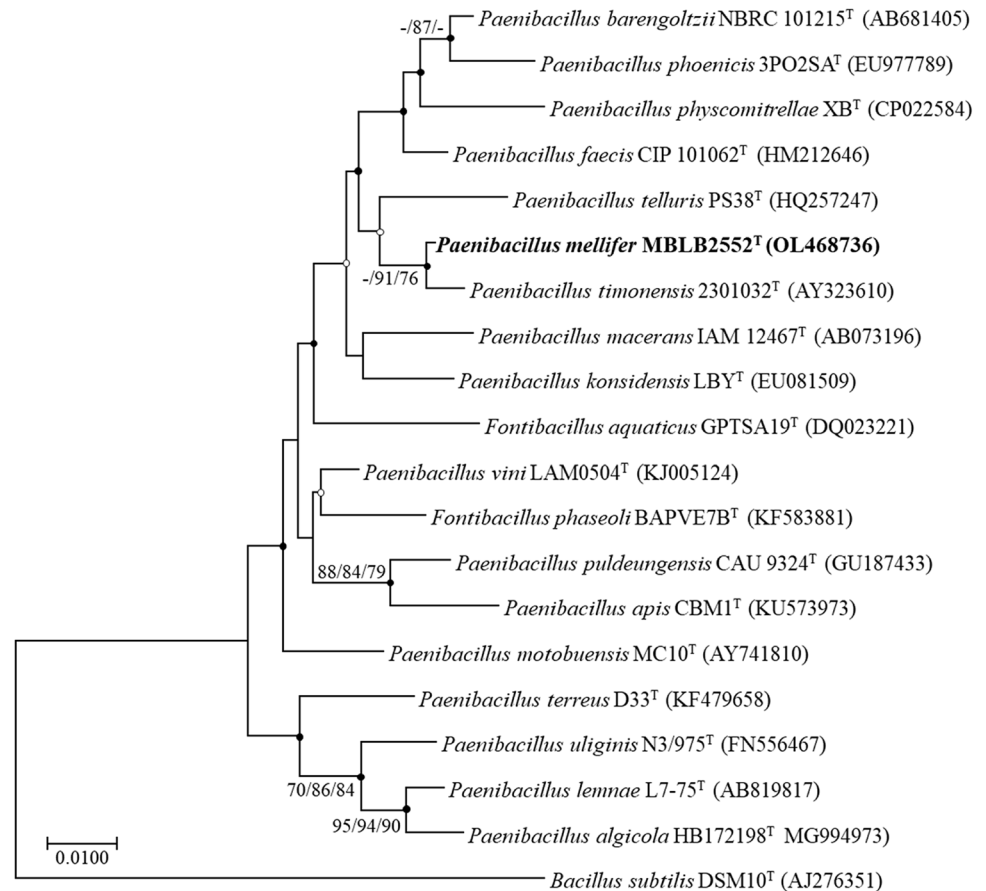


Table 1 General features of the genomes of the strain MBLB2552^T

	Characteristics
Attribute	MBLB2552 ^T
Accession number	JALPRK000000000
Genome size (bp)	5,176,139
Contigs	67
Assembly status	Incomplete
G + C content (%)	52.4
Total genes	4800
Total CDS	4717
- Coding genes	4655
- Pseudo genes	62
RNAs	
- rRNA genes (5S, 16S, 23S)	2, 3, 3
- tRNAs	70
- ncRNAs	5

The genome size was 5,176,139 bp, and the G + C content was 52.4 mol %. Of the 4800 predicted genes, 4655 were coding genes and 62 were pseudogenes (Table 1). The whole-genome sequence of strain MBLB2552^T was deposited into DDBJ/EMBL/GenBank under the accession

number JALPRK000000000. The OrthoANI values between strain MBLB2552^T and other strains in the genus *Paenibacillus* were 89.78% for *P. timonensis* 2301032^T, 82.34% for *P. barengoltzii* NBRC 101215^T, and 76.62% for *P. macerans* IAM 12467^T (Supplementary Fig. S1). The *is*DDH values between strain MBLB2552^T, and *P. timonensis* 2301032^T, *P. polymyxa* G02^T, *P. barengoltzii* NBRC 101215^T, *P. macerans* NBRC 15307^T, *P. physcomitrellae* CGMCC 1.15044^T, and *P. faecis* DSM 23593^T were 39%, 25.3%, 25.0%, 20.9%, 19.9%, and 19.4%, respectively. The values of strain MBLB2552^T were below the thresholds for generally recognized species descriptions (95–96% and 70%, respectively) (Lee et al. 2015; Meier-Kothoff et al. 2013).

