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Qipengyuania spongiae sp. nov., isolated from marine sponge *Cinachyrella kuekenthali*

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Abstract

A novel bacterial strain, designated as PHS-Z21^T, was isolated from the marine sponge *Cinachyrella kuekenthali* collected from PG Dave's Rock, Philippines. Cells of PHS-Z21^T are Gram-stain-negative, non-motile, pale-yellow-pigmented, short rods. PHS-Z21^T is able to grow at 10–40 ℃ (optimum, 30 ℃), pH 5.5–9.0 (optimum, pH 8.5) and with 3–9% (w/v) NaCl (optimum, 4%). Its 16S rRNA gene sequence shows 98.6% similarity to *Qipengyuania nanhaisediminis* CGMCC 1.7715^T, 98.5% similarity to *Qipengyuania vulgaris* 022-2-10^T and 98.4% similarity to *Qipengyuania flava* SW-46^T, respectively. The phylogenetic tree based on 16S rRNA gene sequences reveals that PHS-Z21^T is clustered with *Q. flava* SW-46^T. The total genome of PHS-Z21^T is approximately 2932896 bp in size with a DNA G+C content of 64.7%. The average nucleotide identity (ANI) and digital DNA–DNA hybridization (dDDH) values among PHS-Z21^T and other type strains are 70.0–77.3% (ANIb), 83.3–86.8% (ANIm) and 13.0–26.9% (dDDH), respectively. The dDDH and ANI values are below the standard cutoff criteria for delineating bacterial species. Percentage of conserved proteins (POCP) values between the genome of strain PHS-Z21^T and those of members of the genera *Qipengyuania*, *Erythrobacter*, *Altererythrobacter* and *Alteriqipengyuania* were 62.0–74.5%, 55.8–63.2%, 60.7–66.9% and 63.9–66.8%, respectively, while the AAI values were 68.4–74.3%, 63.8–65.9%, 66.3–68.3% and 64.7–66.9%, respectively. The major fatty acids of PHS-Z21^T are composed of summed feature 8 ($C_{18:1}\omega7c$ and/or $C_{18:1}\omega6c$), $C_{18:1}\omega7c$ 11-methyl, $C_{16:0}$ and summed feature 3 ($C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$). The polar lipids of PHS-Z21^T mainly consist of diphosphatidylglycerol, glycolipid, phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol and glycophospholipid. The respiratory lipoquinone was identified as Q-10. On the basis of the phenotypic and phylogenetic data, strain PHS-Z21^T represents a novel species of the genus *Qipengyuania*, for which the name *Qipengyuania spongiae* sp. nov. is proposed. The type strain is PHS-Z21^T (=MCCC 1K07849^T=KCTC 92590^T).

The genus *Qipengyuania* was described by Feng *et al.* in 2015 [1] with the proposal of type species *Qipengyuania sediminis* belonging to the family *Erythrobacteraceae*. In 2020, an up-to-date taxonomic classification of the family *Erythrobacteraceae* was established based on the phylogenomic reconstruction of core genes and the results of genomic similarity analyses. On the basis of the new taxonomic framework of the family *Erythrobacteraceae*, eight species of the genus *Erythrobacter*, one species of the genus *Altererythrobacter* and one species of the genus *Porphyrobacter* were proposed to be transferred to the genus *Qipengyuania* [2, 3]. Soon after, the species *Erythrobacter favus* was also reclassified as *Qipengyuania fava* [4]. Liu *et al.* in 2022 [5] provided sufficient genotypic and phenotypic data to enable the differentiation of 15 novel species from the known species of the genus *Qipengyuania*, almost doubling the number of described species. At the time of writing, the genus *Qipengyuania* comprises 29 species with validly published names, which can be found on the updated LPSN website (https://lpsn.dsmz.de/genus/qipengyuania) [6]. Bacteria of the genus *Qipengyuania* have been isolated from various habitats, such as sediments [1, 7], freshwater [8], rhizosphere mud [4], tidal flats [9] and seawater [10]. Members of this genus are Gram-stain-negative, orange- or yellow-pigmented bacteria and aerobic chemoorganotrophs. Some species of the genus *Qipengyuania* can produce sulfur-containing carotenoids [11], poly-β-hydroxybutyrate [12] and halotolerant thermoalkaliphilic esterase [13], thus exhibiting great potential for application

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Abbreviations: AAI, average amino acid identity; ANI, average nucleotide identity; dDDH, digital DNA–DNA hybridization; GBDP, genome blast distance phylogeny method; GGDC, Genome-to-Genome Distance Calculator; POCP, percentage of conserved proteins; Q, ubiquinone.
The GenBank accession numbers for the 16S rRNA gene sequence and the whole-genome sequence of strain PHS-Z21^T are OM970863.1 and CP092471, respectively.
Six supplementary figures and two supplementary tables are available with the online version of this article.

in biotechnology and industry. The present study was intended to determine the exact taxonomic position of strain PHS-Z21^T using a polyphasic approach.

During an investigation of bacterial diversity associated with marine sponges, strain PHS-Z21^T was isolated from *Cinachyrella kuekenthali* which was collected in July 2019 at 55 m water depth from PG Dave's Rock, 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 98.9% and 99.6% similarity to *Cinachyrella kuekenthali*, respectively. The sponge sample's 28S rRNA and 18S rRNA were deposited into the GenBank database under the accession numbers OP236538 and OP236537. The sponge was rinsed 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) several times to remove organisms loosely attached to the sponge surface. Sponge surface tissue was removed using a sterile surgical blade. Clean sponge internal tissues were ground with a mortar and pestle aseptically. For microbial culture, the ground sponge sample was diluted in cold sterile artificial seawater free of $Ca^{2+}$and Mg^{2+} 1:200 as bacterial inoculum. A 50 µl bacterial inoculum was transferred into 10 ml Ca^{2+}- and Mg^{2+}- free sterile artificial seawater and filtered onto 0.2 µm PC membrane using a syringe filter holder. The tissue culture insert (TCI) was filled with 3 g ground sponge tissue. A TCI was then placed upside down in a sterile six-well multi-dish. Inoculated PC membranes were then placed on top of a sterile 25 mm TCIs. The culture vessels were incubated at 30 °C in the dark for 7 to 10 days. After incubation, the PC membrane was carefully placed into a centrifuge tube containing 5 ml of ultrapure Milli-Q water with sterile tweezers. Then the sample was homogenized and diluted 10- to 1000- fold and spread on agar plates with 2216E medium (0.5% tryptone, 0.1% yeast extract and 0.01% $FePO_4$, pH 7–7.5; artificial seawater). After incubation for 2 weeks at 30 °C, each colony was selected and spread on marine agar (MA; Difco). The purified strain was stored at −80 °C in marine broth (MB; Difco) with 30% (v/v) glycerol.

