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ORIGINAL PAPER

Sphingomonas hominis sp. nov., isolated from hair of a 21-year-old girl

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Abstract A Gram-stain-negative, aerobic, motile strain, HHU CXW^T, was isolated from hair of a healthy 21-year-old female student of Hohai University, Nanjing, China. The 16S rRNA gene sequence analysis indicated that HHU CXW^T represents a member of the genus *Sphingomonas* with the highest sequence similarity (97.6%) to the type strain *S. aquatilis* JSS7^T. HHU CXW^T grew at 4–35 °C and pH 6–8, with optimum growth at 28 °C and pH 7. Tolerance to NaCl was up to 2% (w/v) with optimum growth in 0.5–1.0% NaCl. The major fatty acids were $C_{16:0}$, $C_{17:1}\omega6c$, $C_{18:1}\omega7c$11-methyl, summed feature 3 ($C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$), and summed feature 8 ($C_{18:1}\omega7c$ and/or $C_{18:1}\omega6c$). The predominant isoprenoid quinone was ubiquinone-10. The polar lipids were diphosphatidylglycerol (DPG), phosphatidylethanolamine (PE), phosphatidylglycerol (PG), sphingoglycolipid (SGL), phosphatidylinositol mannosides (PIM), and an unidentified glycolipid (GL). The DNA G + C content was 67.1%. The average nucleotide identity (ANI) values and digital DNA–DNA hybridization (dDDH) between HHU CXW^T and closely related members of the genus

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Sphingomonas were all below the cut-off level (95–96% and 70%, respectively) for species delineation. On the basis of the phenotypic, phylogenetic and chemotaxonomic characterizations, HHU CXW^T represents a novel species of the genus *Sphingomonas*, for which the name *Sphingomonas hominis* sp. nov. is proposed. The type strain is HHU CXW^T (= KCTC 72946^T = CGMCC 1.17504^T = MCCC 1K04223^T).

Keywords *Sphingomonas hominis* sp. nov · Sphingoglycolipid · bac120 tree

Introduction

Members of *Sphingomonas* within *Alphaproteobacteria* have been isolated from a variety of environments, including human-associated niches, water (fresh and sea), endophytes, terrestrial habitats, sediment (river and subsurface), and rhizosphere soil (Asaf et al. 2020; Aylward et al. 2013). Many isolates possess multifaceted functions ranging from remediation of environmental contaminations to synthetizing highly beneficial phytohormones, such as polycyclic aromatic hydrocarbon (PAH) degradation and sphingan producing. Some species of the genus have also been noted to improve plant-growth during stress conditions such as drought, salinity, and heavy metals in agricultural soil, because of their potential to produce plant growth hormones e.g. gibberellins and indole acetic acid (Asaf et al. 2020; Khan et al. 2014; Yang et al. 2014)

Currently (June, 2020), the genus *Sphingomonas* includes 131 species with validly published and correct names according to the List of Prokaryotic names with Standing in Nomenclature (LPSN) (Parte 2018). A punctiform, orange strain designated HHU CXW^T was isolated from the hair of a healthy 21-year-oldfemale student in class of microbiology experiment, Hohai University, Nanjing, China. The 16S rRNA gene analysis indicated strain HHU CXW^T possibly represent a novel species in genus *Sphingomonas*, and polyphasic taxonomy was performed on this strain subsequently.

Materials and methods

Isolation of the bacterial strain and culture conditions

The hair was collected from a female student in class, and was cut into pieces (< 1 cm in length) before being bespattered on the trypticase soy agar (TSA) medium (BD Diagnostics, Maryland, USA). The plate was incubated at 28 °C for 5 days, and single colonies were selected and cultivated on TSA plates. The strain HHU CXW^T was maintained on TSA medium and stored as aqueous glycerol suspensions (20%, v/v) at − 80 °C.