Based on COG database analysis, the predicted functional genes of the strain MBLB2552^T mainly belonged to Carbohydrate transport and metabolism (G:487 orthologs), transcription (K:464 orthologs), and carbohydrate transport and metabolism (G:353 orthologs), excluding an unknown functional category (S:946 orthologs) (Supplementary Table S1). According to the KEGG database, most annotated genes of strain MBLB2552^T were predicted to be involved in environmental information processing (453), protein families: signaling and cellular processes (320), and protein families:

genetic information processing (312) (Supplementary Fig. S2).

Based on the pan-genomic analysis, strain MBLB2552^T and other genus *Paenibacillus* species had 13,958 POGs, 1448 core POGs, 3831 accessory POGs, 8122 unique POGs, and 557 POGs that were exclusively absent (Fig. 2, Supplementary Table S2). Most of the core POGs were related to translation, ribosomal structure, and biogenesis (J), distributed by the COG database and related to carbohydrate metabolism, distributed by the KEGG database. According to the KEGG database, the number of unique POGs in strain MBLB2552^T was 232, of which 130 were annotated (Supplementary Table S3). The most annotated unique POGs of strain MBLB2552^T were predicted to be ABC transporter pathways (Supplementary Table S3). Based on the COG database, the number of unique POGs of strain MBLB2552^T was 234, and unique POGs were related to carbohydrate transport and metabolism (G, 35, 14.96%), transcription (K, 29, 12.40%), and signal transduction mechanisms (T, 24, 10.30%). (Supplementary Fig. S3). The concatenated core POG-based phylogenomic tree showed that MBLB2552^T was clustered with the other six *Paenibacillus* strains (Supplementary Fig. S4). To provide more distinctive evidence of the strain within the genus *Paenibacillus*, the genome-based phylogenetic tree using TYGS also showed similar aspects, confirming the relatedness between strain MBLB2552^T and other genus *Paenibacillus* species (Fig. 3). Identification of species and subspecies clustering showed that there were 7 species and subspecies, including strain MBLB2552^T. In fact, the current genomic analysis of strain MBLB2552^T revealed no genomic evidence of its presence in the gut of

honey bee. However, it should be noted that some species of the genus *Paenibacillus* have been reported including *P. larvae* as a representative pathogen of honeybee, and *P. apis* and *P. intestini* isolated from the intestine of *Apis mellifera* (Ebeling et al. 2016; Yun et al. 2017). The further research on metagenomic analysis of the honey bee gut will be needed to track the genomic evidence for the plausible presence of strain MBLB2552^T in the gut of honey bee.

Phenotype, biochemical, and chemotaxonomic characteristics

Strain MBLB2552^T cells were Gram stain positive, motile, spore forming, and rod shaped, 1.0–2.0 μm in width by 5.0–10.7 μm in length (Supplementary Fig. S5). Strain MBLB2552^T was resistant to erythromycin and penicillin G, whereas it was sensitive to ampicillin, cephalothin, ciprofloxacin, gentamicin, kanamycin, lincomycin, neomycin, norfloxacin, novobiocin, polymyxin B, streptomycin, and tetracycline. The detailed results of physiological and biochemical characteristics are provided in Table 2, Supplementary Table S4, and the species description.

The cellular fatty acid profile of strain MBLB2552^T was analyzed to anteiso-C_{15:0} (54.7%), C_{16:0} (11.2%), and iso-C_{16:0} (10.4%) as major fatty acids (> 10%). The major fatty acid of strain MBLB2552^T were similar to those of the type strains of the genus *Paenibacillus* except for minor qualitative and quantitative differences. Compared with the data of other reference strains, iso-C_{15:0} was the major fatty acid of *P. timonensis* 2301032^T, but minor fatty acid (< 10%) of others (Supplementary Table S5).