Genomic DNA was extracted using a TIANamp DNA Kit (Tiangen Biotech) according to the manufacturer's instructions. PCR amplification was performed using primers 27F (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′). The PCR conditions were as follows: denaturation at 95 °C for 10 min, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s and extension at 72 °C for 120 s. At the end of the cycles, the reaction mixture was kept at 72 °C for 10 min and then cooled to 4 °C. The amplified gene fragments were recovered by agarose gel electrophoresis and then connected with plasmid pEASY -T5 Zero vector (TransGen Biotech). The related plasmids were introduced into DH5*α* cells for blue–white screening. The positive white clones on the plate were selected for culture, and the plasmids were extracted and sequenced at Sangon Biotech (Shanghai, PR China) [14] to obtain the complete 16S rRNA gene sequence of PHS-Z21^T. A 1409 bp 16S rRNA gene sequence of PHS-Z21^T was obtained, and the 16S rRNA gene similarity was compared using EzBioCloud (www.ezbiocloud.net) [15]. Phylogenetic analysis was carried out using three tree-making algorithms (neighbor-joining, maximum-likelihood and minimum-evolution) using MEGA version X [16] after multiple alignments of sequences using the CLUSTAL_X programme [17]. Regions including any gaps were removed. Evolutionary distance matrices of phylogenetic trees were calculated according to Kimura's two-parameter model [18]. Bootstrap analysis was performed with 1000 replications [19].

EzBioCloud results indicated that PHS-Z21^T probably represented a novel species of the genus *Qipengyuania*, sharing 98.6% similarity to *Qipengyuania nanhaisediminis* CGMCC 1.7715^T, 98.5% similarity to *Qipengyuania vulgaris* 022 2-10^T and 98.4% similarity to *Qipengyuania flava* SW-46^T, respectively. The neighbor-joining tree (Fig. 1) based on 16S rRNA gene sequences indicated that PHS-Z21^T represents a member of the genus *Qipengyuania* and forms a distinct subline at the base of *Qipengyuania mesophila* and a *Qipengyuania qiaonensis*–*Q. flava* cluster. The cluster was further found to be stable when trees were reconstructed using the maximum-likelihood and minimum-evolution algorithms (Figs S1 and S2, available in the online version of this article). On the basis of the results of phylogenetic analysis, together with 16S rRNA gene sequencing, PHS-Z21^T was affiliated to the genus *Qipengyuania*.

Whole-genome sequencing of PHS-Z21^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 rRNAs and tRNAs were predicted using RNAmmer [20] and tRNAscan-SE [21], respectively. The gene prediction was carried out using Glimmer (version 3.02) [22–24]. The genome sequence data of PHS-Z21^T and related members of the genus *Qipengyuania* were uploaded to the Type Strain Genome Server (https://tygs.dsmz.de/) [25] to reconstruct a phylogenomic tree. The single-copy orthologous cluster protein sequences with the command '- e=1e−5 - cov=50 - identity=50' were extracted by Proteinortho version 6. After multiple comparisons of single-copy orthologous cluster protein sequences using the MUSCLE programme [26], phylogenetic analysis was conducted using the neighbor-joining tree-making algorithms using MEGA version X. The average nucleotide identity (ANI) values were calculated using the EZBioCloud web service and digital DNA–DNA hybridization (dDDH) values were calculated with the Genome-to-Genome Distance Calculator 3.0 (GGDC 3.0) [27, 28]. The percentage of conserved proteins (POCP) between the genome of PHS-Z21^T and the genomes of selected type strains of members of the family *Erythrobacteraceae* were calculated [29, 30]. Average amino acid identity (AAI) values were also calculated as a mean similarity of orthologous genes as described previously [31].

