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Paenibacillus sinensis sp. nov., a nitrogen-fixing species isolated from plant rhizospheres

Qin Li · Yashi Li · Xiaomeng Liu · Sanfeng Chen

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Abstract Two strains HN-1^T and 39 were isolated from rhizospheres of different plants grown in different regions of PR China. The two strains exhibited high nitrogenase activities and possessed almost identical 16S rRNA gene sequences. The average nucleotide identity (ANI) and digital DNA–DNA hybridization (dDDH) values between the two strains were 99.9 and 99.8%, respectively, suggesting that they belong to one species. Phylogenetic analysis based on the 16S rRNA gene sequence showed that strains HN-1^T and 39 are the members of the genus *Paenibacillus* and both strains exhibited 99.5% similarity to *Paenibacillus stellifer* DSM 14472^T and the both strains represented a separate lineage from all other *Paenibacillus* species. However, the ANI of type strain HN-1^T with *P. stellifer* DSM 14472^T was 90.69, which was below the recommended threshold value (< 95–96% ANI). The dDDH showed 42.1% relatedness between strain HN-1^T and *P. stellifer* DSM 14472^T, which was lower than the recommended threshold value (dDDH < 70%). The strain HN-1^T contain anteiso-$C_{15:0}$ as major fatty acids and MK-7 as predominant isoprenoid quinone. The major polar lipids were diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, four aminophospholipids and an unidentified glycolipid. Unlike the most closely related *P. stellifer* DSM 14472^T, strain HN-1^T or 39 was positive for catalase reaction. Distinct phenotypic and genomic characterisations from previously described taxa support the classification of strains HN-1^T or 39 as representatives of a novel species of the genus *Paenibacillus*, for which the name *Paenibacillus sinensis* is proposed, with type strains HN-1^T (=CGMCC 1.18902, JCM 34,620), and reference strain 39 (=CGMCC 1.18879, JCM 34,616), respectively.

Keywords Genomic characterisations · Nitrogen-fixing bacteria · *Paenibacillus sinensis* sp. nov. · Rhizosphere of plant

Introduction

The genus *Paenibacillus* was proposed by Ash et al. (1993) and its description was emended by Shida et al. (Shida et al. 1997). Some species of the genus *Bacillus* were transferred to the genus *Paenibacillus* (Ash et al. 1993; Heyndrickx et al. 1996; Shida et al. 1997; Lee et al. 2004; Hu et al. 2010), and further descriptions of novel members increased the number of species of the genus *Paenibacillus*. At the time of writing, the genus

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Q. Li · Y. Li · X. Liu · S. Chen (✉)
State Key Laboratory for Agrobiotechnology, China Agricultural University, Beijing 100193, People's Republic of China
e-mail: chensf@cau.edu.cn

comprises 256 species and four subspecies with validly published names (www.bacterio.net/paenibacillus.html). Members of the genus *Paenibacillus* are rod-shaped, aerobic or facultatively anaerobic, spore-forming bacteria with anteiso-$C_{15:0}$ as the major cellular fatty acid and menaquinone 7 (MK-7) as the major menaquinone, and their DNA G + C contents range from 45 to 54 mol% (Ash et al. 1993; Priest 2009).

Species of the genus *Paenibacillus* have been isolated from diverse environment habitats (https://lpsn.dsmz.de/genus/paenibacillus) and have diverse physiological characteristics. Presently, there are only a few species with nitrogen fixation ability in these bacteria, including *Paenibacillus azotofixans* (Seldin et al. 1984), *Paenibacillus borealis* (Elo et al. 2001), *Paenibacillus brasilensis* (von der Weid et al. 2002), *Paenibacillus graminis* (da Mota et al. 2004), *Paenibacillus massiliensis* (Ding et al. 2005), *Paenibacillus zanthoxyli* (Ma et al. 2007a), *Paenibacillus sabinae* (Ma et al. 2007b), *Paenibacillus forsythiae* (Ma and Chen 2008), *Paenibacillus donghaensis* (Choi et al. 2008), *Paenibacillus sonchi* (Hong et al. 2009), *Paenibacillus riograndensis* (Beneduzi et al. 2010), *Paenibacillus jilunlii* (Jin et al. 2011a), *Paenibacillus sophorae* (Jin et al. 2011b), *Paenibacillus stellifer* (Jin et al. 2011c), *Paenibacillus triticisoli* (Wang et al. 2013a), *Paenibacillus polymyxa* (Wang et al. 2013b), *Paenibacillus beijingensis* (Gao et al. 2012), *Paenibacillus taohuashanense* (Xie et al. 2012), *Paenibacillus brassicae* (Gao et al. 2013), *Paenibacillus bryophyllum* (Liu et al. 2018), *Paenibacillus helianthi* (Ambrosini et al. 2018), *Paenibacillus maysiensis* (Wang et al. 2018), *Paenibacillus rhizophilus* (Ripa et al. 2019), *Paenibacillus durus* (Guella et al. 2019), *Paenibacillus apii* (Tong et al. 2020). Most of N_2-fixating *Paenibacillus* species isolated from plant roots have been shown to play an important role in promoting plant growth by nitrogen fixation, phosphate solubilization, production of plant phytohormones and various enzymes (Xie et al. 2016; Grady et al. 2016; Li et al. 2019). Comparative genomic analysis revealed the conservation of *nif* cluster comprising 9 genes (*nifB nifH nifD nifK nifE nifN nifX hesA nifV*) in nitrogen-fixating *Paenibacillus* strains (Xie et al. 2014). In addition, some N_2-fixing *Paenibacillus* strains with additional *nif* and *nif*-like genes exhibited higher nitrogenase activities (Li et al. 2014, 2021). Such traits make the study of the regulation of the multiple *nif* genes of N_2-fixing *Paenibacillus* under different environmental conditions and their adaptation to varying ecological niches interesting.