Phenotypic and biochemical characterization

Colony properties of strain HHU CXW^T were observed on TSA medium. Cell morphology was examined using optical microscopy (Axio Vert A1, Zeiss, German) after 2-day-incubation in trypticase soy broth (TSB) (BD Diagnostics, Maryland, USA) at 28 °C with vibration (180 rpm). Gram staining was determined by using a Gram Stain kit (G1060, Solarbio, China) according to the manufacturer's instructions. Growth was tested at 4, 10, 20, 28, 35 and 37 °C on TSA medium. The pH range for growth was determined by measuring the optical densities (at 600 nm) of TSB cultures after 2 days. The pH was adjusted to pH 4–10 (at intervals of 1.0 pH unit) in a vertical flow clean bench after sterilization, using NaOH and HCl solutions. NaCl tolerance was determined on TSA plates adjusted to 0%, 0.5%, 1%, 2%, 3%, 4% and 5% concentrations (w/v). Motility was determined by observing growth of cells in test tubes containing semisolid TSA medium with 0.5% agar after 3 days of incubation at 28 °C (Cowan and Steel 1996). Additional physiological and biochemical characterization were performed using the Biolog GEN III microtest system (Biolog, USA), API 20NE and API ZYM systems (bioMérieux, France) according to the manufacturer's instructions.

Chemotaxonomic characterisation

The biomass used for analysis of cellular fatty acids, polar lipids, and quinones, were obtained from cultures grown in TSB medium for 2 days at 28 °C. Cellular fatty acids were extracted, methylated and

analyzed by using the Sherlock Microbial Identification System (MIDI) according to previous method (Sasser 1990) and the manufacturer's instructions. Quinones were extracted (Collins et al. 1977) and detected by HPLC (Tamaoka 1986). Polar lipids were determined according to published procedures (Collins and Jones 1980; Minnikin et al. 1979).

Phylogenetic and genotypic analysis

Genomic DNA was isolated by using the Ezup Column Bacteria Genomic DNA Purification Kit (Sangon Biotech, China), and PCR amplification of the 16S rRNA gene sequence were performed with the universal primers 27F and 1492R (Lane 1991). The obtained 16S rRNA gene sequence was analyzed on the EzBioCloud server (https://www.ezbiocloud.net/) (Yoon et al. 2017). Phylogenetic analysis was carried out based on the neighbor-joining (Saitou and Nei 1987), maximum-likelihood (Felsenstein 1981) and maximum-parsimony (Fitch 1971) methods by using the MEGA X software package (Kumar et al. 2018) after multiple alignments of sequences using Clustal version 2.1 program (Larkin et al. 2007). Evolutionary distance matrices of phylogenetic trees were calculated according to Kimura's two-parameter model (Kimura 1980). Bootstrap analysis was performed with 1000 replications (Felsenstein 1985).

Whole-genome sequencing of HHU CXW^T was performed using paired-end sequencing method with Hiseq X platform (Illumina) at Magigene Company, Guangzhou, PR China. Reads of each data set were filtered, and high-quality paired-end reads were assembled using SPAdes version 3.13.0 (Nurk et al. 2013) in UGENE software package (Okonechnikov et al. 2012). Sequences with coverage < 5 or length $<$ 500 bp were excluded from the output genome sequences. The average nucleotide identity (ANI) based on BLAST (ANIb) was determined using JSpecies version 1.2.1 (Richter and Rossello-Mora 2009) while the digital DNA-DNA hybridization (dDDH) values were calculated using the Genome-to-Genome Distance Calculator (GGDC) available at https://ggdc.dsmz.de (Meier-Kolthoff et al. 2013). Formula 2 was applied for the dDDH analysis. Genome annotation was performed by the NCBI Prokaryotic Genome Annotation Pipeline (Tatusova et al. 2016) and eggNOD online server (https://eggnog-mapper.embl.de/) (Huerta-Cepas et al. 2017). The DNA G + C content of the genome was calculate based on the genome sequences.

A gene set called bac120 was used to infer a super phylogeny based on multilocus sequence analysis, which includes 120 universal single-copy genes among domain Bacteria (Parks et al. 2018). This genome-based tree was referred as 'bac120 tree'. Generally, the protein sequences of bac120 from a *S. ginsenosidimutans* strain (Assembly No. GCF_002374835.1) were retrieve from the Genome Taxonomy Database (https://gtdb.ecogenomic.org/); local tblastn program in blast-2.9.0 + software package (Altschul et al. 1997) was used to search homologs in the *Sphingomonas* genomes of interest with 1e-5 evalue and 50% identity as cutoffs; the gene sequences of each gene cluster were aligned by using Clustal Omega version 1.2.1 (Sievers et al. 2011); a maximum-likelihood tree was inferred by applying FastTree 2.1 (Price et al. 2009) to the common genes of the bac120 set among all the genomes of interest after alignment trimming and concatenation. The bac120 tree was constructed on a set of genomes as input data, which was implement by using an in-house Perl script under Ubuntu operation system.

