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ORIGINAL PAPER

Paenibacillus hamazuiensis sp. nov., a bacterium isolated from Hamazui hot spring in Yunnan province, south-west China

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Abstract

A bacterial strain, Gram-positive, aerobic, rod-shaped, motile, designated YIM B00624^T which was isolated from a Hamazui hot spring in Tengchong, Yunnan province, south-west China. The strain grew well on International Streptomyces Project (ISP) 2 medium and colonies were creamy yellow, flat and circular. The results of 16S rRNA gene sequence similarity analysis showed that strain YIM B00624^T was closely related to the type strain of *Paenibacillus filicis* S4^T (95.9%). The main menaquinone of strain YIM B00624^T was menaquinone-7 (MK-7) and major fatty acids were anteiso-$C_{15:0}$, anteiso-$C_{17:0}$ and $C_{16:0}$. The isolate contained *meso*-diaminopimelic acid as the diagnostic diamino acid and the major polar lipids were diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylmonomethylethanolamine and four unidentified glycolipids. The DNA G+C content of strain YIM B00624^T was 53.4 mol%. Based on physiological, phenotypic and chemotaxonomic data, strain YIM B00624^T belongs to a novel species of the genus *Paenibacillus*, for which the name *Paenibacillus hamazuiensis* sp. nov. is proposed. The type strain is YIM B00624^T (=CGMCC 1.19245^T=KCTC 43365^T).

Keywords *Paenibacillus hamazuiensis* · Hot spring · Polyphasic taxonomy

Abbreviations

MK Menaquinone
ISP 2 Yeast extract–malt extract agar

Introduction

On the basis of the analysis of the 16S rRNA gene sequences, the genus *Paenibacillus* was proposed by Ash et al. (1993), and the description was later amended by Shida et al. (1997). At the time of writing, the genus comprises 277

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species with validly published names (https://lpsn.dsmz.de/genus/paenibacillus). Strains of the genus have MK-7 as the predominant quinone and anteiso-$C_{15:0}$ as the major fatty acid. Bacteria belonging to the genus *Paenibacillus* have been isolated from a variety of sources that were relevant to our humans, animals, plants and environment (Yoon et al. 2005; Hwang and Ghim 2017; Simon et al. 2017; Yun et al. 2017; Yang et al. 2021). Many *Paenibacillus* species have specific functions, such as promoting crop growth, producing antimicrobial compounds which can be used as medicine or pesticides, yielding enzymes including carboxylesterases, lipases, pectinases, cellulases, etc., which could be utilized for bioremediation, detergents, biofuel or healthcare (Grady et al. 2016). Thermotolerant enzymes are widely used in industry or academic studies due to their better stable characteristics under high-temperature processes, and hot springs are excellent sources for screening thermostable enzymes or microorganisms. In this study, a bacterial strain YIM B00624^T isolated from a hot spring which represents a novel *Paenibacillus* species.

Materials and methods

Bacterial isolation and growth conditions

Strain YIM B00624^T was isolated from the Tengchong Hamazui hot spring using T3 medium after 14 days of incubation at 45 ℃. The components of the medium include 1 g microcrystalline cellulose, 1 g casein acid hydrolyzed, 0.5 g $CaCO_3$, 1 g $FeSO_4$, 20 g agar, 1 L H_2O, the pH is set to 6. The strain was maintained on ISP 2 agar at 4 ℃ and at 20% glycerol (w/v) at −80 ℃. Biomass for molecular and chemical research is on ISP2 without agar (pH 6.0) at 50 ℃ and 180 r.p.m. for 1 week.

Phylogenetic analysis

The extraction of genomic DNA, PCR amplification and sequencing of the 16S rRNA gene were carried out as described by Yang et al. (2021). Multiple alignments of the most closely related bacterial sequences were followed out using the CLUSTAL X 1.8 program (Thompson et al. 1997). Using Molecular Evolutionary Genetics Analysis 7.0 (Kumar et al. 2016), the phylogenetic tree was reconstructed by the neighbour-joining (Saitou and Nei 1987), maximum parsimony (Fitch 1971) and maximum-likelihood (Felsenstein 1981) algorithms. The stability of strain relationships was evaluated by bootstrap analysis of 1000 resamplings (Felsenstein 1985). Sequence similarity values between the most closely related strains were determined using the EzBioCloud 16S rRNA gene sequence database (https://www.ezbiocloud.net/; Yoon et al. 2017). The whole genome of strain YIM B00624^T was sequenced using PacBio and Illumina Hiseq 2000 sequencers at Shanghai Majorbio Bio-pharm Technology Co., Ltd (Shanghai, China). Using SOAPdenovo software version 2.04 to assemble the sequenced reads (Li et al. 2010). The DNA G+C mol% value was obtained from the genomic sequences.