In this study, two nitrogen-fixing strains HN-1^T and 39 isolated from the rhizosphere of plant were charactered by a polyphasic taxonomic approach, one more presumably novel species strain belonging to the genus *Paenibacillus*.

Materials and methods

Isolation of the bacterial strains and culture conditions

Strain 39 was isolated from a soil sample collected from arbor rhizosphere in Beijing of China (39°57'N, 116°17'E). 1 g soil sample was suspended in 9 mL sterile water, stirred for 30 min and heated at 80 °C for 15 min. After that, 100 µL suspension was spread on nitrogen-free medium agar plates in triplicate. After incubation at 30 °C for 3 days, single colonies were isolated by streaking plating. The nitrogen-free medium consisted 20 g sucrose, 0.1 g K_2HPO_4, 0.4 g KH_2PO_4, 0.2 g $MgSO_4{\cdot}7H_2O$, 0.1 g NaCl, 0.01 g $FeCl_3$, and 0.002 g Na_2MoO_4 per liter water. The strain HN-1^T was previously isolated from the rhizosphere of rice on nitrogen-free medium agar plates (Liu et al. 2019). Strains were routinely cultured in LD medium (per liter contains 2.5 g NaCl, 5 g yeast, and 10 g tryptone) at 30 °C for further identification and study. The type strain of the genus *Paenibacillus*, *P. polymyxa* DSM 36^T, *P. sabinae* DSM 17841^T, *P. stellifer* DSM 14472^T, *P. zanthoxyli* JH29^T, *P. graminis* RSA19, *P. triticisoli* BJ-18^T and *P. azotofixans* ATCC 35681^T were obtained from our bacterial collection. Bacteria were freeze-dried or frozen using a sterile glycerol solution in cryogenic tubes to preserve the samples (30% v/v), and stored at − 80 °C and − 20 °C.

Nitrogenase activity assay

To determine nitrogenase activity, strains HN-1^T and 39 and reference *Paenibacillus* strains, including *P. polymyxa* DSM 36^T, *P. sabinae* DSM 17841^T, *P. stellifer* DSM 14472^T, *P. zanthoxyli* JH29^T, *P. graminis* RSA19^T, *P. triticisoli* BJ-18^T and *P.*

azotofixans ATCC 35681^T were grown in 20 mL of LB broth medium in 50 mL flasks shaken overnight at 30 °C. The cultures were collected by centrifugation, precipitations were washed three times with sterilized water and then resuspended in nitrogen-limited medium (per liter contains 10.4 g Na_2HPO_4, 3.4 g KH_2PO_4, 26 mg $CaCl_2{\cdot}2H_2O$, 30 mg $MgSO_4$, 0.3 mg $MnSO_4$, 36 mg Ferric citrate, 7.6 mg $Na_2MoO_4{\cdot}2H_2O$, 10 µg p-aminobenzoic acid, 5 µg biotin, 0.3 g glutamate and 4 g glucose). The nitrogenase activity was determined using the acetylene reduction assay and expressed as nmol C_2H_4 mg^{-1} protein h^{-1} as described previously (Wang et al. 2013b).

