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Paenibacillus spongiae sp. nov. isolated from deep-water marine sponge *Theonella swinhoei*

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Abstract

A novel bacterial strain, designated as PHS-Z3^T, was isolated from a marine sponge belonging to the genus *Theonella* on the Puerto Galera Deep Monkey, Philippines. Cells of PHS-Z3^T were Gram-stain-positive, motile, oxidase- and catalase-positive, white-pigmented, spore-forming, short rods that could grow at 10–40°C (optimum, 20°C), pH 6.0–9.5 (optimum, pH 7.5) and with 2–16% (w/v) NaCl (optimum, 7%). The 16S rRNA gene sequence of PHS-Z3^T showed 97.9%, 96.7%, and 96.2% identities to *Paenibacillus mendelii* C/2^T, *Paenibacillus oenotherae* DT7-4^T and *Paenibacillus aurantiacus* RC11^T, respectively. The results of phylogenetic analysis based on 16S rRNA gene sequences indicated that PHS-Z3^T formed an independent cluster with *Paenibacillus mendelii* C/2^T. The total genome of PHS-Z3^T was approximately 7613364 bp in size with a DNA G+C content of 51.6%. The average nucleotide identity (ANI) and digital DNA–DNA hybridization (dDDH) values between PHS-Z3^T and other type strains of species of the genus *Paenibacillus* were 68.0–81.4% [ANI by BLAST (ANIb)], 83.0–88.0% [ANI by MUMmer (ANIm)] and 12.7–32.1% (dDDH). The dDDH and ANI values were below the standard cut-off criteria for delineation of bacterial species. The percentage of conserved proteins (POCP) values between the genome of PHS-Z3^T and those of members of the genus *Paenibacillus* were 39.7–75.7%, while the average amino acid identity (AAI) values were 55.9–83.7%. The sole respiratory quinone in the strain was MK-7, and the predominant fatty acids were anteiso-$C_{15:0}$ and $C_{16:0}$. The major polar lipids of PHS-Z3^T consisted of diphosphatidylglycerol, phospholipid and phosphatidylglycerol. The characteristic amino acid in the cell wall of PHS-Z3^T was diamino heptanoic acid (*meso*-DAP). On the basis of the molecular, physiological, biochemical and chemotaxonomic features, strain PHS-Z3^T represents a novel species of the genus *Paenibacillus*, for which the name *Paenibacillus spongiae* sp. nov. is proposed, with the type strain PHS-Z3^T (=MCCC 1K07848^T=KCTC 43443^T).

The genus *Paenibacillus*, the type genus of the family *Paenibacillaceae* was originally proposed by Ash *et al.* in 1993 [1], and, at the time of writing, contains 296 species with validly published names, and eight synonyms (https://lpsn.dsmz.de/genus/paenibacillus) [2]. Species of the genus have been isolated from various sources, including mural paintings [3], gut [4], plants [5, 6], soil [7, 8, 9], water [10] and plant rhizosphere [11, 12]. They are generally Gram-stain-positive, facultatively anaerobic or aerobic bacterial strains [12, 13]. Species of the genus *Paenibacillus* are known for their plant growth-promoting properties. The model species *Paenibacillus polymyxa* plays important roles in plant growth promotion and biocontrol [14, 15]. In addition, some members of the genus *Paenibacillus* have been reported to produce different enzymes, plant hormones and antibacterial substances and can promote bioremediation by degrading compounds such as phenol, polycyclic aromatic hydrocarbons and lignin [16, 17]. The symbiotic microorganisms in sponges mainly include members of the phyla *Pseudomonadota*, *Actinomycetota*, *Bacteroidota* and *Bacillota* [18]. At present, the microorganisms isolated from sponges are mainly obtained through traditional isolation and cultivation methods, but most of the microorganisms isolated are known bacteria. With the continuous development of sequencing technology, some uncultured microorganisms among sponge symbiotic microorganisms are receiving more attention. These microorganisms often have more activity, including anti-tumour, antibacterial, insecticidal and enzyme

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Abbreviations: AAI, average amino acid identity; ANI, average nucleotide identity; ANIb, ANI by BLAST; ANIm, ANI by MUMmer; CDS, coding sequence; dDDH, digital DNA–DNA hybridization; GBDP, genome blast distance phylogeny method; GGDC, genome-to-genome distance calculator; MK, menaquinone; POCP, percentage of conserved proteins; sRNA, small RNA.
The GenBank accession numbers for the 16S rRNA gene sequence and the whole-genome sequence of the strain PHS-Z3^T are OM970862 and CP091430, respectively
Three supplementary tables and six supplementary figures are available with the online version of this article.

inhibitory activities [19–22]. Therefore, it is of great significance to screen more uncultured microorganisms and further explore the potential applications of natural products in medicine. The present study aims to determine the exact taxonomic position of strain PHS-Z3^T using a polyphasic approach.