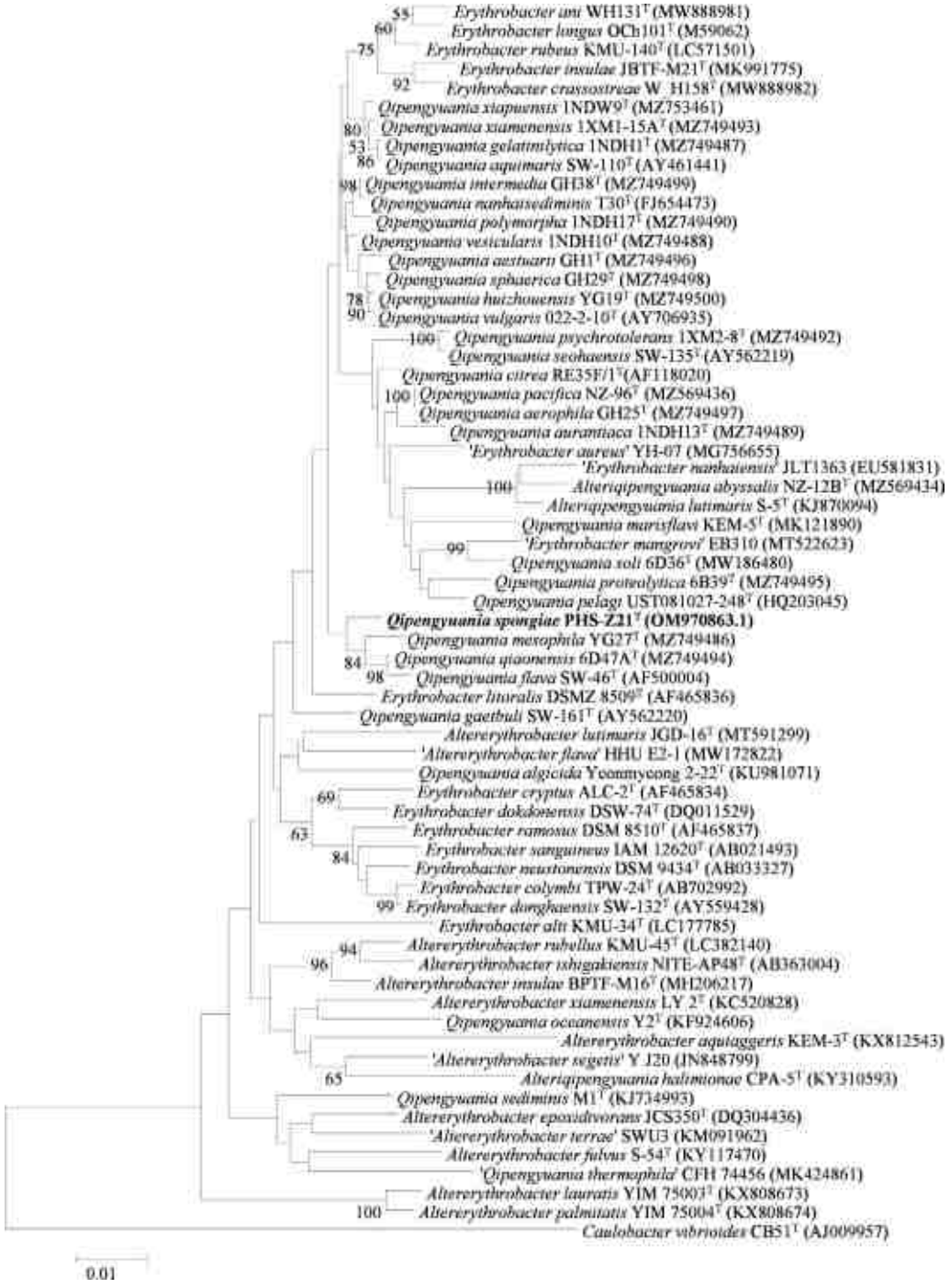

Fig. 1. Neighbor-joining tree showing the phylogenetic position of PHS-Z21^T based on 16S rRNA gene sequences. The numerals (values>50% are noted) indicate percentages of bootstrap samplings as derived from 1000 replications. Bar, 0.01 substitutions per nucleotide position. GenBank accession numbers are given in parentheses. The sequence of *Caulobacter vibrioides* CB51^T serves as an outgroup.

The whole genome sequence of PHS-Z21^T is 2932 896 bp. The genomic sequence of the strain was uploaded to NCBI GenBank with the accession number CP092471. The genome contained 2861 coding sequences, 3 rRNA genes, 7 sRNA genes and 45 tRNA genes, and the DNA G+C content was 64.7%. The DNA G+C contents of the members of the genus of *Qipengyuania* are between 60.5% and 66.5% (Table 1). Carotenoids are ubiquitous natural pigments, belonging to non-enzymatic system for oxidative stress defence [32]. The results of genomic analysis of PHS-Z21^T indicated that four genes (*crtB*, *crtI*, *crtY* and *crtZ*) are responsible for carotenoid biosynthesis. A variety of genes encoding enzymes for oxidative stress [33], such as superoxide dismutase, catalase,

Table 1. The ANI and dDDH values between PHS-Z21^T and related type strains of species of the genera *Qipengyuania*, *Erythrobacter* and *Alteriqipengyuania*