Genome sequencing and analysis

The whole genomic DNA of the strains HN-1^T and 39 was extracted using the TIANamp Bacteria DNA Kit, evaluated by gel electrophoresis, and estimated using a NanoDrop 2000 (Thermo Scientific, MA, USA). The draft genome sequence was produced by using Illumina paired-end sequencing technology at the mega genomics. Assembly was conducted using SOAP de-novo v. 1.04 assembler (Li et al. 2008). Gene prediction was made using Glimmer v. 3.0 (Delcher et al. 2007). Annotation of protein-coding sequence was performed by using the Basic Local Alignment Search Tool (BLAST) against the COG, Kyoto Encyclopedia of Genes and Genomes (KEGG) databases and NCBI nr protein database.

Average nucleotide identity (ANI) was calculated in EZBioCloud [https://www.ezbiocloud.net/tools/ani] (Yoon et al. 2017a), using the algorithm published by Lee et al. (2016). Digital DNA–DNA hybridization (dDDH) values were computed at GGDC (Genome-to-Genome Distance Calculator) using GGDC 2.0 BLAST + and recommended formula 2 (Meier-Kolthoff et al. 2013).

Phylogenetic analysis of 16S rRNA gene and *gyrB* gene

The 16S rRNA gene sequences of 39 were acquired from a PCR product using highly specific forward primer 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and universal reverse primer 1492R (5′-GGTTACCTTGTTACGACTT-3′). The DNA sequence obtained was compared to reference 16S rRNA gene sequences available in the Genbank database using BLASTN software (Altschul et al. 1990) and the EzBioCloud server (https://www.ezbiocould.net) (Yoon et al. 2017b). The sequences of the *gyrB* gene were obtained from the genome of HN-1^T and 39 and other type strains. Multiple sequence alignments were analysed using CLUSTAL X (Thompson et al. 1997). The phylogenetic tree calculating evolutionary distance matrices was constructed by the maximum likelihood method (Felsenstein 1981) using MEGA (version 7.0) (Kumar et al. 2016). Bootstrap analysis was conducted on 1000 replications (Felsenstein 1985).

Phenotypic characterization

Colony shape and size of strains were observed after 72 h of incubation on LD medium at 30 °C. For endospore staining, the strains grown on LD agar for 2 days at 30 °C, following 7 days at 4 °C was stained using schaeffer-fulton method (Mormak et al. 1985) and visualized by light microscopy. Cell morphology was also obtained by scanning electrical microscopy (SEM), after incubated on endospore-forming medium agar plate [yeast extract 0.07%, tryptone 0.1%, glucose 0.1%, $(NH_4)_2SO_4$, 0.02%, $MgSO_4{\cdot}7H_2O$, 0.02%, K_2HPO_4, 0.1% (w/v), pH 7.2] for 72 h. The flagellation type was determined by transmission electron microscopy (TEM) after 48 h incubation of strain HN-1 on LD medium. Cell motility was evaluated in semi-solid (0.3% agar) LD medium after incubation at 30 °C for 24 h. Physiological and biochemical characteristics were determined in comparison with *P. sabinae* DSM 17841^T and *Paenibacillus stellifer* DSM 14472^T. Most physiological and biochemical tests, including activities of catalase and oxidase, nitrate reduction, hydrolysis of starch, aesculin and tween 20, production of dextrin and indole, methy red reaction, Voges-Proskauer reaction, lysozyme test and production of acid from fermentation of different substrates were performed according to Zhao et al. (2014). Temperature range for growth were determined after incubation at 4, 10, 15, 25, 28, 30, 37, 40 and 45 °C on LD agar. The pH range for growth was determined in LD broth adjusted to pH 4.0–10.0 (using increments of 1.0 pH unit) by using HCl and NaOH buffers. Growth in the absence of NaCl and in the presence of 0, 0.2, 0.5, 1.0, 2.0, 3.0 and 4.0% (w/v) NaCl was investigated by using LD broth. A spectroscopic method of monitoring turbidity at OD_{600} was

used to assess the growth at various temperature, pH values and NaCl concentration. The ability of strains to assimilate different substrates were tested using Biolog GEN III MicroPlate system (Biolog Microstation™, CA, USA) following the manufacturer's instructions.

Chemotaxonomic characterization

Strains were incubated in LD medium at 30 °C for 2 days. The compositions of cellular fatty acid were analyzed according to the method described by Komagata and Suzuki (1987) using Sherlock Identification System (MIDI) (Sasser et al. 2005). Cellular menaquinones and respiratory quinones were extracted, purified, and analyzed by HPLC according to the method described by Collins (1980). Polar lipid was extracted by the method of Minnikin et al. (1979), and was identified by two-dimensional TLC as described by Collins et al. (1980).