Physiology and morphology

Light microscopy (BX41, Olympus) and transmission electron microscopy (JEM-2100, JEOL) were used to observe the morphology, motility and size of cell, and the cells were grown exponentially. Gram staining was confirmed by KOH lysis test using standard Gram reaction (Cerny 1978). The temperature range of bacterial growth was determined by culture at 4, 10, 15, 20, 25, 30, 37, 40, 45, 50, 55 and 60 ℃ on ISP 2 for 1 week. The pH range for growth was determined by culture in ISP 2 without agar adjusted to pH 4.0–11.0 (at 1.0 intervals using the buffer system described by Xu et al. 2005). NaCl concentrations (0–10%, w/v, at 0.5% intervals) for growth of the strain were measured over a week. Oxidase activity was determined by dimethyl-*p*-phenylenediamine hydrochloride. Catalase activity was determined by titrating H_2O_2 solution to produce bubbles. Urease, lipase, gelatinase activities and H_2S and melanin production were tested as described by Dong and Cai (2001). Substrate utilization and chemical sensitivity were determined using BIOLOG GEN III MicroPlate. The other enzyme activities and acid production of carbohydrates were tested using the API ZYM, API 20NE and API 50CH systems (bioMérieux) according to the manufacturer's instructions.

Chemotaxonomic characteristics

Amino acids in cell walls and sugars in whole cell hydrolysates were analyzed according to the procedures described by Hasegawa et al. (1983); Lechevalier and Lechevalier (1970) and Tang et al. (2009). The extraction of polar lipids was determined by two-dimensional TLC and identified using previously described procedures (Collins and Jones 1980; Minnikin et al. 1979). Menaquinones were extracted according to Collins et al. (1977) and separated by HPLC (Tamaoka et al. 1983). The Sherlock Microbial Identification System (MIDI) was used to extract, methylate and analyze cellular fatty acids according to the manufacturer's instructions. For fatty acid analysis, strain YIM B00624^T was cultured at 45 ℃ on tryptic soy agar (Difco) for 4 days. The Microbial Identification software package (Sherlock Version 6.1; MIDI databaseTSBA6) was used to analyze fatty acid methyl esters (Sasser 1990).

Results and discussion

Phylogenetic analysis

An almost complete 16S rRNA gene sequence (1552 bp) of strain YIM B00624^T was obtained. According to the comparative analysis of gene sequences, strain YIM B00624^T was most similar to *Paenibacillus filicis* S4^T (95.9%). These values were below the 98.7% cutoff point recommended for recognition of genomic species which need not to calculate overall genome related index (OGRI) (Stackebrandt and Ebers 2006; Kim et al. 2014). Based on the 16S rRNA gene sequences, the phylogenetic tree of strain YIM B00624^T was constructed by clustering neighbour-joining algorithms, maximum-parsimony algorithms and maximum-likelihood algorithms at values (Fig. 1; Fig. S1, S2). The phylogeomic tree also indicated that strain YIM B00624^T formed a stable phylogenetic lineage within the genus *Paenibacillus* (Supplementary Fig. S3). The whole genome of strain YIM B00624^T contained 2 contigs, with a total length of 8,795,693 bp and the N50 length of 8,770,504 bp. Some other genome characteristics of strain YIM B00624^T and other reference genomes are summarized in Table S1. Based on the preliminary analysis of the genome sequence of YIM B00624^T, the peripheral pathways for metabolism of aromatic compounds such as biphenyl and benzoate using acetaldehyde dehydrogenase (EC 1.2.1.10), 4-hydroxy-2-oxovalerate aldolase (EC 4.1.3.39), etc., were found. Esterases including carboxylesterases and lipases are usually used as biocatalysts in a variety of industrial processes, including biochemical, food, pharmaceutical, and biological purposes