PHS-Z21^T vs	Accession number	Genome size (Mbp)	DNA G+C content (%)	POCP	AAI (%)	ANI (%)		GGDC	
						ANIb	ANIm	dDDH (%)*	Model C.I. (%)
Qipengyuania sediminis CGMCC 1.12928^T	CP037948	2.4	66.5	65.1	68.4	73.6	83.6	16.9	13.8–20.4
Qipengyuania vulgaris 022-2-10^T	WTYC00000000	3.2	60.5	69.7	71.0	74.2	85.0	18.4	15.3–22.0
Qipengyuania mesophila YG27^T	JAIGNU000000000	3.1	64.5	69.2	71.5	76.1	85.0	23.7	20.4–27.3
Qipengyuania qiaonensis 6D47A^T	JAIGNO000000000	3.4	61.5	64.8	70.5	74.3	84.3	18.1	15.0–21.6
Qipengyuania flava SW-46^T	CP032228	3.2	63.5	71.3	72.6	75.6	85.3	21	17.8–24.6
Qipengyuania gaetbuli SW-161^T	WTYF00000000	2.8	64.0	66.5	71.1	75.2	84.3	21.2	18.0–24.9
Qipengyuania pelagi JCM 17468^T	WTYD00000000	3.0	64.0	74.5	74.3	77.3	86.3	26.9	23.6–30.6
Qipengyuania nanhaisediminis CGMCC 1.7715^T	FOWZ00000000	2.9	61.5	69.2	70.7	74.3	84.6	19.2	16.0–22.7
Qipengyuania citrea CIP 107092^T	JALJZT000000000	3.0	64.0	70.2	72.4	75.8	85.1	22.0	18.7–25.6
Qipengyuania aquimaris SW-110^T	WTYI00000000	2.7	61.5	67.8	71.0	74.0	84.2	18.8	15.7–22.4
Qipengyuania algicida KEMB 9005-328^T	WTYA00000000	3.2	60.5	62.0	69.5	73.5	83.9	16.5	13.5–20.0
Qipengyuania soli 6 D36^T	CP064654	2.9	63.0	64.8	70.3	74.4	84.0	19.1	16.0–22.7
Qipengyuania seohaensis SW-135^T	CP024920	2.9	61.5	68.7	70.7	74.2	84.7	18.7	15.6–22.3
Qipengyuania marisflavi KEM-5^T	VCAO00000000	2.7	61.5	69.0	70.9	73.7	83.8	17.9	14.8–21.4
Qipengyuania pacifica NZ-96^T	JAHWXO000000000	3.5	60.5	70.3	73.1	74.9	86.8	18.3	15.2–21.8
Qipengyuania oceanensis MCCC 1A09965^T	WTYN00000000	2.9	63.5	67.6	69.5	74.7	85.6	19.5	16.4–23.1
Erythrobacter sanguineus JCM 20691^T	MUYH00000000	3.0	63.5	63.1	65.9	72.5	83.6	15.7	12.8–19.2
Erythrobacter rubeus KMU-140^T	JACXLC000000000	3.0	60.5	59.9	65.4	72.0	83.5	14.5	11.7–17.9
Erythrobacter ramosus DSM 8510^T	JACICE000000000	3.3	64	59.0	64.8	72.4	83.5	15.5	12.6–18.9
Erythrobacter neustonensis DSM 9434^T	CP016033	3.1	65.0	63.2	64.9	72.5	83.5	15.9	12.9–19.4
Erythrobacter longus DSM 6997^T	JMIW00000000	3.6	57.0	59.8	63.8	70.0	83.3	13.0	10.3–16.3
Erythrobacter litoralis DSM 8509^T	CP017057	3.3	65.0	62.2	65.8	73.4	83.7	16.6	13.6–20.1
Erythrobacter insulae JBTF-M21^T	VHJK00000000	3.0	56.5	62.8	65.3	69.9	83.5	13.2	10.5–16.5
Erythrobacter donghaensis DSM 16220^T	MUYG00000000	3.4	66.0	59.6	64.7	72.9	83.8	16.0	13.1–19.5
Erythrobacter colymbi JCM 18338^T	MUYK00000000	4.3	66.0	55.8	64.1	73.2	84.1	15.5	12.6–19.0
Erythrobacter dokdonensis DSM 17193^T	MUYI00000000	3.0	64.5	61.3	65.7	72.8	83.7	16.5	13.5–20.0
Erythrobacter cryptus DSM 12079^T	AUHC00000000	3.0	67.5	61.6	65.4	72.6	83.4	15.7	12.8–19.2

Continued

Table 1. Continued

PHS-Z21^T vs	Accession number	Genome size (Mbp)	DNA G+C content (%)	POCP	AAI (%)	ANI (%)		GGDC	
						ANIb	ANIm	dDDH (%)*	Model C.I. (%)
Altererythrobacter lutimaris JGD- 16^T	JABWTA000000000	3.0	57.5	60.7	66.3	70.7	83.5	13.8	11.0–17.2
Altererythrobacter epoxidivorans JCS350^T	CP012669	2.8	61.5	66.9	68.3	73.1	84.2	16.7	13.7–20.2
Altererythrobacter ishigakiensis NBRC 107699^T	CP015963	2.7	56.5	61.6	67.1	70.6	83.8	13.5	10.7–16.8
Alteriqipengyuania halimionae LMG 29519^T	WTYR00000000	2.8	63.5	63.9	64.7	72.7	84.3	16.8	13.8–20.3
Alteriqipengyuania abyssalis NZ-12B^T	JAHWXP000000000	3.1	65.0	66.8	66.9	74.1	84.9	18.0	14.9–21.6
Altererythrobacter xiamenensis CGMCC 1.12494^T	FXWG00000000	3.1	61.5	66.6	68.2	73.3	84.0	17.0	13.9–20.5

Note: *Formula 2 based on a generalized linear model (identities/high-scoring segment pair) was used for dDDH.
AAI, average amino acid identity; ANIb, Average nucleotide identity based on blast; ANIm, average nucleotide identity based on MUMmer; C.I., confidence interval; dDDH, digital DNA–DNA hybridization; GGDC, Genome-to-Genome Distance Calculator; POCP, percentage of conserved proteins.