Result and discussion

Bacterial isolation and acetylene-reduction assay

The two strains were isolated from rhizospheres of different plants grown in different regions of PR China. The designated type strain HN-1^T was previously isolated from rhizosphere soil of rice collected from Xiangtan City, Hunan Province (Liu et al. 2019); Strain 39 was isolated from rhizosphere soil of arbor collected from Haidian District of Beijing. Since bacteria in the soil sample were cultured in nitrogen-free medium on the purpose of isolating nitrogen-fixing strain, strain 39 is possible to have nitrogen-fixing capability. Strains HN-1^T isolated from the rhizosphere of rice was detected by acetylene reduction to have nitrogen-fixing capacity (Liu et al. 2019). Acetylene reduction assays were performed to verify the nitrogenase activity of HN-1^T and 39. As shown in Table 1, strains HN-1^T and 39 exhibited very high nitrogenase activity compared to other nitrogen-fixing *Paenibacillus* species, suggesting a high efficiency of the nitrogen fixation process.

Phylogenetic analysis of 16S rRNA gene and *gyrB* gene

The almost-complete 16S rRNA gene sequence of strain 39 was obtained and used for initial BLAST searches of the GenBank database. Comparisons of 16S rRNA gene sequences revealed that strain 39 was shown to belong to the genus *Paenibacillus* and share 99.9% 16S rRNA gene sequence identity with strain HN-1^T. These two strains showed highest 16S rRNA gene similarity to *P. stellifer* DSM 14472^T (99.5%), followed by *P. azotofixans* ATCC 35681 T (97.1%) and *P. sabinae* DSM 17841^T (97.0%). According to EzBiocloud database, high level of similarities included 99.5% (*P. stellifer* DSM 14472^T), 97.1% (*P. azotofixans* ATCC 35681^T). Others were below 97%: 96.9% (*P. bryophyllum* L201^T), 96.7% (*P. albidus* Q4-3^T), 96.7% (*P. apii* 7124^T), etc. Phylogenetic trees were inferred using the maximum-likelihood (ML) methods in the software MEGA7. Phylogenetic analysis based on 16S rRNA gene sequences revealed that strains HN-1^T and 39 clustered with species of the genus *Paenibacillus* and formed a monophyletic cluster with *P. stellifer* DSM 14472^T, as the three strains formed a separate phylogenetic branch within the genus *Paenibacillus* with a high bootstrap resampling value of 100% (Fig. 1).

Generally, 98.7% sequence identity on the 16S rRNA gene are considered to be within the same species (Kim et al. 2014). However, several reports have been published showing that *Paenibacillus* species with > 99% 16S rRNA gene sequence similarity may not belong to the same species (Kamfer et al. 2017; Kim and Cha 2018; Ghio et al. 2019; Guella et al. 2019; Velazquez et al. 2020). Thus, housekeeping genes are now routinely used to complement the 16S rRNA gene analysis for species level determination (da Mota et al. 2004; Holmes et al. 2004; Rodriguez et al. 2019). Due to the low level of discrimination based on 16S rRNA gene between closely related species, the *gyrB* gene (coding for the b subunit of DNA gyrase) was used as an alternative phylogenetic marker (Wang et al. 2007). The *gyrB* genes were retrieved from the HN-1^T and 39 genomes. The *gyrB* gene clearly distinguishes HN-1^T and 39 from other *Paenibacillus* species with only 93.04% gene sequence identity to *P. stellifer* DSM 14472^T (Fig. S1). Based on the 95–96% *gyrB* gene sequence similarity as the interspecies gap (Lee et al. 2008; Liu

Table 1 Nitrogenase activity of strains HN-1^T and 39 in comparison with some nitrogen-fixing species of the genus *Paenibacillus*

Strain	Nitrogenase activity [nmol C_2H_4 (mg protein h)$^{-1}$]
P. polymyxa DSM 36^T	1355.1 ± 152.4
P. stellifer DSM 14472^T	6099.5 ± 497.3
P. zanthoxyli JH29^T	6282.4 ± 307.7
P. graminis RSA19^T	4272.9 ± 207.9
P. sabinae DSM 17841^T	7749 ± 371.8
P. azotofixans ATCC 35681^T	5511.5 ± 260.2
P. triticisoli BJ-18^T	743.6 ± 82.9
39	7160.3 ± 584.6
HN-1^T	6937.2 ± 625.1