Fig. 2 Flower plot indicating core and strain-specific POGs of novel strains MBLB2552^T, and six closely related taxa

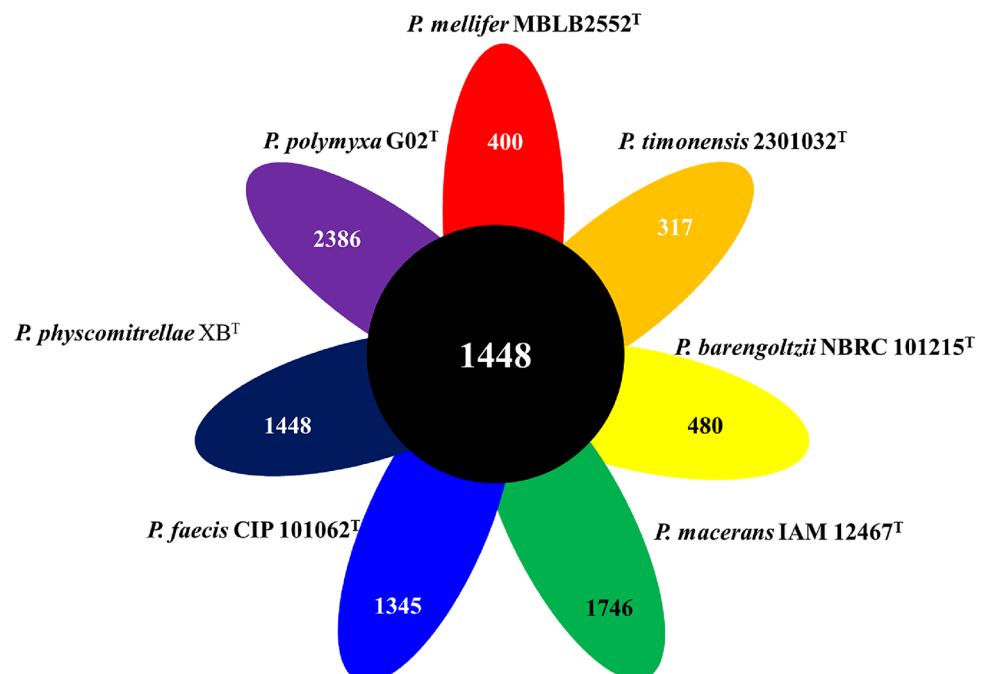
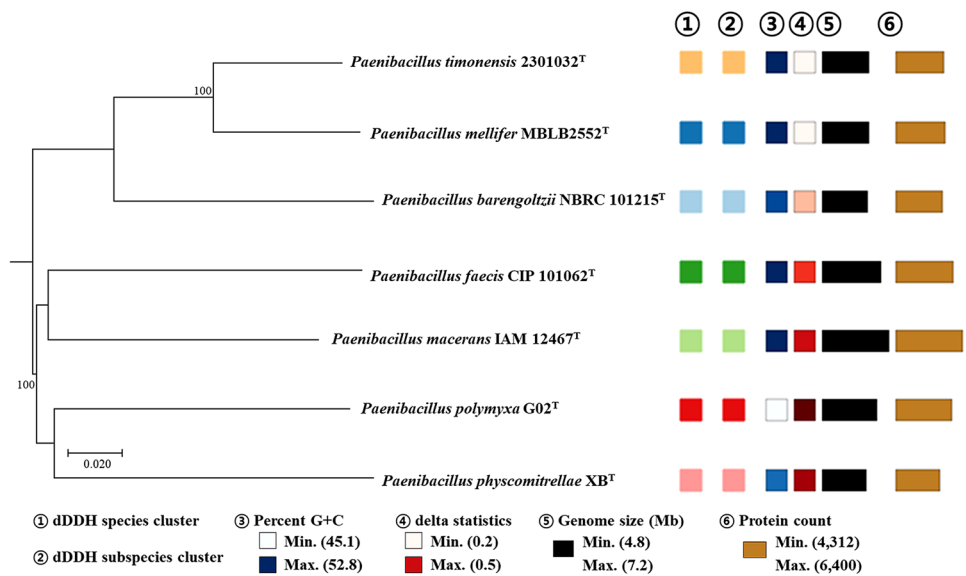


Fig. 3 Phylogenomic tree based on TYGS results showing the relationship between strain MBLB2552^T with related type strains in genus *Paenibacillus*. The whole-genome sequence-based tree was inferred with FastME 2.1.6.1 (Lefort et al. 2015) from GBDP distances. The branch lengths are scaled in terms of GBDP distance formula d5