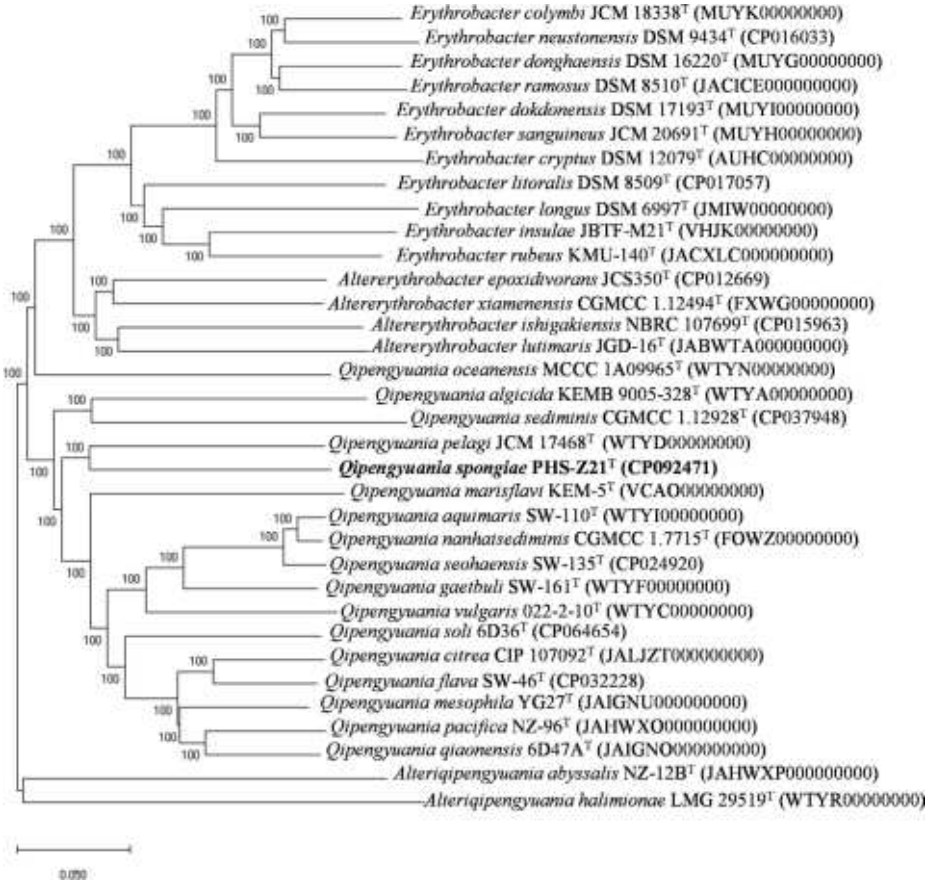

Fig. 2. A neighbor-joining tree based on the partition of 976 single-copy orthologous cluster protein sequences showing the phylogenetic relationships of PHS-Z21^T. Bootstrap values are based on 1000 replicates. Bar, 0.05 substitutions per nucleotide position. GenBank accession numbers are given in parentheses.

peroxiredoxin, peroxidase, dioxygenase and glyoxalase, were detected in this genome. Aerobic anoxygenic photosynthesis is encoded by a series of genes found in PHS-Z21^T (Table S1). As predicted by the antiSMASH 6.0 programme [34], the genome of PHS-Z21^T has four secondary metabolite biosynthetic gene clusters (Table S2). Consistent with *Qipengyuania pelagi* JCM 17468^T, *Qipengyuania algicida* KEMB 9005-328^T, *Qipengyuania qiaonensis* 6D47A^T and *Qipengyuania pacifica* NZ-96^T, PHS-Z21^T also has the potential to produce lasso peptide. The lasso peptides have been recognized as a potential source for biopharmaceutical development because of their antibacterial, antiviral or other biological activities [35].

The phylogenetic trees (Fig. S3 and Fig. 2) indicate that *Qipengyuania pelagi* JCM 17468^T forms a stable cluster in the evolutionary tree with PHS-Z21^T. As shown in Table 1, among the genomes of PHS-Z21^T and 31 type strains of related species, the ANIb values are 70.0–77.3% and the ANIm values are 83.3–86.8%, which are lower than the recommended threshold values for species delineation [36]. The dDDH values between PHS-Z21^T and its neighbouring type strains range from 13.0 % to 26.9%, far below the 70.0% threshold for species delineation [37]. POCP values between the genome of PHS-Z21^T and those of members of the genera *Qipengyuania*, *Erythrobacter*, *Altererythrobacter* and *Alteriqipengyuania* are 62.0–74.5%, 55.8–63.2%, 60.7–66.9% and 63.9–66.8%, respectively, while the AAI values are 68.4–74.3%, 63.8–65.9%, 66.3–68.3% and 64.7–66.9%, respectively. Although there is no generally recognized genus boundary for AAI, there is a proposed genus boundary (70%) for the family *Erythrobacteraceae* [2]. Except for *Qipengyuania sediminis* CGMCC 1.12928^T, *Qipengyuania oceanensis* MCCC 1A09965^T and *Qipengyuania algicida* KEMB 9005-328^T, the AAI values between PHS-Z21^T and other strains of members of the genus *Qipengyuania* are greater than 70% (Table 1). According to the results in the AAI analysis, PHS-Z21^T clearly represents a member of the genus *Qipengyuania*. On the basis of the results of 16S rRNA gene sequence and genome sequence phylogenetic analyses, *Q. flava* SW-46^T, *Q. gaetbuli* SW-161^T, *Q. sediminis* CGMCC 1.12928^T, *Q. pelagi* UST081027-248^T, *Q. mesophila* YG27^T and *Q. qiaonensis* 6D47A^T which were obtained from the Korean Collection for Type Cultures (KCTC) and the Japan Collection of Microorganisms (JCM), were selected as experimental controls.

Gram-staining was performed using a Gram stain kit (Hangzhou Tianhe Microorganism Reagent) as instructed by the manufacturer. Growth at various temperatures (4, 10, 15, 20, 30, 37, 40 and 50 °C) was measured on MA medium after 7 days of incubation. The requirement for and tolerance of NaCl for growth were tested in MB medium 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 (at intervals of pH 0.5 units) was examined in MB buffered with sodium acetate/acetic acid (pH 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 MB after incubation for 7 days by measuring the OD_{600} (Multifunctional enzyme marker-Spark; Tecan). According to the manufacturer's instructions, anaerobic growth was determined in MA medium at 30 °C after 7 days of incubation using the AnaeroPack-Anaero (MGC) [38]. The cells were grown in MB at 30 °C after 7 days of incubation, and cell morphology was observed with a scanning electron microscope (Sirion 200, FEI). Colony appearance was observed on MA medium after 7 days of incubation at 30 °C.

PHS-Z21^T is Gram-stain-negative and strictly aerobic. Scanning electron microscopy reveals that the cells of PHS-Z21^T are about 0.8–0.9 µm long and 0.3–0.4 µm wide, appearing to be rod-shaped without flagella (Fig. S4). Colonies are pale-yellow-pigmented, circular, convex with entire margins and 0.6 mm in diameter after incubation for 7 days on MA at 30 °C. PHS-Z21^T grows at 10–40 °C and shows fast growth at 30 °C. It is able to grow at pH 5.5–9.0 with the highest cell concentration at pH 8.5, and in the presence of 3–9% (w/v) NaCl, with the highest cell concentration at 4% (w/v) NaCl.