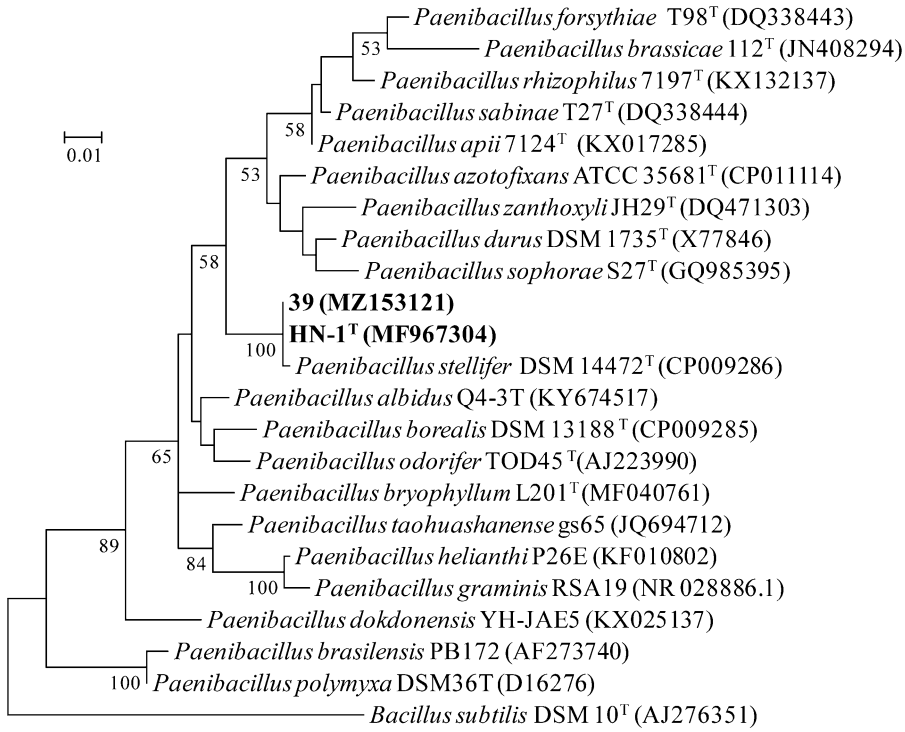

Fig. 1 Maximum-likelihood phylogenetic tree based on 16S rRNA gene sequences showing the position of strains 39 and HN-1^T among species of the genus *Paenibacillus*. Bootstrap analyses were performed with 1000 cycles. Numbers (50%) at nodes are bootstrap values. Bar 0.01 substitutions per nucleotide positions

et al. 2013), strains HN-1^T and 39 could be assigned to novel species.

Genome sequence and similarity analysis

Genome sequencing was performed to evaluate the genomic relatedness of the strains HN-1^T and 39 to its closely related recognized species in the genus *Paenibacillus*. Genomes of strains HN-1^T and 39 were approximately 6.32 and 6.45 Mb, respectively. The DNA G + C content of the strains HN-1^T and 39 were 53.36 and 52.99%, respectively. The total number of protein coding genes in HN-1^T and 39 were 5631 and 5782, respectively. While, the related strain *P. stellifer* DSM 14472^T had a complete genome of 5.66 Mb, comprising 5007 protein coding genes with a DNA G + C content of 53.5%. An overview of the genome sequences of strains HN-1^T and 39 and other genome sequences from related species was given in Table 2. The high-quality draft genomes of

Table 2 Genome characteristics of strains HN-1^T and 39 and the related species in the genus *Paenibacillus*

Species	Strain	Genome size (Mb)	GC%	Protein	Gene	tRNA	rRNA	DDBJ/EMBL/GenBank accession number	ANI value with strain HN-1^T	dDDH value with strain HN-1^T	References
P. sinensis	HN-1^T	6.32	53.36	5646	5843	87	17	JAHCMB000000000	/	/	This study
P. sinensis	39	6.45	52.99	5720	5941	85	54	JAHBAZ000000000	99.93	99.8	This study
P. stellifer	DSM 14472^T	5.66	53.5	5007	5260	87	33	CP009286	90.69	42.1	Suominen et al. (2003))
P. sabinae	DSM 17841^T	5.27	52.6	4702	4903	82	26	CP004078	76.96	22.0	Ma et al. (2007b))
P. *apii*	7124^T	5.40	52.3	4892	5050	68	5	JAAKGU000000000	76.90	22.1	Tong et al. (2020)
P. azotofixans	ATCC 35681^T	5.58	51.0	5064	5310	96	29	CP011114	76.80	22.0	Seldin et al. (1984))
P. *albidus*	Q4-3^T	7.67	49.9	6692	6972	83	10	BMKR00000000	73.31	19.4	(Zhuang et al. (2017))

strains HN-1^T and 39 were deposited in GenBank under accession numbers JAHCMB000000000 and JAHBAZ000000000, respectively.