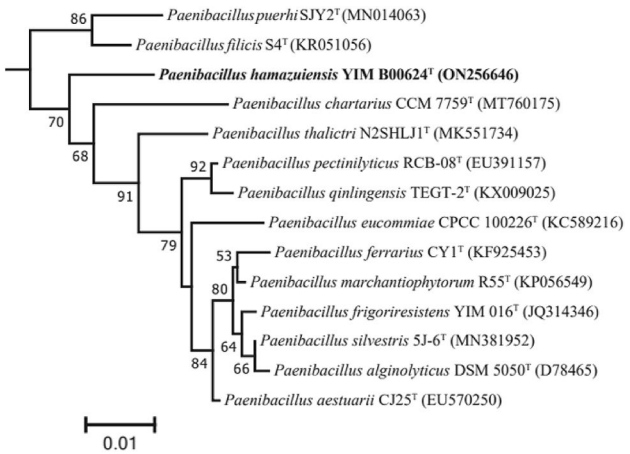

Fig. 1 Neighbour-joining tree based on 16S rRNA gene sequences showing the relationships between strain YIM B00624^T and other member of the genus *Paenibacillus*. Bootstrap values (> 50%) based on 1000 replicates are indicated at the branch nodes. *Lactobacillus delbrueckii* subsp. *sunkii* YIT 11221^T was used as an outgroup. Bar, 0.01 changes per nucleotide position

(Nakamura et al. 2018). Three putative acetyl xylan esterases are acquired in the genome sequence of YIM B00624^T and detailed characterizations will be proceeded in the future work. The DNA G+C content of strain YIM B00624^T was 53.4 mol%, which comes from the genomic sequences. Analysis of these data showed that strain YIM B00624^T was a new species of the genus *Paenibacillus*.

Physiology and morphology

Strain YIM B00624^T was Gram-positive, aerobic and motile. The shape of the cells was rod-shaped with flagella and the endospores located terminally within a swollen sporangium (Fig. S4). Colonies were creamy yellow, flat and circular. Strain YIM B00624^T was positive for catalase, oxidase, nitrate reduction, urease and H_2S production, but negative for gelatin liquefaction. Tweens 20, 40, 60 and 80 are hydrolyzed. Other physiological characteristics of the strain are summarized in Table 1, Table S2 and in the species description.

Chemotaxonomic characteristics

The predominant menaquinone of strain YIM B00624^T was MK-7. Cell wall amino acids of the strain contained *meso*-diaminopimelic acid as the diamino acid. Galactose (34.4%), glucose (23.8%), mannose (11.3%) and rhamnose (10.7%) as the major sugars, while ribose (7.9%), xylose (2.2%), arabinose (0.5%), as minor whole-cell sugar. The polar lipids of strain YIM B00624^T were diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylmonomethylethanolamine and four unidentified glycolipids (Fig. S5). The strain was found to contain anteiso-$C_{15:0}$ (49.2%), anteiso-$C_{17:0}$ (15.9%) and $C_{16:0}$ (11.3%) as major fatty acids, iso-$C_{16:0}$ (9.4%), iso-$C_{15:0}$ (5.3%), iso-$C_{17:0}$ (5.0%), $C_{17:0}$ (2.2%) as minor fatty acids (Table S3).

Some chemotaxonomic characteristics, for example, major fatty acids and major menaquinone of strain YIM B00624^T are consistent with other members of the genus *Paenibacillus*. On the basis of the phylogenetic and chemotaxonomic data described here, strain YIM B00624^T should belong to the genus *Paenibacillus*. However, some distinct characteristics were easily distinguished from other strains of the genus *Paenibacillus* (Table 1). In summary, strain YIM B00624^T is considered a novel species of the genus *Paenibacillus*, for which the name *Paenibacillus hamazuiensis* sp. nov. is proposed.

Description of *Paenibacillus hamazuiensis* sp. nov

Paenibacillus hamazuiensis (ha.ma.zui.en'sis. N.L. masc. adj. *hamazuiensis* pertaining to Hamazui Hot Spring).

Table 1 Differential characteristics of strain YIM B00624^T and related *Paenibacillus* species

Characteristic	1	2	3	4
Temperature range for growth (℃)	25–50	15–37	15–45	10–37
NaCl range (%, w/v)	0–2.5	0–3	1–3	0–2
pH range	6.0–8.0	5.5–9.0	5.5–7.5	5.0–8.0
Nitrate reduction	+	−	−	−
Urease	+	−	−	−
Hydrolysis of				
Starch	−	+	+	−
Gelatin	−		+	−
Catalase	+	+		+
Acid production from				
D-rarbinose	−	−	−	+
L-arabinose	+	−	−	+
D-ribose	+	−	NR	+
L-rhamnose	+	−	−	+
Inositol	+	−	−	+
D-mannitol	+	+	−	−
D-celiobiose	+	+	−	+
Sucrose	+	+	−	+
Inulin	+	−	NR	−
D-meleZitose	+	−	NR	−
Xylitol	−	−	NR	w
D-lyxose	−	−	NR	+
D-tagatose	+	−	NR	−
L-fucose	+	−	NR	−
Potassium 5-Ketogluconate	+	−	NR	−
DNA G + C content (mol%)	53.4	53.2	53.9	50.8