Catalase activity was determined by assessing the production of bubbles after adding a drop of 3% (v/v) H_2O_2 to bacterial cultures grown on MA plates at 30 °C for 7 days. Oxidase activity was determined based on the oxidation of tetramethyl-p-phenylenediamine [39] after the bacteria had been cultured on MA plates at 30 °C for 7 days. The cells were cultured on MB medium at 30 °C for 3 days and pigments were extracted with methanol. Then the supernatant was analysed by spectrophotometry to detect the presence of carotenoid pigments and bacterial chlorophyll a. After PHS-Z21^T had been cultured on MB medium at 30 °C for 7 days, the acid production and enzyme activity from 49 carbohydrates were determined using API 50CH and API ZYM systems (bioMérieux). According to the manufacturer's instructions, API 20E and API 20NE systems (bioMérieux) and Biolog GEN III microporous plates were used to determine other biochemical characteristics and carbon source utilization. All these were carried out in duplicate and repeated twice to ensure reproducibility. The susceptibility of PHS-Z21^T to 22 different types of antibiotics was tested. Discs (Bio-Rad) containing the following concentrations 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 and 100 µg piperacillin. The cell suspensions were spread and the discs placed onto MA plates. The plates were incubated at 30 °C for 7 days and the radii of the growth-inhibition zones was measured. The sensitivity of bacteria to antibacterial agents is based on the diameter of the inhibition ring around the paper. The larger the inhibition ring, the more sensitive the bacteria are to the antibacterial agents. The general criteria are: a diameter of the inhibition ring exceeding 20 mm corresponds to extremely sensitive, >15–20 mm to highly sensitive, 10–15 mm to moderately sensitive and less than 10 mm to resistant [40].

PHS-Z21^T is positive for catalase, which is consistent with the phenotypes of *Q. flava* SW-46^T, *Q. gaetbuli* SW-161^T, *Q. sediminis* CGMCC 1.12928^T, *Q. mesophila* YG27^T and *Q. qiaonensis* 6D47A^T. PHS-Z21^T is positive for oxidase, which is inconsistent with the phenotype of *Q. sediminis* CGMCC 1.12928^T. No reduction of nitrate to nitrite or indole production was found in PHS-Z21^T. One absorption peak was detected at 450 nm for PHS-Z21^T, and two absorption peaks were detected at 454 nm and 478 nm for *Q. flava* SW-46^T, *Q. gaetbuli* SW-161^T, *Q. sediminis* CGMCC 1.12928^T, *Q. pelagi* UST081027-248^T, *Q. mesophila* YG27^T and *Q. qiaonensis* 6D47A^T, which indicates that carotenoids exist in these strains. However, the characteristic absorption peak of bacteriochlorophyll a was not detected (Fig. S5). PHS-Z21^T is negative for the hydrolysis of gelatin, which is inconsistent with the phenotype of *Q. sediminis* CGMCC 1.12928^T. It is negative for the assimilation of L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, capric acid, trisodium citrate and phenylacetic acid. The results of the Biolog GENIII microporous plate test indicate that PHS-Z21^T is positive for D-fructose 6-phosphate, gelatin, glycyl L-proline, L-aspartic acid, L-glutamic acid, L-histidine, glucuronamide and *β*-hydroxy-DL butyric acid. Other phenotypic characteristics and those of the closest phylogenetic relatives are listed in Table 2. PHS-Z21^T is resistant to (µg per disc unless otherwise stated) gentamicin (10), kanamycin (30), clindamycin (2), levofloxacin (5), aztreonam (30), ciprofloxacin (5) and tobramycin (10), but susceptible to streptomycin (10), chloramphenicol (30), ceftazidime (30), rifampicin (5), erythromycin (15), clarithromycin (15), tetracycline (30), mezlocillin (5), vancomycin (30), amoxicillin (10), penicillin (10 U), cefotaxime (30), piperacillin (100), ceftriaxone (30) and ampicillin (10).

After cultivation on MB for 7 days at 30 °C, cells of PHS-Z21^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 previously described methods [41, 42]. Polar lipids were extracted and separated on silica gel 60 aluminium-backed thin-layer plates according to the methods of Minnikin *et al.* [43]. α-Naphthol, ninhydrin and molybdatophosphoric acid were used to detect other polar lipids according to the methods described by Tindall [44]. After incubation for 7 days at 30 °C on MA medium, well-grown cells were harvested and fatty acid methyl esters were prepared as described previously [45]. 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) [46, 47].

Table 2. Differential characteristics of PHS-Z21^T and type strains of species of the genus *Qipengyuania*

Strains: 1, PHS-Z21^T; 2, *Q. flava* SW-46^T; 3, *Q. gaetbuli* SW-161^T; 4, *Q. sediminis* CGMCC 1.12928^T; 5, *Q. pelagi* UST081027-248^T; 6, *Q. mesophila* YG27^T; 7, *Q. qiaonensis* 6D47A^T. Data are from this study, except where indicated. +, Positive; –, negative; w, weakly positive; NA, no data available.

Characteristic	1	2	3	4	5	6	7
Growth temperature (°C):							
Range	10–40	10–40	15–40	10–37	20–30	15–40	15–40
Optimum	30	30	30	30	30	30	30
Growth pH:							
Range	5.5–9.0	5.5–9.0	5.5–9.0	5.5–8.5	5.5–9.0	5.5–10.0	5.5–10.0
Optimum	8.5	6.5	5.5	6.0	8.0	7.0	7.0
NaCl concentration (%):							
Range	3–9	0–10	0–10	0–10	0–10	0–10	0–10
Optimum	4	5	1	0	2	3	3
Oxidase activity	+	+	w	–	+	+	+
Assimilation of:							
D-Glucose	–	–	+	–	+	–	–
Maltose	–	+	+	–	+	+	+
Potassium gluconate	w	–	–	–	–	+	–
Adipic acid	+	w	w	+	+	+	w
Malic acid	–	–	w	–	–	+	–
Hydrolysis of:							
Gelatin	–	–	–	+	–	–	–
β-Glucosidase	w	w	+	–	–	+	+
Enzyme activity:							
α-Chymotrypsin	+	w	w	+	+	+	+
α-Glucosidase	–	+	+	–	+	+	+
Acid production form:							
Cellobiose	–	–	–	+	+	–	–
Glycogen	–	–	–	w	–	–	–
Gentiobiose	–	–	–	+	–	–	–
Melezitose	–	–	–	+	–	–	–
Aesculin ferric citrate	–	–	+	–	–	–	–
Inulin	–	–	–	+	–	–	–
D-Fructose	–	+	–	+	+	–	–
D-Xylose	–	–	–	w	+	–	–
D-Ribose	–	–	–	w	+	–	–
D-Arabitol	–	–	–	+	–	–	–
DNA G+C content (%)	64.7	63.3*	64.5†	66.5‡	64.0§	64.5‖	61.5‖

*Data from Lee *et al.* [4].
†Data from Yoon *et al.* [8].
‡Data from Feng *et al.* [1].
§Data from Wu *et al.* [9].
‖Data from Liu *et al.* [5].