The average nucleotide identity (ANI) and digital DNA–DNA hybridization (dDDH) values are widely used to define bacterial species (Konstantinidis and Tiedje 2005; Varghese et al. 2015; Chun et al. 2018; Ciufo et al. 2018). The ANI and dDDH value of genomes for strain HN-1^T and strain 39 were 99.8% and 99,93%, respectively, meaning that the two strains belong to one species (Table2). But the ANI values between strain HN-1^T and reference strains *P. stellifer* DSM 14472^T, *P. sabinae* DSM 17841^T, *P. apii* 7124^T and *P. azotofixans* 35681^T were 90.69, 76.96, 76.90 and 76.80%, respectively (Table 2). The dDDH values between strain HN-1^T and the reference strains *P. stellifer* DSM 14472^T, *P. sabinae* DSM 17841^T, *P. apii* 7124^T and *P. azotofixans* 35681^T were 42.1, 22.0, 22.1 and 22.0%. These values are lower than the proposed and accepted species threshold value of 95–96% ANI and 70% dDDH for differentiating bacterial species (Chun et al. 2018; Richter and Rossello´-Mo´ra 2009), suggesting that the new isolate HN-1^T represents a distinctive species.

Analysis of nitrogen fixation and nitrogen metabolism genes

The nitrogen fixation genes of strains HN-1^T and 39 were extracted by using Prokka software from the genome sequences (Seemann 2014). The genome of strains HN-1^T and 39 contain a compact *nif* cluster comprising ten genes *nifB, nifH, nifD, nifK, nifE, nifN, nifX, orf1, hesA* and *nifV* encoding Mo-nitrogenase, which is unique features of the *Paenibacillus* nitrogen fixation system. In addition to the *nif* cluster, the two strains have *anfHDGK* encoding Fe-nitrogenase and linked to additional copies of *nifBENX* genes, while the closely related species *P. stellifer* DSM 14472^T contains *anfHDGK* preceding additional *nifV* gene. Beyond the *nif* and *anf* cluster, there are multiple *nifHDK*-like genes located at different sites in their genomes. The organization of *nif*, *anf* and *nif*-like genes in type strain HN-1^T and the closely related species *P. stellifer* DSM 14472^T was shown in Fig. S2. Previous studies showed that 3 *nifH* genes of *P. sabinae* DSM 17841^T are functional by complementing *K. oxytoca* Δ*nifH* mutant (Hong et al. 2012). Thus,

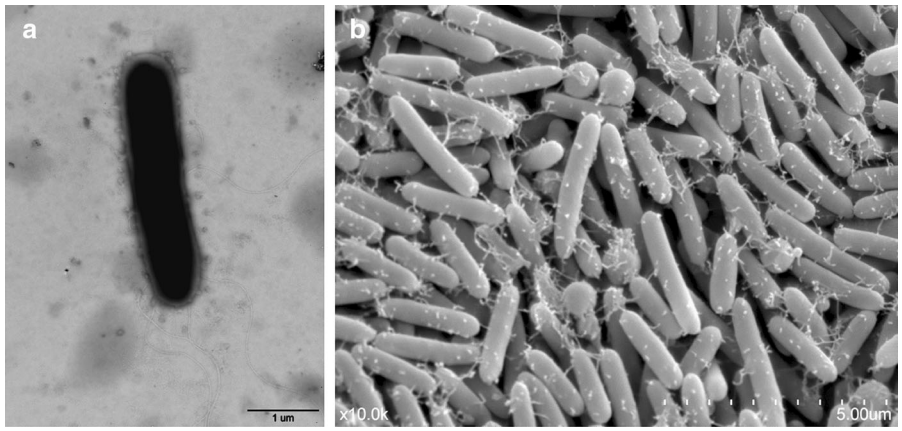

Fig. 2 Morphology of flagella and endospores, (**a**) Transmission electron microscopic image of flagella of strain HN-1^T and (**b**) scanning electron microscopic image of vegetative cells and spores of strain HN-1^T

the high nitrogenase activity exhibited by these strains may be due to their additional *nif* genes.

Paeniacillus azotofixans ATCC 35681^T can fix nitrogen even in the presence of nitrate due to the absence of nitrate reductase (Seldin et al. 1984). Whole genome sequence analysis strains HN-1^T and 39 revealed that nitrate reductase gene cluster *narIJHG* were not detected, which suggested these two strains can also fix nitrogen in the nitrate-enriched medium. The draft genome of strains HN-1^T and 39 harbor two sets of NAD(P)H-nitrite reductases (*nirBD*) which are involved in the reduction of nitrite to ammonium in both assimilatory and dissimilatory reduction processes. Additional searches for genes associated with nitric oxide (*nirS* or *nirK*) and nitrous oxide reduction (*norBC*) were performed, but these genes were not detected in their genomes. Therefore, strains HN-1^T and 39 may possess dissimilatory nitrate reduction to ammonium pathway, but lack denitrification pathway.