The major polar lipids in strain MBLB2552^T were identified as diphosphatidylglycerol (DPG), phosphatidylglycerol (PG), phosphatidylcholine (PC), phosphatidylserine (PS), unidentified polar lipids (PL), unidentified glycolipids (GL), and unidentified lipids (L) (Supplementary Fig. S6). According to available polar lipid data, DPG, PG, and phosphatidylethanolamine (PE) are the major polar lipids in the genus *Paenibacillus*. However, the polar lipid profile of isolate MBLB2552^T was distinguished from other related genus *Paenibacillus* strains by differences in the minor proportion of PE (Supplementary data Fig. S6) (Jiang et al. 2015; Li et al. 2016). The major respiratory quinone analyzed was MK-7, which is the major respiratory quinone found in the genus *Paenibacillus* strains (Clermont et al. 2015; Li et al. 2016). The diagnostic diamino acid in the cell wall was meso-diaminopimelic acid. In summary, the combination of morphological, physiological, chemotaxonomic, phylogenetic, and genomic characteristics was typical of the genus *Paenibacillus* with a slight difference, which indicates that MBLB2552^T belongs to the genus *Paenibacillus*. Therefore, we conclude that strain MBLB2552^T represents a novel genus *Paenibacillus* species, for which the name *Paenibacillus mellifer* sp. nov. is proposed.

Description of *Paenibacillus mellifer* sp. nov.

Paenibacillus mellifer (*mel'li.fer*. L. masc. adj. *mellifer*, honey-producing, referring to the origin from the stomach and digestive tract of honey bees *Apis mellifera*).

Cells are Gram-positive, facultative anaerobic, motile, spore-forming, rod-shaped (1.0–2.0 × 5.0–10.7 μm)

bacteria. Colonies on TSA are non-pigmented, circular, convex, bright and cream colored. Grows at 20–45 °C, in 0–2.0% (w/v) NaCl, and at pH 6.0–9.0, with optimal growth at 30 °C, pH 8.0, and 0.5% (w/v) NaCl. Positive for catalase and oxidase. Hydrolysis of casein and starch are positive, but Tweens 20, and 80 and gelatin are negative. H₂S production is positive. Using API 20NE kit, activity of nitrate reduction, β-glucosidase, β-galactosidase, utilization of glucose, arabinose, mannose, mannitol, *N*-acetyl-glucosamine, maltose and gluconate is positive. The API 50CH test shows positive for the utilization of D-arabinose, L-arabinose, D-xylose, D-galactose, D-glucose, D-fructose, D-mannose, L-rhamnose, D-mannitol, methyl-α-D-glucoside, *N*-acetylglucosamine, amygdalin, arbutin, esculin, salicin, D-cellobiose, D-maltose, D-lactose, D-melibiose, sucrose, D-trehalose, inulin, D-raffinose, starch, gentiobiose, D-turanose, D-lyxose, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol, and gluconate. The predominant fatty acid components are anteiso-C_{15:0}, C_{16:0}, and iso-C_{16:0}. The major polar lipids are diphosphatidylglycerol (DPG), phosphatidylglycerol (PG), phosphatidylcholine (PC), phosphatidylserine (PS), unidentified polar lipids (PL), unidentified glycolipid (GL), and unidentified lipids (L). The diagnostic diamino acid in the cell wall was meso-diaminopimelic acid. The MK-7 is the predominant menaquinone. The DNA G + C content of the type strain is 52.4 mol%.

The type strain is MBLB2552^T (=JCM 35371^T =KCTC 43386^T), isolated from the honey bee gut. The NCBI GenBank accession numbers for the 16S rRNA gene

Table 2 Characteristics that differentiate *Paenibacillus mellifer* sp. nov. from closely related *Paenibacillus* species