Table 3. Differential chemotaxonomic characteristics of PHS-Z21^T and closely related species of the genus *Qipengyuania*

Strains: 1, PHS-Z21^T; 2, *Q. flava* SW-46^T; 3, *Q. gaetbuli* SW-161^T; 4, *Q. sediminis* CGMCC 1.12928^T; 5, *Q. pelagi* UST081027-248^T; 6, *Q. mesophila* YG27^T; 7, *Q. qiaonensis* 6D47A^T. Data are from this study, except where indicated. TR, trace amount (< 1 %); –, not detected; NA, no data available. Q, ubiquinone; DPG, diphosphatidylglycerol; GL, unidentified glycolipid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PL, phospholipid; GPL, glycophospholipid; SGL, sphingoglycolipid.

Component	1	2	3	4	5	6	7
Major menaquinones:							
	Q-10	Q-10*	Q-10†	Q-10‡	Q-10§	Q-10‖	Q-10‖
Fatty acids							
Saturated:							
$C_{15:0}$	–	–	–	7.9	–	1.7	TR
$C_{16:0}$	18.76	9.72	11.64	5.2	4.1	6.8	11.6
$C_{17:0}$	TR	TR	TR	3.2	1.5	1.4	TR
$C_{18:0}$	TR	1.44	TR	–	1.3	TR	1.1
Branched:							
iso-$C_{19:1}$	–	–	–	3.6	–	–	–
Unsaturated:							
$C_{16:1}\omega5c$	TR	–	1.36	–	–	1.4	1.0
$C_{16:1}\omega7c$	–	–	–	5.1	–	–	–
$C_{17:1}\omega6c$	3.96	1.45	10.58	44.2	39.2	20.2	2.6
$C_{17:1}\omega8c$	–	1.66	1.09	5.0	4.1	1.8	–
$C_{18:1}\omega7c$ 11-methyl	9.27	7.41	5.76	–	–	–	4.3
$C_{18:1}\omega5c$	TR	TR	1.26	–	–	TR	TR
$C_{18:1}\omega9c$	–	1.01	TR	–	1.6	–	–
Hydroxylated:							
$C_{14:0}$ 2-OH	2.61	1.75	4.39	1.4	5.1	3.4	4.3
$C_{15:0}$ 2-OH	TR	1.87	1.69	5.4	10.6	5.6	TR
$C_{16:0}$ 2-OH	–	1.26	TR	–	–	4.0	4.6
Summed features¶:							
3	16.4	10.53	10.57	–	2.4	11.4	10.9
8	43.6	54.59	44.4	–	28.1	37.8	54.3
Major polar lipids:							
	DPG, GL, PC, PE, PG, GPL	PC, PE, PG, SG*	NA	PE, PC, PG, SGL, DPG‡	PC, PE, DPG, PG§	PE, PC, PG, SGL, DPG, GL‖	PE, PC, PG, SGL, DPG, GL, PL‖

*Data from Lee *et al.* [4].
†Data from Yoon *et al.* [8].
‡Data from Feng *et al.* [1].
§Data from Wu *et al.* [9].
‖Data from Liu *et al.* [5].
¶Summed features are fatty acids that cannot be resolved reliably from another fatty acid using the chromatographic conditions chosen. The MIDI system groups these fatty acids together as one feature with a single percentage of the total [46]. Summed feature three is comprised of $C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$. Summed feature eight is comprised of $C_{18:1}\omega7c$ and/or $C_{18:1}\omega6c$.

The fatty acid composition of the isolates and the related type strains are shown in Table 3. The main fatty acid components of PHS-Z21^T (>5%) were $C_{18:1}\omega7c$ 11-methyl (9.3%), summed feature 3 ($C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$) (16.4%), $C_{16:0}$ (18.8%) and summed feature 8 ($C_{18:1}\omega7c$ and/or $C_{18:1}\omega6c$) (43.6%). The main components of fatty acids of PHS-Z21^T are generally identical to those of *Q. flava* SW-46^T, *Q. gaetbuli* SW-161^T, *Q. sediminis* CGMCC 1.12928^T, *Q. pelagi* UST081027-248^T, *Q. mesophila* YG27^T and *Q. qiaonensis* 6D47A^T, but differences are observed in the relative proportions of individual components among these strains. In particular, the proportions of of $C_{16:0}$ and $C_{18:1}\omega7c$ 11-methyl in PHS-Z21^T are higher than those in *Q. flava* SW-46^T,

Q. gaetbuli SW-161^T, *Q. sediminis* CGMCC 1.12928^T, *Q. pelagi* UST081027-248^T, *Q. mesophila* YG27^T and *Q. qiaonensis* 6D47A^T. The predominant respiratory quinone of PHS-Z21^T was Q-10, which is typical of members of the genus *Qipengyuania*. The major polar lipids of PHS-Z21^T were composed of diphosphatidylglycerol, glycolipid, phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol and glycophospholipid (Fig. S6). The polar lipids of PHS-Z21^T include diphosphatidylglycerol, phosphatidylcholine, phosphatidylethanolamine and phosphatidylglycerol, which are consistent with those of *Q. sediminis* CGMCC 1.12928^T, *Q. pelagi* UST081027-248^T, *Q. mesophila* YG27^T and *Q. qiaonensis* 6D47A^T. The polar lipids of PHS-Z21^T include an unidentified glycolipid and an unidentified glycophospholipid, which are inconsistent those of with *Q. flava* SW-46^T, *Q. gaetbuli* SW-161^T, *Q. sediminis* CGMCC 1.12928^T and *Q. pelagi* UST081027-248^T (Table 3).