Results and discussion

Phylogenetic and genotypic characteristics

Based on the results from EzBioCloud server, HHU CXW^T shared the highest 16S rRNA gene sequence similarity (99.1%) with two non-type strains *Sphingomonas* sp. JS21-1 (FNZZ01000011) and *Sphingomonas* sp. Mn802worker (AORY01000018) (Aylward et al. 2013), while *S. aquatilis* JSS7^T (AF131295) from the valid published species of *Sphingomona* showed the highest similarity (97.6%) with strain HHU CXW^T. All three phylogenetic trees using neighbor-joining, maximum-likelihood and maximum-parsimony algorithms, indicated that HHU CXW^T clustered with *Sphingomonas* sp. JS21-1 and *Sphingomonas* sp. Mn802worker, forming a distinct but not well supported clade in the genus *Sphingomona* (Fig. 1, S1 and S2). Based on the 16S rRNA gene analysis, *S. aquatilis* KCTC 2881^T (= JSS7^T) was used as a reference for the subsequent tests.

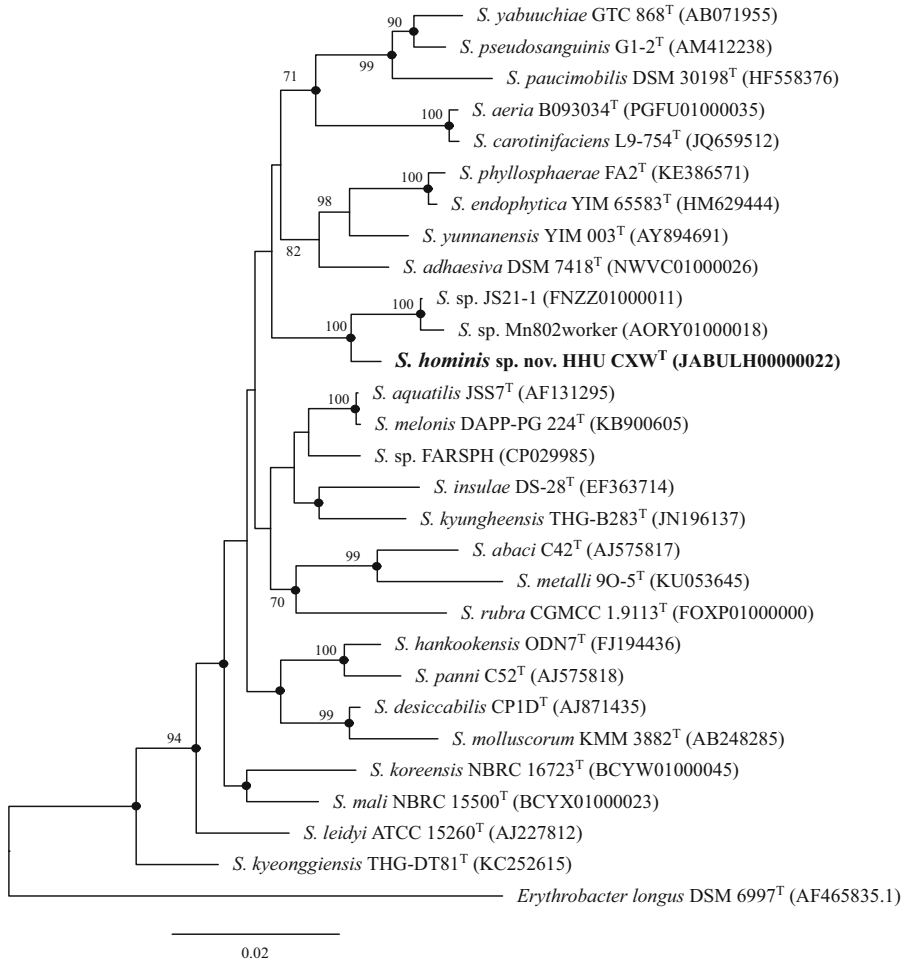

Fig. 1 Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the position of strain HHU CXW^T. Bootstrap values (expressed as percentages of 1000 replications) of above 70% are shown at the branch nodes. The GenBank accession numbers are indicated in the brackets at the end of the tip labels. The black dots denote nodes that were also recovered using the maximum-likelihood and maximum-parsimony methods. *Erythrobacter longus* DSM 6997^T is used as out group. Bar, 0.02 substitutions per nucleotide position