Characteristics	1	2	3	4	5	6	7
Range of growth temperature (°C)	20–45	30–50	10–50	30–50	20–50	25–32	10–40
Range of growth NaCl concentration (% w/v)	0–2	<5	<5	<5	<4	<4	<4
Range of growth pH	6.0–9.0	ND	4.5–9.0	5.0–9.0	6.0–9.0	5.0–9.0	5.0–9.0
Catalase	+	+	+	+	+	–	+
Oxidase	+	+	+	+	+	+	–
Hydrolysis of							
Casein	+	ND	–	–	ND	–	+
Tweens 80	–	+	ND	ND	–	–	–
Gelatin	–	–	–	–	–	+	+
H ₂ S production	+	–	–	–	ND	–	ND
Reduction of nitrate to nitrite	+	w	+	+	+	–	+
Glucose fermentation	–	+	–	–	–	–	–
Arginine dihydrolyase	–	–	–	–	–	–	+
Urease	–	+	+	+	–	–	–
β-glucosidase (esculin)	+	+	+	–	–	+	+
Acid production from							
Glycerol	–	–	–	+	w	–	–
D-Arabinose	w	–	–	+	–	–	–
L-Arabinose	+	+	–	+	+	+	+
D-Ribose	–	–	–	+	+	+	+
Methyl-β-D-xyloside	–	–	–	+	+	–	+
D-Glucose	+	+	–	+	+	+	+
D-Mannose	+	–	–	+	+	+	+
L-Sorbose	–	–	–	–	+	–	–
L-Rhamnose	w	–	–	+	+	–	–
D-Mannitol	+	–	+	+	+	+	+
Methyl-α-D-mannoside	–	–	–	+	–	–	–
Methyl-α-D-glucoside	+	+	+	+	–	+	+
N-acetylglucosamine	+	+	+	–	+	+	–
Arbutin	+	+	–	+	+	+	+
Salicin	+	+	–	+	+	+	+
D-Maltose	+	+	+	+	+	–	+
D-Melibiose	+	+	+	+	+	+	–
Sucrose	+	–	+	+	+	+	–
D-Trehalose	+	–	+	+	+	+	+
Inulin	+	–	+	+	–	–	v
D-Melezitose	–	–	–	+	–	+	v
D-Raffinose	+	–	–	+	+	+	+
Starch	+	+	+	+	+	–	+
Glycogen	–	+	+	+	+	–	+
Gentiobiose	+	+	–	+	+	+	v
D-Turanose	–	–	–	+	+	+	+
L-Fucose	+	–	–	–	–	–	–
D-Arabitol	–	–	–	+	–	–	–
Gluconate	+	+	+	+	+	+	v
Utilization of							
Glucose	+	+	–	+	+	+	+
Arabinose	+	+	+	+	+	+	–
Mannose	+	+	–	+	+	+	+
Mannitol	+	–	+	+	+	+	+

Table 2 (continued)

Characteristics	1	2	3	4	5	6	7
<i>N</i> -acetyl-glucosamine	+	+	+	–	+	+	–
Maltose	+	+	–	+	+	–	+
Genomic DNA G + C content (mol%)	52.4	52.6	51.6	52.6	52.8	50.5	45.5

+ positive, – negative

Taxa: strains: 1, *Paenibacillus melifer* sp. nov. MBLB2552^T (data were obtained from this study); 2, *P. timonensis* 2301032^T (Roux and Raoult 2004; Clermont et al. 2015); 3, *P. barengoltzii* NBRC 101215^T (Osman et al. 2006; Clermont et al. 2015); 4, *P. macerans* IAM 12467^T (Zhou et al. 2012; Clermont et al. 2015); 5, *P. faecis* 656.84^T (Clermont et al. 2015); 6, *P. physcomitrellae* XB^T (Zhou et al. 2015); 7, *P. polomyxa* CIP 66.22^T (Clermont et al. 2015; Kwak et al. 2020). All strains were positive for β-galactosidase, hydrolysis starch, utilization of amygdalin, D-cellobiose, esculin, D-fructose, D-galactose, D-lactose, D-xylose, and gluconate. All species were found negative for indole production, utilization of adipate, D-adonitol, L-arabitol, caprate, citrate, dulcitol, erythritol, D-fucose, inositol, 2-ketogluconate, 5-ketogluconate, D-lyxose, malate, D-sorbitol, D-tagatose, phenyl-acetate, L-xylose, and xylitol

sequence and complete genome are OL468736 and JAL-PRK000000000, respectively.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s00203-022-03178-0>.

Author contributions ESC and CYH performed all the experiments, analyzed the data, and drafted the manuscript. HWK acquired funding. MJS designed all the experiments and supervised the manuscript.

Funding This work was carried out with the support of Cooperative Research Program for Agriculture Science and Technology Development (Project No. PJ015755022022). This work was also supported by Research Assistance Program (2021) in the Incheon National University.

Declarations

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

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