During a study of symbiotic uncultured microorganisms of sponges, strain PHS-Z3^T was isolated from a marine sponge which was collected in July 2019 at 90 m water depth from Puerto Galera Deep Monkey, Philippines (13° 19′ 43.8″ N, 120° 35′ 35.1″ E) and was stored at −80°C until use. The sponge sample was identified according to 28S ribosomal ribonucleic acid (rRNA) and 18S rRNA gene sequences with 99.8% and 99.8% similarity to *Theonella swinhoei*, respectively. The 28S rRNA and 18S rRNA genes sequences of the sponge sample were deposited into the GenBank database under the accession numbers OP236534 and OP236533. The sponge microbial microcultivation was carried out according to the methods of Ferrari *et al*. [23]. As shown in Fig. S1, available in the online version of this article, firstly, the sponge sample was rinsed several times with ice-cold Ca^{2+}- and Mg^{2+}- free sterile artificial seawater (400 mM NaCl, 27.6 mM Na_2SO_4, 2.3 mM $NaHCO_3$, 8.9 mM KCl, 0.8 mM KBr, 0.4 mM H_3BO_3, 0.15 mM $SrCl_2$, 0.07 mM NaF) to remove organisms loosely attached to the sponge surface. A 3 g sample of clean sponge tissue was placed into a sterile mortar with 6 ml of cold $Ca^{2+}$and Mg^{2+}-free sterile artificial seawater and aseptically ground with a sterile pestle and then filtered through a 40 µm nylon cell strainer. The entrapped sponge tissue was filled into a sterile 25 mm tissue culture insert (TCI) with a 0.4 µm aperture. The filtrate was subsequently subjected to centrifugation at 100 ***g***, resulting in the supernatant containing sponge microorganisms. Subsequently, approximately 500 µl of the supernatant were filtered onto a 0.22 µm polycarbonate (PC) membrane. These PC membranes were placed on the top of the TCIs, which were then inserted into a sterile six-well multidish and cultured at 20°C in the dark for 7 days. After 7 days of incubation, the PC membranes containing the sponge microbial supernatant from the six-well multidish were carefully taken out using sterile tweezers and then placed into a centrifuge tube containing 1 ml ultrapure Milli-Q water. Subsequently, the solution with sponge microorganisms was vortexed for 10 min and diluted 10- to 1000-fold before being spread onto agar plates containing marine agar 2216E (Difco). After 14 days of incubation at 20°C, an individual colony was selected and streaked onto marine agar 2216E (Difco). The purified strain was then stored at −80°C in marine broth 2216E (Difco) with 30% (v/v) glycerol. A PC membrane without sponge microbial supernatant was used as a control.

Genomic DNA was extracted using a TIANamp DNA Kit (Tiangen Biotech) according to the manufacturer's instructions. The amplified DNA fragments were cloned into plasmid pEASY-T5 Zero vector (TransGen Biotech) and sequenced with primers 27F (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′). The almost full-length 16S rRNA gene sequence of PHS-Z3^T (1474 bp) was determined. The 16S rRNA gene similarity was compared using EzBioCloud (www.ezbiocloud.net) [24]. Phylogenetic analysis was carried out using three tree-making algorithms (neighbor-joining, maximum-likelihood and minimum-evolution) using MEGA version X [25] after multiple alignments of sequences using the CLUSTAL_X programme [26]. Regions including any gaps were removed. Evolutionary distance matrices of phylogenetic trees were calculated according to Kimura's two-parameter model [27]. Bootstrap analysis was performed with 1000 replications [28].

On the basis of the results of phylogenetic analysis of the 16S rRNA gene sequences PHS-Z3^T is included in the cluster of the genus *Paenibacillus*. The neighbor-joining tree (Fig. 1) indicated that PHS-Z3^T was clustered with *P. mendelii* C/2^T. The cluster was further found to be stable when trees were reconstructed using the maximum-likelihood and minimum-evolution algorithms (Figs S2 and S3). On the basis of the results of phylogenetic analyses and 16S rRNA gene sequencing, PHS-Z3^T was found to be affiliated with the genus *Paenibacillus*.