Whole Genome Shotgun project of HHU CXW^T has been deposited at GenBank/EMBL/DDBJ under the accession number JABULH000000000. The draft genome consists of 36 contigs with a total size of 3,558,003 bp and genomic DNA G + C content 67.1%. A total of 3345 coding genes was predicted, of which 736, 1904 and 2802 genes were assigned to Gene Ontology (GO) (The Gene Ontology Consortium 2019), Kyoto Encyclopedia of Genes and Genomes (KEGG) (https://www.kegg.jp/) and Cluster of Orthologous Groups (COG) (Galperin et al. 2015) database, respectively. Future analysis of the KEGG annotation results from eggNOD server suggested that there were 28 genes associated with flagellar assembly (ko02040), 35 with biofilm formation (ko05111, ko02025 and ko02026), 7 with carotenoid biosynthesis (ko00361), respectively. Dramatically, strain HHU CXW^T harbors a *crtX* gene (locus tag HRV97_09920), which could encode an enzyme catalyzing zeaxanthin to produce zeaxanthin diglucoside, and is rare present among *Sphingomonadales* (Fig. 2) (Siddaramappa et al. 2018).

According to the taxa of 16S rRNA gene tree, 15 *Sphingomonas* genomes together with genomes of strain HHU CXW^T and *Erythobacter longus* DSM 6997^T (JMIW01), were used to infer a robust tree based on the bac120 gene set, which was named bac120 tree in this study. A total of 69 genes from

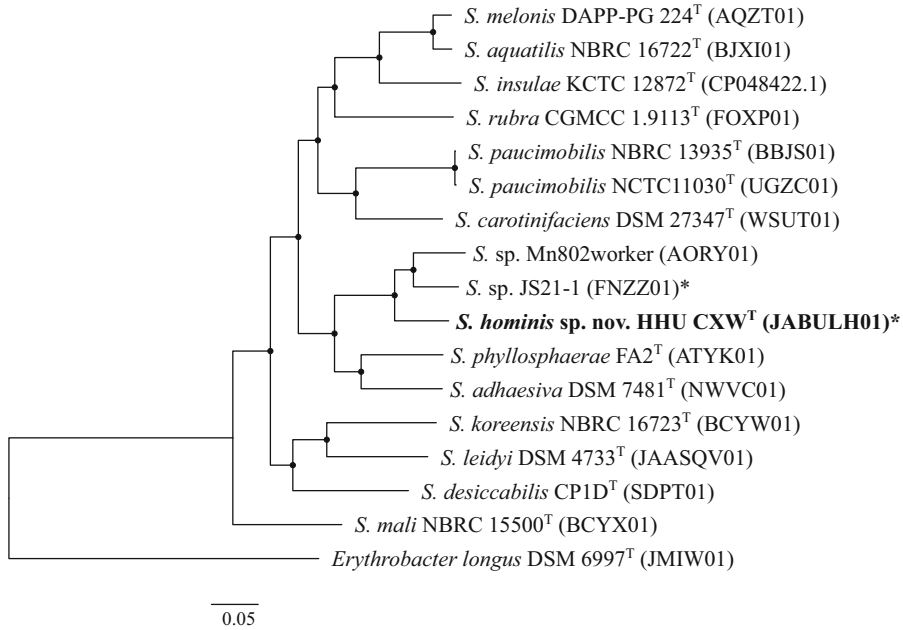

Fig. 2 Maximum-likelihood phylogenetic tree based on 69 common bac120 gene sequences showing the position of strain HHU CXW^T. Bootstrap values of 100% are marked with black dots at the branch nodes. Tip labels followed by a asterisk indicated a *crtX* homolog was found in the genome, and the GenBank accession number or whole genome shotgun (WGS) project number is indicated in the bracket. *Erythrobacter longus* DSM 6997 ^{T} is used as out group. Bar, 0.05 substitutions per nucleotide position

bac120 gene set was detected in all the 17 genomes and used in further analysis. The bac120 tree was well supported on all the branches (Fig. 2). The same as shown in the 16S rRNA gene tree, strains HHU CXW^T is close related to isolates *Sphingomonas* sp. JS21-1 and *Sphingomonas* sp. Mn802worker. Additionally, the bac120 tree clarified that *S. phyllosphaerae* (Huang et al. 2012; Rivas et al. 2004) and *S. adhaesiva* (Feng et al. 2018) were the currently known valid species sharing the most recent ancestor with strain HHU CXW^T.