Phenotypic characteristics

Strains HN-1^T and 39 were found to be Gram-positive, facultatively anaerobic, motile and rod-shaped. Colonies grown on LD medium after 72 h of incubation at 30 °C were usually 0.8–1.2 mm in diameter, circular, moist, milky and convex (Fig. S3a). Endospores were stained with malachite green and observed under light microscope (Fig. S3b). The transmission electron micrographs of type strain HN-1^T showed the presence of peritrichous flagella on cell surface (Fig. 2a). Strain HN-1^T produced ellipsoidal spores in swollen sporangia in the terminal region of the cell by scanning electron microscope (Fig. 2b).

In order to determine physiological and biochemical characteristics of HN-1^T and 39 in comparison with *P. stellifer* DSM 14472^T and *P. sabinae* DSM 17841^T, a series of tests were carried out following the proposed minimal standards for describing new taxa of facultatively anaerobic, endospore-forming bacteria (Logan et al. 2009). The strains HN-1^T and 39 grew well in up to 4% NaCl (w/v), however, strain *P. stellifer* DSM 14472^T tolerated only 3% NaCl. The pH range for growth was 5.0–9.0 and the temperature range for growth is 15–42 °C. Strains HN-1^T and 39 was determined to be negative for the Voges–Proskauer reaction, and positive for the methyl red reaction. Strains HN-1^T and 39 were positive for catalase reaction and can produce acid from rhamnose and sorbitol, which differentiated HN-1^T and 39 from the most related *P. stellifer* DSM 14472^T. The ability of strains to assimilate different substrates were tested using GEN III microplates by Biolog system (Biolog Microstation TM, CA, USA) (Kiran et al. 2017; Ripa et al. 2019). Strain HN-1^T and *P. stellifer* DSM 14472^T differed in the metabolization of D-Fucose, D-Maltose, 3-Methyl glucose, D-Sorbitol, Stachyose, Citric acid, α-Keto-butyric acid, Mucic acid, Methyl pyruvate, Gelatin, Inosine, D-Glucose-6-PO4, Pectin, Aztreonam, Fusidic acid, Nalidixic acid, Vancomycin, Lithium chloride, Sodium bromate, Sodium lactate 1%, Rifamycin sv and Troleandomycin as a sole carbon source. Strain HN-1^T and 39 exhibited nearly

Table 3 Phenotypic characteristics that differentiate strains HN-1^T and 39 from their closely related species *P. stellifer* DSM 14472^T and *P. sabinae* DSM 17841^T

Characteristic	1	2	3	4
4%NaCl	+	+	−	−
Catalase	+	+	−	+
Voges-Proskauer	−	−	−	+
Nitrate reduction	−	−	−	+
Hydrolysis of Starch	+	+	+	−
Production of Dextrin	+	+	+	−
Production of acid from				
Rhamnose	+	+	−	−
Glycerol	−	−	−	−
Arabinose	+	+	+	−
Mannitol	−	−	−	+
Xylose	+	+	+	−
Sorbitol	+	+	−	+
Oxidation of (Biolog GENIII)				
α-D-Lactose	+	+	+	−
D-Fucose	−	−	w	−
D-Mannitol, D-Gluconic acid, Lincomycin, Minocycline, Troleandomycin	−	−	−	+
D-Melibiose	w	w	+	+
D-Mannose, 3-Methyl glucose, Pectin, Aztreonam, Vancomycin, Lithium chloride, Sodium lactate 1%	+	+	w	+
D-Sorbitol, Nalidixic acid	+	+	−	+
L-Fucose, L-Rhamnose, D-Galacturonic acid, D-Glucuronic acid, L-Galactonic acid lactone, Glucuronamide, D-Fructose-6-PO_4, α-Keto-glutaric acid	w	w	w	−
Stachyose	+	+	w	+
Acetoacetic acid	w	w	w	+
Citric acid, Gelatin, Inosine, D-Glucose-6-PO_4, Mucic acid	w	w	−	−
α-Keto-butyric acid	w	w	−	w
Methyl pyruvate, Fusidic acid	w	w	−	+
Rifamycin sv	−	−	+	+
Sodium bromate	+	+	−	w
Tetrazolium blue	−	−	w	w
Tetrazolium violet	w	w	+	w

Strains: 1. HN-1^T; 2. 39; 3. *P. stellifer* DSM 14472^T; 4, *P. sabinae* DSM 17841^T; +, Positive reaction; −, negative reaction; w, weak reaction

identical phenotypic characteristics, indicating that they belong to one species. Table 3 shows the phenotypic properties that distinguishes the novel strains HN-1^T and 39 from the other *Paenibacillus* species.