The genome sequence of *P. mendelii* C/2^T as well as that of PHS-Z3^T were determined. Whole-genome sequencing of PHS-Z3^T was performed on the DNBSEQ (BGI) and Nanopore (ONT) platforms. Reads of each data set were filtered, and high-quality paired-end reads were assembled using Canu v1.5 and GATK v1.6–13. The draft genome sequence of C/2^T was determined by using the DNBSEQ (BGI) platform, assembled into draft genomes using SOAPdenovo. The rRNAs and tRNAs of PHS-Z3^T and C/2^T were predicted using RNAmmer [29] and tRNAscan-SE, respectively [30]. The gene prediction of PHS-Z3^T and C/2^T was carried out using Glimmer (version 3.02) [31–33]. The genome sequence data of PHS-Z3^T and related members of the genus *Paenibacillus* were uploaded to the Type Strain Genome Server (https://tygs.dsmz.de/) [34] to reconstruct a phylogenomic tree. The average nucleotide identity (ANI) values were calculated using the EZBioCloud web service and digital DNA–DNA hybridisation (dDDH) values were calculated using the Genome-to-Genome Distance Calculator 3.0 (GGDC 3.0) [35, 36]. The percentage of conserved proteins (POCP) between the genome of PHS-Z3^T and those of the selected type strains of species of the genus *Paenibacillus* were calculated [37]. The average amino acid identity (AAI) values were also calculated as a mean similarity of orthologous genes as described previously [38].

The whole genome sequence of PHS-Z3^T had a total length of 7613364 bp. The accession number of the genomic sequence of strain PHS-Z3^T is CP091430, and genome annotation was carried out using the NCBI Prokaryotic Genome Annotation Pipeline. The genome contained 6702 coding sequences (CDSs), 27 rRNA genes, 7 small RNA (sRNA) genes and 77 tRNA genes, and the DNA G+C content was 51.6%. The draft genome sequence of strain C/2^T had a total length of 8136757 bp. The accession number of the genome sequence of strain C/2^T is JANHOF000000000. The genome contained 7417 CDSs, 6 rRNA genes, 17 sRNA genes and 58 tRNA genes, and the DNA G+C content was 51.5%. As predicted using the antiSMASH 6.0 programme [39], the genome