Thus, on the basis of the results described, strain PHS-Z21^T represents a novel species of the genus *Qipengyuania*, for which the name *Qipengyuania spongiae* sp. nov. is proposed.

DESCRIPTION OF *QIPENGYUANIA SPONGIAE* SP. NOV.

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

Cells are Gram-stain-negative, strictly aerobic, non-motile, short rods (0.8–1.0 µm long and 0.3–0.5 µm wide). Colonies are pale-yellow-pigmented, circular, convex with entire margins and 0.6 mm in diameter after incubation for 7 days on MA at 30 °C. Can grow at 10–40 °C (optimum, 30 °C), pH 5.5–9.0 (optimum, pH 8.5) and with 3–9% (w/v) NaCl (optimum, 4%). Catalase and oxidase activity are positive. In the API 20NE system, weakly positive reactions are obtained for glucose fermentation, aesculin hydrolysis and assimilation of potassium gluconate. The assimilation of adipic acid is positive. Negative for the reduction of nitrate to nitrite, denitrification reduction, indole production, arginine dihydrolysis, hydrolysis of urease, gelatin and *β*-galactosidase and assimilation of glucose, L-arabinose, D-mannose, D-mannitol, N-acetyl-glucosamine, maltose, capric acid, malic acid, trisodium citrate and phenylacetic acid. In the API ZYM system, positive reactions are obtained for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, *α*-chymotrypsin, acid phosphatase and naphthol-AS-BI-phosphohydrolase. Lipase (C14) is weakly positive. Negative for *α*-galactosidase, *β*-galactosidase, *β*-glucuronidase, *α*-glucosidase, *β*-glucosidase, *N*-acetyl-*β*-glucosaminidase, *α*-mannosidase and *α*-fucosidase. In the API 50 CH system, no acid is produced from glycerol, erythritol, D- and L-arabinose, D-ribose, D- and L-xylose, D-adonitol, methyl *β*-D-xylopyranoside, D-galactose, D-mannose, L-sorbose, L-rhamnose, dulcitol, inositol, D-mannitol, D-sorbitol, methyl *α*-D-mannopyranoside, methyl *α*-D-glucopyranoside, *N*-acetylglucosamine, amygdalin, arbutin, aesculin ferric citrate, salicin, cellobiose, lactose, melibiose, sucrose, inulin, melezitose, raffinose, starch, glycogen, xylitol, gentiobiose, turanose, lyxose, D-tagatose, D- and L-fucose, D- and L-arabitol, potassium gluconate, potassium 2-ketogluconate, glucose, fructose, maltose and trehalose and potassium 5-ketogluconate. Can use D-fructose-6-phosphate, gelatin, glycyl L-proline, L-aspartic acid, L-glutamic acid, L-histidine, glucuronamide, *β*-hydroxy-DL butyric acid, dextrin (weakly), D-galacturonic acid (weakly), D-glucuronic acid (weakly), *α*-ketoglutaric acid (weakly), tween 40 (weakly), acetoacetic acid (weakly) and acetic acid (weakly) as sole carbon and energy sources, but can't use maltose, trehalose, cellobiose, gentiobiose, sucrose, turanose, stachyose, raffinose, lactose, melibiose, methyl *β*-D-glucoside, D-salicin, *N*-acetyl-D-glucosamine, *N*-acetyl-*β*-D-mannosamine, *N*-acetyl-D-galactosamine, *N*-acetyl neuraminic acid, *α*-D-glucose, D-mannose, D-fructose, D-galactose, 3-methyl D-glucose, D- and L-fucose, L-rhamnose, inosine, D-sorbitol, D-mannitol, D-arabitol, *myo*-inositol, glycerol, D-glucose 6-phosphate, D-aspartic acid, D-serine, L-alanine, L-arginine, L-pyroglutamic acid, L-serine, pectin, L-galactonic acid lactone, D-gluconic acid, mucic acid, quinic acid, D-saccharic acid, *p*-hydroxyphenylacetic acid, methyl pyruvate, L-lactic acid, citric acid, D- and L-malic acid, bromosuccinic acid, *γ*-aminobutryric acid, *α*-hydroxybutyric acid, *α*-ketobutyric acid, propionic acid and formic acid. Resistant to (µg per disc unless otherwise stated) gentamicin (10), kanamycin (30), clindamycin (2), levofloxacin (5), aztreonam (30), ciprofloxacin (5) and tobramycin (10), but susceptible to streptomycin (10), chloramphenicol (30), ceftazidime (30), rifampicin (5), erythromycin (15), clarithromycin (15), tetracycline (30), mezlocillin (5), vancomycin (30), amoxicillin (10), penicillin (10U), cefotaxime (30), piperacillin (100), ceftriaxone (30) and ampicillin (10). The predominant respiratory quinone is Q-10, which is typical of members of the genus *Qipengyuania*. The main fatty acid components (>5%) are $C_{18:1}$ω7c 11-methyl, summed feature 3 ($C_{16:1}$ω7*c* and/or $C_{16:1}$ω6*c*), $C_{16:0}$ and summed feature 8 ($C_{18:1}$ω7*c* and/or $C_{18:1}$ω6*c*). The major polar lipids mainly consist of diphosphatidylglycerol, glycolipid, phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol and glycophospholipid.

The type strain, PHS-Z21^T (=MCCC 1K07849^T= KCTC 92590^T), was isolated from marine sponge *C. kuekenthali* collected from PG Dave's Rock, Philippines. The DNA G+C content of the type strain is 64.7%. The GenBank accession numbers of the 16S rRNA gene and whole genome sequences of PHS-Z21^T are OM970863.1 and CP092471, respectively.

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Author contribution
Z.L. designed the research and project outline. Q.S. identified the sponge sample. M.Z. and J.S. performed 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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