Strains: 1, YIM B00624^T (this study); 2, *P. filicis* S4^T (data from Kim et al. 2009); 3, *P. chartarius* CCUG 55240^T (data from Kämpfer et al. 2012); 4, *P. thalictri* N2SHLJ1^T (data from Tuo et al. 2019).

+ positive, − negative, *w* weak, *NR* not reported

A gram-positive, aerobic and motile bacterium. Colonies are creamy yellow, flat and circular. Cells are rods (width of 0.2–1.2 μm and length of 2.0–4.8 μm) and the endospores located terminally within a swollen sporangium. Growth occurs at 25–50 °C (with an optimum at 45 °C), at pH 6.0–8.0 (with an optimum pH range of 6.0) and NaCl tolerance range for growth is 0.0–2.5% (w/v) NaCl. The following substrates are utilized for growth (Biolog GENIII systems): dextrin, D-cellobiose, gentiobiose, sucrose, D-turanose, stachyose, α-D-lactose, D-melibiose, β-methyl-D-glucoside, D-salicin, *N*-acetyl-D-glucosamine, *N*-acetyl-β-D-mannosamine, *N*-acetyl-D-galactosamine, *N*-acetylneuraminic acid, α-D-glucose, D-mannose, D-fructose, D-galactose, 3-methyl glucose, L-fucose, L-rhamnose, inosine, D-sorbitol, D-mannitol, myo-inositol, glycerol, gelatin, glycyl-L-proline, L-alanine, L-arginine, L-aspartic acid, L-glutamic acid, L-histidine, L-pyroglutamic acid, L-serine, pectin, D-galacturonic acid, L-galactonic acid, D-gluconic acid, D-glucuronic acid, glucuronamide, mucic acid, quinic acid, D-saccharic acid, D-lactic acid methyl ester, L-lactic acid, citric acid, α-keto-glutaric acid, D-malic acid, L-malic acid, bromo-succinic acid, tween 40, γ-amino-butryric acid, α-hydroxy-butyric acid, β-hydroxy-D, L butyric acid, acetoacetic acid, propionic acid, acetic acid, formic acid. Nitrate reduction, arginine dihydrate enzyme, urease, hydrolysis of aesculin, ρ-nitro-β-D-methylgalactosidase and the assimilation of glucose, arabinose, mannose, mannitol, maltose, malic acid were positive in tests using API 20NE. The results from API 50CH tests showed that acids were produced from L-arabinose, D-xylose, methyl-β-D-xylopyranoside, D-galactose, D-glucose, fructose, D-mannose, methyl-α-D-glucopyranoside, arbutin, esculin ferric citrate, salicin, D-celiobiose, D-maltose, D-L actose, D-melibiose, sucrose, D-trehalose, D-raffinose, starch, glycogen, gentiobiose, D-turanose and D-tagatose. In the API ZYM system, alkaline phosphatase (weak), esterase (C4), esterase lipase (C8), acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase (weak) and β-glucosidase activities are present. The diagnostic diamino acid of peptidoglycan is *meso*-diaminopimelic acid. Galactose, glucose, mannose and rhamnose are the major whole-cell sugars. The polar lipids consist of diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylmonomethylethanolamine and four unidentified glycolipids. The predominant menaquinone is MK-7. Major fatty acids are anteiso-$C_{15:0}$, anteiso-$C_{17:0}$ and $C_{16:0.}$ The DNA G+C content of the strain is 53.4 mol%.

The type strain, YIM B00624^T (= CGMCC 1.19245^T = KCTC 43365^T), was isolated from a Hamazui hot spring in Tengchong, Yunnan province, south-west China.

The GenBank accession numbers for the 16S rRNA gene sequence and genomic sequence are ON256646 and GCA_023276405.1, respectively.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00203-022-03282-1.

Author contributions JW, E-MZ and S-KT carried out the data analysis and wrote the manuscript. JW, MD and LR performed the experiments. C-PM and Y-QL participated in the data analysis. J-MD and S-KT supervised the project. All authors reviewed and approved the final version of the paper.

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Declarations

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

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