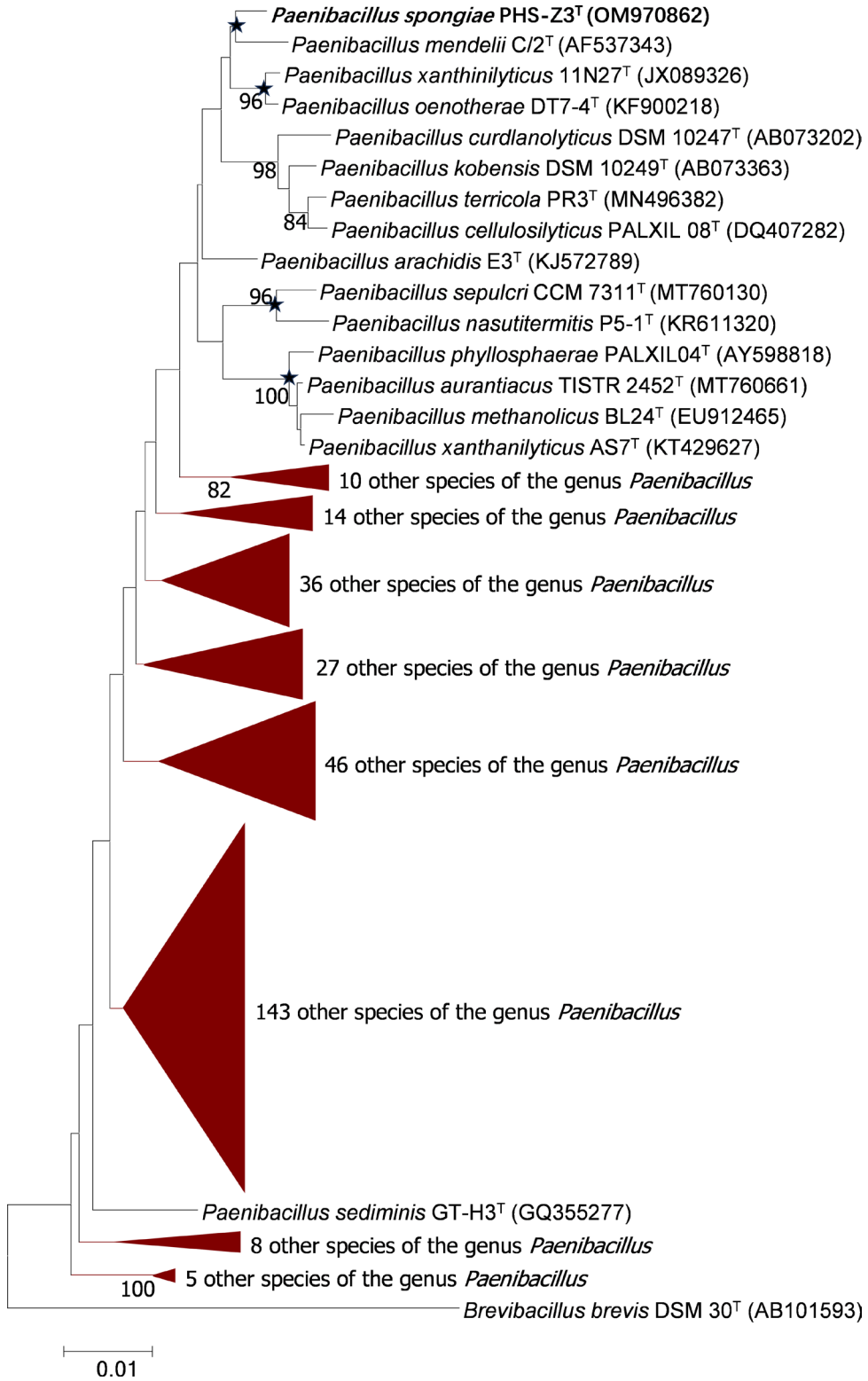

Fig. 1. Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences showing the position of strain PHS-Z3^T within the genus *Paenibacillus*. Values above each branch indicate bootstrap values (>70%) from the neighbor-joining method as percentages of 1000 replicates. *Brevibacillus brevis* ATCC 8246^T (X60612) was used as an outgroup. Nodes which are present in all trees are indicated by stars. Bar, 0.01 substitutions per nucleotide position.