The ANIb values between HHU CXW^T and the close related strains *Sphingomonas* sp. JS21-1 (FNZZ01), *Sphingomonas* sp. Mn802worker (AORY01) and *S. aquatilis* NBRC 16722^T (BJXI01) were all below 85%, while the dDDH values were 28.5%, 27.9%, and 21.1%, respectively (Table 1). These values are lower than the proposed and generally accepted species boundaries of 70% for dDDH and 95–96% for ANI (Goris et al. 2007; Richter and Rossello-Mora 2009), which suggest that strain HHU CXW^T represents a novel species of the genus *Sphingomona*. Furthermore, *Sphingomonas* sp. JS21-1 and *Sphingomonas* sp. Mn802workers should represent two novel species of *Sphingomonas*, too, according to the ANIb and dDDH analysis (Table 1).

Table 1 The average nucleotide identity based on BLAST (ANIb) and digital DNA-DNA hybridization (dDDH) analysis among HHU CXW^T and closely related strains

Strain	1	2	3	4
1	–	84.3/84.2	83.9/83.1	75.6/75.2
2	28.5	–	86.4/87.2	75.6/74.9
3	27.9	33.3	–	74.4/74.6
4	21.1	20.7	20.5	–

Taxa: 1, HHU CXW^T; 2, *Sphingomonas* sp. JS21-1; 3, *Sphingomonas* sp. Mn802worker; 4, *Sphingomonas aquatilis* KCTC 2881 ^{T}

The ANIb results are shown above diagonal, and two reciprocal analysis are separated by a solidus. The dDDH results are shown below diagonal

Phenotypic characteristics

Cells of HHU CXW^T were Gram-staining negative, motile, aerobic and rod shaped. The colonies on TSA

medium were orange, circular, smooth, and opaque. Growth of HHU CXW^T was observed at 4–35 °C (optimum temperature, 28 °C) and at pH 6–8 (optimum, pH 7). The tolerance to NaCl was up to 2% (w/v) with optimum growth in the presence of 0.5–1.0% NaCl. HHU CXW^T was catalase-positive and oxidase-negative. In the API ZYM tests, HHU CXW^T was positive for acid phosphatase, alkaline phosphatase, cystine arylamidase, esterase (C4), esterase lipase (C8), leucine arylamidase, naphthol-AS-BI-phosphohydrolase, trypsin, valine arylamidase, and α-glucosidase, but negative for lipase (C14), *N*-acetyl-β-glucosaminidase, α-chymotrypsin, α-fucosidase, α-galactosidase, α-mannosidase, β-galactosidase, β-glucuronidase, and β-glucosidase. In the API 20NE tests, HHU CXW^T was positive for hydrolysis of esculin and nitrate reduction, but negative for arginine dihydrolase, D-glucose fermentation, indole production, and hydrolysis of 4-nitrophenyl β-D-galactopyranoside, gelatin, and urea. Additionally, HHU CXW^T was negative for assimilation of adipic acid, capric acid, D-glucose, D-maltose, D-mannitol, D-mannose, L-arabinose, malic acid, *N*-acetylglucosamine, phenylacetic acid, potassium gluconate, and trisodium citrate. In the BIOLOG GEN III tests, HHU CXW^T was negative for all the tests, while the reference strain *S. aquatilis* KCTC 2881^T was positive for utilization of α-D-glucose, glycyl-L-prolin, D-glucuronic acid, and L-glutamic acid, and was resistant to pH 6, 4% NaCl, lincomycin, and tetrazolium blue.