Chemotaxonomic characteristics

In order to determine the composition of cellular fatty acid, strains HN-1^T, 39, *P. stellifer* DSM 14472^T and *P. sabinae* DSM 17841^T were incubated in LD medium at 30 °C for 2 days. Whole cell fatty acid analysis revealed that anteiso-$C_{15:0}$, $C_{16:0}$, iso-$C_{14:0}$, iso-$C_{16:0}$ and iso-$C_{15:0}$ are present as major (> 5%) fatty acids, and anteiso-$C_{17:0}$, iso-$C_{17:0}$ and $C_{18:1\omega9c}$ are present as minor (< 5 but > 1%) fatty acids (Table S1). Anteiso-$C_{15:0}$ is the predominant fatty acid of members of the genus *Paenibacillus* (Ash et al. 1993), consistent with strains HN-1^T and 39 being a member of this genus. However, in the closely related type strains *P. stellifer* DSM 14472^T, the fatty acid $C_{16:0}$ was found to be more abundant than anteiso-

$C_{15:0}$. The major menaquinone of strains HN-1^T and 39 was MK-7, in conformity to genus *Paenibacillus*. The polar lipids of strains HN-1^T and 39 detected by two-dimensional TLC are diphosphatidylglycerol (DPG), phosphatidylethanolamine (PE), phosphatidylglycerol (PG), four aminophospholipids (APL) and unidentified glycolipid (Fig. S4).

In summary, the phylogenetic, genomic, phenotypic and chemotaxonomic data of strains HN-1^T and 39 showed that they are different from all other closely related species of genus *Paenibacillus*. Therefore, we conclude that strain HN-1^T or 39 should be recognised as a novel species of the genus *Paenibacillus*, for which the name *Paenibacillus sinensis* sp. nov. is proposed.

Description of *Paenibacillus sinensis* sp. nov.

Paenibacillus sinensis (sin. en'sis. L.gen. n. sinensis of China, where the type strain HN-1^T was isolated).

Cells are Gram-positive, facultative anaerobic, rod-shaped (0.4–0.5 μm × 2.0–3.2 μm) and motile by means of peritrichous flagella. In slightly swollen sporangia, an ellipsoidal spore is formed and located in terminal position of cells. Colonies on LD medium are circular, convex, cream white, with diameter 1.0–2.0 mm. Nitrogen fixation positive and multiple *nifH* genes are present. The growth temperature is 15–42 °C, optimal at 30 °C. The growth pH range is 5.0–9.0, optimal at pH 7.0. NaCl concentration of 0–4% (w/v) is tolerable for growth, optimal at 0–0.2%. Positive tests for catalase, methyl red test, starch and aesculin hydrolysis, but negative for oxidase, Voges–Proskauer reaction, nitrate reduction. The various substrates are assimilated examined using Biolog GEN III microplates: dextrin, D-maltose, D-trehalose, D-cellobiose, D-gentiobiose, sucrose, D-turanose, stachyose, D-raffinose, α-D-lactose, D-melibiose, β-Methyl-D-glucoside, D-salicin, α-D-glucose, D-mannose, D-fructose, D-galactose, 3-methyl glucose, 1% sodium lactate, D-serine, D-sorbitol and pectin were utilized. Strains are resistant to inhibitory chemicals: aztreonam, nalidixic acid, vancomycin, lithium chloride, potassium tellurite, sodium bromate, sodium butyrate, sodium lactate 1% and sensitive to troleandomycin, lincomycin, guanidine HCl, niaproof 4, tetrazolium blue. The major menaquinone is MK-7. The predominant fatty acid is anteiso-$C_{15:0}$. The major polar lipids are DPG, PE, and PG. The DNA G + C contents for strains HN-1^T and 39 are 53.36 and 52.99 mol%, respectively.

The type strain, HN-1^T (= CGMCC 1.18902, JCM 34,620), was isolated from the rhizosphere soil of rice in Hunan P. R. China. The GenBank (EMBL) accession number for the 16S rRNA gene sequence of strain HN-1^T is MF967304 and the GenBank accession number for the draft genome sequence is JAHCMB000000000.

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Availability of data and material The GenBank accession numbers for 16S rRNA gene sequences of strains HN-1^T and 39 are MF967304 and MZ153121, respectively. The draft genome sequences of strains HN-1^T and 39 have been deposited at NCBI under the accession no. JAHCMB000000000 and JAHBAZ000000000.

Declaration

Conflict of interest The authors declare no conflict of interest.

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