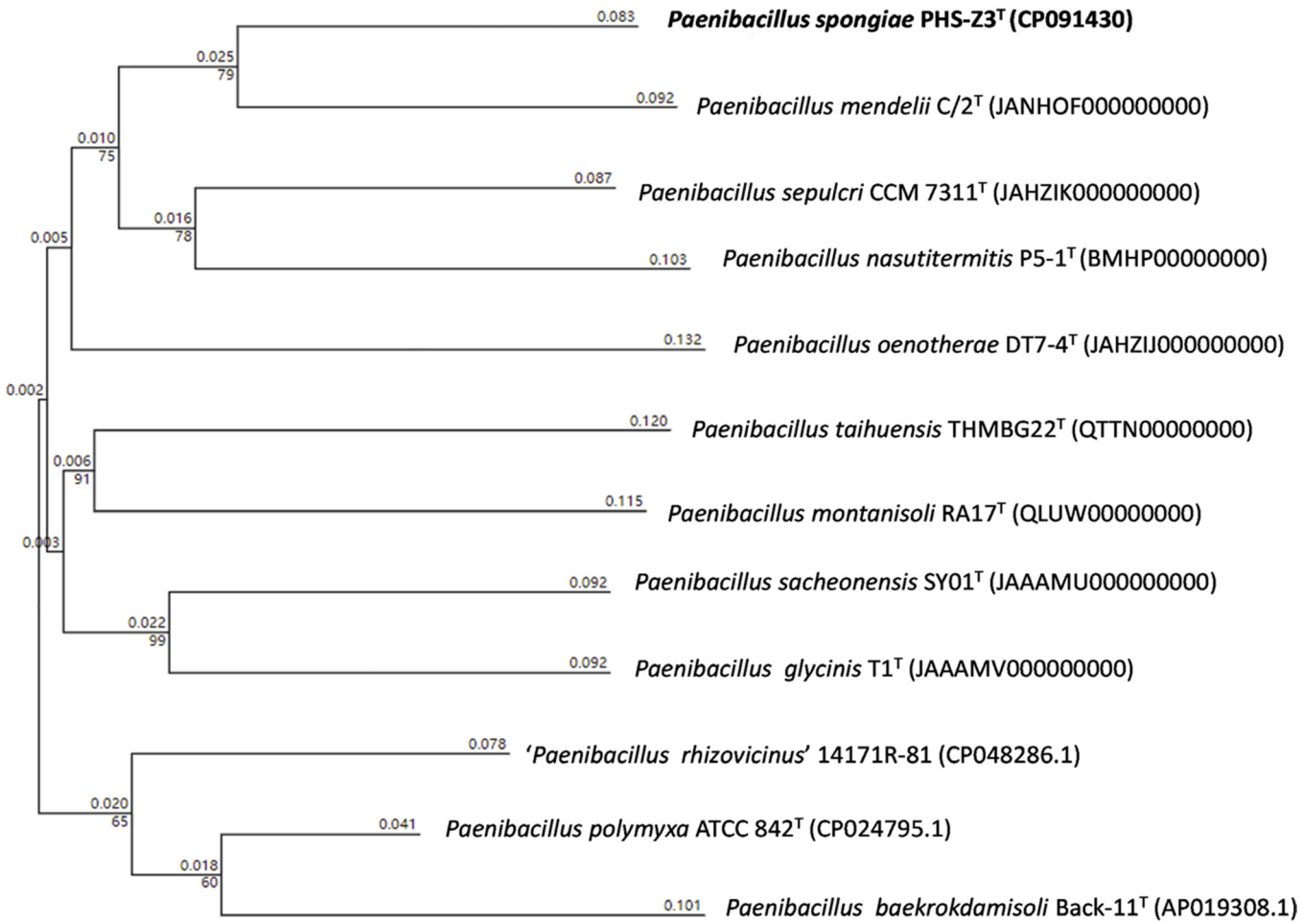

Fig. 2. The phylogenomic tree based on genome sequences of PHS-Z3^T and the related strains using the genome BLAST distance phylogeny (GBDP) method. The numbers above the branches are GBDP pseudo-bootstrap support values>60% from 100 replications, with an average branch support of 69.9%. GenBank accession numbers are included in parentheses.

of PHS-Z3^T harbours ten secondary metabolite biosynthetic gene clusters including lassopeptides (Table S1). Other strains of species of the genus *Paenibacillus*, with the exception of *Paenibacillus taihuensis* THMBG22^T, *Paenibacillus montanisoli* RA17^T and '*Paenibacillus rhizovicinus*' 14171 R-81, also have the potential to produce lassopeptide. The lassopeptides have been recognised as a potential source for biopharmaceutical development because of their antibacterial, antiviral or other biological activities [40].

The phylogenomic tree (Fig. 2) indicates that PHS-Z3^T forms a stable cluster in the evolutionary tree with *Paenibacillus mendelii* C/2^T. As shown in Table S2, among the genomes of PHS-Z3^T and 10 strains of related species, the ANI values by BLAST (ANIb values) are 68.0–81.4% and the ANI values by MUMmer (ANIm values) are 83.0–88.0%, which are lower than the recommended threshold values for species delineation [41]. The dDDH values between PHS-Z3^T and its neighbouring type strains ranged from 12.7% to 32.1%, which are far below the 70% threshold for species delineation [41]. POCP values between the genome of PHS-Z3^T and those of members of the genus *Paenibacillus* were 39.7–75.7%, while the AAI values were 55.9–83.7%. Although there is no generally recognised genus boundary, it has been suggested recently that AAI (60.0–80.0%) and POCP (50.0%) could be thresholds for distinguishing genera [37, 42]. The results of the phylogenetic analyses of the 16S rRNA gene and whole genomes provide strong evidence in favour of recognising PHS-Z3^T as representing a novel species of the genus *Paenibacillus*. On the basis of the 16S rRNA gene sequence and the results of genome sequence phylogenetic analyses, *P. mendelii* C/2^T (=DSM 19248^T), *Paenibacillus sepulcri* CCM 7311^T (=LMG 19508^T), *P. polymyxa* ATCC 842^T (=LMG 13294^T), *Paenibacillus nasutitermitis* P5-1^T (=CGMCC 1.15178^T), *P. oenotherae* DT7-4^T (KCTC 33186^T) and *Paenibacillus xanthinilyticus* 11N27^T (=NBRC 109108^T) were selected as experimental controls.

Gram-staining was performed using a Gram stain kit (Hangzhou Tianhe Microorganism Reagent) according to the manufacturer's instructions. Growth at various temperatures (4, 10, 15, 20, 28, 37, 40 and 50 °C) was measured on the marine agar

2216E (Difco) medium. The requirement and tolerance of NaCl for growth were tested in marine broth 2216E (Difco) with NaCl omitted. The final NaCl concentrations supplemented in the above broth were 1–16% (w/v; intervals of 1%). Growth at pH 5.5–10.0 (intervals of pH 0.5 units) was examined in marine broth 2216E (Difco) buffered with sodium acetate/acetic acid (pH 4.0–5.5), phosphate/NaOH (pH 6.0–8.5) and bicarbonate/carbonate (pH 9.0–10.0). Growth at different salt concentrations and pH levels were determined in marine broth 2216E (Difco) by measuring the OD_{600} (Multifunctional enzyme marker-Spark; Tecan). According to the manufacturer's instructions, anaerobic growth was determined in marine agar 2216E (Difco) medium using the AnaeroPack-Anaero (MGC) [43]. The cells were grown in marine broth 2216E (Difco), and cell morphology was observed using a scanning electron microscope (Sirion 200, FEI). Colony appearance was observed after growth on marine agar 2216E (Difco) medium at 20°C for 7 days. The motility of cells for growth and diffusion was observed in a test tube containing semi solid marine agar 2216E (Difco). Unless otherwise specified, the above experimental conditions were conducted with cells cultured at 20°C for 14 days.

PHS-Z3^T was Gram-stain-positive, facultatively anaerobic and motile. A photomicrograph of PHS-Z3^T was produced by phase-contrast microscopy at 1000× magnification. As shown in Fig. S4a, PHS-Z3^T was spore-forming. The spores were spherical, terminal spores, not swelling the sporangia. Under a scanning electron microscope, the cell of PHS-Z3^T were about 1.0–1.5 µm long and 0.5–0.8 µm wide, appearing to be rod-shaped and lacking flagella (Fig. S4b). Colonies of PHS-Z3^T were punctiform, circular and white–cream on marine agar 2216E (Difco) medium when incubated at 20°C for 14 days. PHS-Z3^T grew at 10–40°C and showed fast growth at 20°C. It was able to grow at pH 6.0–9.5 with maximum growth at pH 7.5 and in the presence of 2–16% (w/v) NaCl with maximum growth at 7% (w/v) NaCl.

Catalase activity was determined by assessing the production of bubbles after the addition of a drop of 3% (v/v) H_2O_2 onto the bacterial culture on marine agar 2216E (Difco) plates at 20°C for 7 days. Hydrolysis of xylan, casein, starch and carboxymethyl cellulose were examined as described by Tindall *et al.* [44]. Oxidase activity was determined based on the oxidation of tetramethyl-*p*-phenylenediamine [45] after the strains had been cultured on marine agar 2216E (Difco) plates at 20°C for 7 days. After PHS-Z3^T was cultured on marine broth 2216E (Difco) at 20°C for 7 days, the acid production and enzyme activity from 49 carbohydrates were determined using the API 50 CH and API ZYM systems (bioMérieux). According to the manufacturers' instructions, API 20E, API 20NE system (bioMérieux) and GEN III microporous plates (Biolog) were used to determine other biochemical characteristics and carbon source utilisation. All these were carried out in duplicate and repeated twice to ensure reproducibility. The susceptibility of the strains to 22 different types of antibiotics was tested. Discs (Bio-Rad) containing the following quantities of antibiotics were used: 2 µg (clindamycin), 5 µg (ciprofloxacin, levofloxacin and rifampicin), 10 µg (ampicillin, gentamicin, tobramycin, amoxicillin and streptomycin), 10 U (penicillin), 15 µg (clarithromycin and erythromycin), 30 µg (aztreonam, ceftriaxone, cefotaxime, ceftazidime, chloramphenicol, kanamycin, tetracycline and vancomycin), 75 µg (mezlocillin), 100 µg (piperacillin). The cell suspensions and the discs were placed on marine agar 2216E (Difco) plates. The plates were incubated at 20°C for 3 days and the radii of the growth-inhibition zones were measured. The general criteria for bacterial sensitivity to antimicrobial agents were: diameter of the inhibition ring exceeding 20 mm are extremely sensitive, more than 15 up to 20 mm are highly sensitive, 10–15 mm are moderately sensitive and less than 10 mm are resistant [46].

PHS-Z3^T was positive for catalase, which was consistent with *P. mendelii* C/2^T, *P. sepulcri* CCM 7311^T and *P. polymyxa* ATCC 842^T. PHS-Z3^T was positive for oxidase, which was inconsistent with *P. polymyxa* ATCC 842^T. No reduction of nitrate to nitrite was found in PHS-Z3^T. PHS-Z3^T was positive for the hydrolysis of aesculin, starch and carboxymethyl cellulose but negative for the hydrolysis of xylan and casein. PHS-Z3^T was positive for the assimilation of galactose and maltose. It was negative for the assimilation of gelatin, D-glucose, L-arabinose, D-mannose, D-mannitol and *N*-acetyl-glucosamine. The Biolog GEN III microporous plate test indicated that PHS-Z3^T was positive for maltose, trehalose, gentiobiose, sucrose, stachyose, raffinose, melibiose, D-salicin, inosine, glycerol and pectin. Other phenotypic characteristics and those of their closest phylogenetic relatives are shown in Table 1. Notably, PHS-Z3^T showed a relatively higher salt tolerance of up to 7% NaCl compared with 2% for its related species. PHS-Z3^T was resistant to (µg per disc) gentamicin (10), kanamycin (30), clindamycin (2), levofloxacin (5), aztreonam (30) and ciprofloxacin (5), but susceptible to streptomycin (10), chloramphenicol (30), ceftazidime (30), rifampicin (5), erythromycin (15), clarithromycin (15), tetracycline (30), tobramycin (10), mezlocillin (75), vancomycin (30), amoxicillin (10), penicillin (10 U), cefotaxime (30), piperacillin (100), ceftriaxone (30) and ampicillin (10).

After cultivation in marine broth 2216E (Difco) for 7 days at 20°C, cells of PHS-Z3^T were harvested and washed with sterile distilled water before freeze-drying. Isoprenoid quinones were extracted from freeze-dried cells (100 mg) with chloroform–methanol (2:1, v/v) and analysed using methods described previously [47, 48]. Polar lipids were extracted and separated on silica gel 60 aluminum-backed thin-layer plates according to the methods of Minnikin *et al.* [49]. α-Naphthol, ninhydrin and molybdatophosphoric acid were used to detect other polar lipids according to he method of Tindall [50]. After incubation for 7 days at 20°C on marine agar 2216E (Difco), well-grown cells were harvested and fatty acid methyl esters were prepared as described previously [51]. The fatty acids were analysed by gas chromatography (model 6850, Agilent Technologies) and identified using the TSBA6.0 database of the Microbial Identification System (MIDI) [52, 53].

Table 1. Differential characteristics of PHS-Z3^T with respect to the known type strains of species of the genus *Paenibacillus*. Strains: 1, PHS-Z3^T; 2, *P. mendelii* C/2^T; 3, *P. sepulcri* CCM 7311^T; 4, *P. polymyxa* ATCC 842^T; 5, *P. nasutitermitis* P5-1^T; 6, *P. oenotherae* DT7-4^T; 7, *P. xanthinilyticus* 11N27^T. Data are from this study, except where indicated. +, Positive; –, negative; w, weakly positive.

Characteristic	1	2	3	4	5	6	7
Growth temperature (°C):							
Range	10–40	25–30	10–30	10–40	10–40	15–37	20–35
Optimum	20	30	25	28	30	30	35
Growth pH:							
Range	6.0–9.5	70–8.5	6.0–8.0	6.0–8.0	5.5–9.0	6.0–7.0	6.0–7.0
Optimum	7.5	8.5	7.2–7.4	7.0	7.5	7.0	6.5
NaCl concentration (%):							
Range	2–16	<5	<3	<5	0–4	0–2	0–1
Optimum	7	2	2	2	1	0	0
Oxidase activity	+	+	+	–	+	+	–
Fermentation of:							
D-Glucose	–	–	–	+	+	–	–
Nitrate reduction	–	–	–	+	–	+	–
Assimilation of:							
Galactose	+	w	w	+	–	–	–
D-Glucose	–	–	+	–	+	+	–
L-Arabinose	–	–	+	–	+	+	–
D-Mannose	–	–	w	–	+	–	–
D-Mannitol	–	–	+	–	+	–	–
N-Acetyl-glucosamine	–	w	–	–	–	w	–
Maltose	+	+	+	–	+	–	–
Hydrolysis of:							
Aesculin	+	w	w	+	–	–	–
Starch	+	–	–	+	+	+	+
Xylan	–	–	–	–	–	–	+
Carboxymethyl cellulose	+	–	–	+	–	–	–
Casein	–	–	–	–	+	+	–
Enzyme activity:							

Continued

Table 1. Continued

Characteristic	1	2	3	4	5	6	7
Alkaline phosphatase	−	−	−	+	−	−	−
α-Galactosidase	−	−	−	+	+	+	−
α-Glucosidase	−	−	−	+	+	−	+
β-Glucosidase	−	−	−	+	−	−	−
Acid production form:							
Glycogen	−	−	−	+	+	+	−
Gentiobiose	w	+	w	+	+	−	−
D-Lyxose	−	−	+	−	−	−	−
L-Fucose	−	+	+	−	−	−	−
Melezitose	+	+	+	−	−	+	−
Salicin	w	w	−	+	−	+	−
Methyl-α-D-Glucopyranoside	−	−	−	+	w	+	−
D-Mannitol	−	−	+	+	−	−	+
L-Rhamnose	−	+	−	−	−	−	+
D-Mannose	−	−	w	+	−	−	−
D-Fructose	−	−	−	+	−	−	−
D-Glucose	w	−	+	+	+	+	+
D-Galactose	−	−	+	+	−	+	−
Methyl-β-D-xylopyranoside	+	+	+	w	+	−	−
D-Xylose	−	w	−	+	+	+	−
D-Ribose	+	+	−	+	+	−	−
L-Arabinose	−	−	+	+	−	+	−
DNA G+C content (%)	51.6	50.8*	50.0†	43.0-46.0‡	48.9§	50.1‡	50.3¶

*Data from Šmerda *et al.* [5].
†Data from Šmerda *et al.* [3].
‡Data from Kim *et al.* [12].
§Data from Wang *et al.* [4].
¶Data from Kim *et al.* [7].

As summarised in Table S3, the main fatty acid components of PHS-Z3^T (>10.0%) were anteiso-$C_{15:0}$ (22.2%) and $C_{16:0}$ (16.6%). The main components were generally identical to those of *P. mendelii* C/2^T, *P. sepulcri* CCM 7311^T, *P. polymyxa* ATCC 842^T, *P. nasutitermitis* P5-1^T, *P. oenotherae* DT7-4^T and *P. xanthinilyticus* 11N27^T, but differences were observed in the relative proportions of individual components among the strains. The predominant respiratory quinone of PHS-Z3^T was MK-7, which was typical of members of the genus *Paenibacillus*. The diagnostic polar lipids of the PHS-Z3^T were diphosphatidylglycerol, phosphatidylglycerol and phospholipid. Three unidentified polar lipids were detected as minor components in PHS-Z3^T. The polar lipids of PHS-Z3^T include diphosphatidylglycerol and phosphatidylglycerol, which were consistent with the corresponding phenotypes of *P. nasutitermitis* P5-1^T and *P. oenotherae* DT7-4^T. The polar lipids of PHS-Z3^T include phospholipid, which was consistent with the profile of *P. xanthinilyticus* 11N27^T (Fig. S5). The characteristic amino acid in the cell wall of strain PHS-Z3^T was diamino heptanoic acid (*meso*-DAP) (Fig. S6).

Thus, on the basis of the results described, strain PHS-Z3^T represents a novel species of the genus *Paenibacillus*, for which the name *P. spongiae* sp. nov. is proposed.

DESCRIPTION OF *PAENIBACILLUS SPONGIAE* SP. NOV

Paenibacillus spongiae (spon'gi.ae. L. gen. n. *spongiae* of a sponge, the source of the type strain).

Cells are Gram-stain-positive, facultatively anaerobic, motile, spore-forming, short rods, 0.5–0.8 µm wide and 1.0–1.5 µm long. Colonies on marine agar 2216E (Difco) medium are smooth, circular, transparent with entire margins, white and 0.7–1.0 mm in diameter at 20°C after growth for for 14 days. A flagellum is not present. Can grow at 10–40°C (optimum, 20°C), pH 6.0–9.5 (optimum, pH 7.5) and with 2–16% NaCl (w/v, %) (optimum, 7%). Catalase and oxidase activity are positive. Positive for the hydrolysis of aesculin, starch and carboxymethyl cellulose and the assimilation of galactose and maltose. Negative for nitrite reduction, indole production, D-glucose fermentation, arginine hydrolysis, hydrolysis of urea, xylan and casein and assimilation of gelatin, D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Esterase (C4), esterase lipase (C8), naphthol-AS-BI-phosphohydrolase, β-galactosidase, leucine arylamidase and acid phosphatase are present. Acid production occurs using glycerol, D-ribose, methyl β-D-xylopyranoside, amygdalin, aesculin ferric citrate, cellobiose, maltose, D-glucose (weakly), salicin (weakly), gentiobiose (weakly), lactose, melibiose, sucrose, trehalose, melezitose, raffinose and turanose. Maltose, trehalose, cellobiose (weakly), gentiobiose, sucrose, turanose (weakly), stachyose, raffinose, melibiose, D-salicin, inosine, glycerol, pectin, glucuronamide (weakly) and potassium tellurite can be utilised. The major fatty acids (>10%) are anteiso-$C_{15:0}$ and $C_{16:0}$. The predominant respiratory quinone is MK-7, and the diagnostic polar lipids are diphosphatidylglycerol, phospholipid and phosphatidylglycerol. The characteristic amino acid in the cell wall is diamino heptanoic acid (*meso*-DAP).

The type strain, PHS-Z3^T (=MCCC 1K07848^T=KCTC 43443^T), was isolated from a sponge (*Theonella swinhoei*). The DNA G+C content of strain PHS-Z3^T is 51.63%. The GenBank accession number for the 16S rRNA gene sequence of strain PHS-Z3^T is OM970862. The GenBank accession number for the whole-genome sequence of strain PHS-Z3^T is CP091430.

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Author contributions
Z.L. designed the research and project outline. Q.S. identified the sponge sample. M.Z. and J.S. performed bacterial isolation and polyphasic taxonomy. M.Z. performed genome analysis. M.Z. and Z.L. drafted the manuscript. All authors read and approved the final manuscript.

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

Ethical statement
This article does not contain any studies with human participants or animals performed by any of the authors.

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