Chemotaxonomic characteristics

The major fatty acids (> 5%) of strain HHU CXW^T were $C_{16:0}$, $C_{17:1}\omega6c$, $C_{18:1}\omega7c11$-methyl, summed feature 3 ($C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$), and summed feature 8 ($C_{18:1}\omega7c$ and/or $C_{18:1}\omega6c$) (Table S1). The predominant isoprenoid quinone detected in strain HHU CXW^T was ubiquinone-10, which is the typical quinone reported for the genus *Sphingomonas* (Li et al. 2019). The polar lipids of strain HHU CXW^T mainly consisted of diphosphatidylglycerol (DPG), phosphatidylethanolamine (PE), phosphatidylglycerol (PG), sphingoglycolipid (SGL), and phosphatidylinositol mannosides (PIM), which is similar to that of *S. aquatilis* KCTC 2881^T (Fig. S3). Minor amount of phosphatidylcholine (PC) and an unidentified polar lipid (L) were also detected.

Taxonomic conclusions

Strain HHU CXW^T shared < 98% similarities of 16S rRNA gene with valid described species and formed a distinct clade in the genus *Sphingomonas* (Fig. 1, S1, and S2), and the ANIb and dDDH analysis between HHU CXW^T and closely related species were all below the generally accepted species boundaries 95–96% and 70%, respectively (Table 1). Furthermore, strain HHU CXW^T showed differences from closely related type strains *S. aquatilis* KCTC 2881 ^{T} and *S. phyllosphaerae* FA2^T on colony color, nitrate reduction, assimilation of several carbon resource, diagnostic phospholipids and major fatty acids (Table 2). Based on the genotypic, phenotypic and chemotaxonomic features, strain HHU CXW^T can be considered as a novel species of the genus *Sphingomonas*, for which the name *Sphingomonas hominis* sp. nov. is proposed.

Description of *Sphingomonas hominis* sp. nov.

Sphingomonas hominis (ho'mi.nis L. gen. masc. n. *hominis*, of a human being, named for the host from whose hair this species is found).

Cells are Gram-staining negative, aerobic, rod shaped, and motile. Colonies are orange, circular, smooth and opaque on TSA medium. Growth occurs at 4–35 °C (optimum temperature, 28 °C) and at pH 6–8 (optimum, pH 7). The tolerance to NaCl was up to 2% (w/v) with optimum concentration 0.5–1.0%. Catalase-positive and oxidase-negative. In the API ZYM tests, positive for acid phosphatase, alkaline phosphatase, cystine arylamidase, esterase (C4), esterase lipase (C8), leucine arylamidase, naphthol-AS-BI-phosphohydrolase, trypsin, valine arylamidase, and α-glucosidase; but negative for lipase (C14), *N*-acetyl-β-glucosaminidase, α-chymotrypsin, α-fucosidase, α-galactosidase, α-mannosidase, β-galactosidase, β-glucosidase, and β-glucuronidase. In the API 20NE tests: positive for nitrate reduction and hydrolysis of esculin; negative for arginine dihydrolase, D-glucose fermentation, indole production, and hydrolysis of 4-nitrophenyl β-D-galactopyranosidegelatin, and urea; negative for assimilation of adipic acid, capric acid, D-glucose, D-maltose, D-mannitol, D-mannose, L-arabinose, malic acid, *N*-acetylglucosamine, phenylacetic acid, potassium gluconate, and trisodium citrate. The major fatty acids are $C_{16:0}$, $C_{17:1}\omega6c$, $C_{18:1}\omega7c11$-

Table 2 Differential phenotypic and chemotaxonomic characteristics of strains HHU CXW^T and closely related type strains

Characteristic	1	2	3*
Colony colour	Orange	Yellow	Yellow
Nitrate reduction	+	−	−
Assimilation of			
D-glucose	−	+	+
L-arabinose	−	+	+
D-mannose	−	+	+
N-acetylglucosamine	−	+	+
D-maltose	−	+	+
Trypsin	+	−	n.d
α-chymotrypsin	−	+	n.d
β-galactosidase	−	+	n.d
β-glucosidase	−	+	n.d
Diagnostic phospholipids[a]	DPG, PE, PG, SGL, GL, PIM, (PC), (L)	DPG, PE, PG, SGL, GL, PIM, (2 L)	PE, PG, DPG, PC, SGL, (PME), (PDE), PL1, PL2
DNA G + C content (mol%)	67.1	67.1	67.2

Taxa: 1, strain HHU CXW^T; 2, *Sphingomonas aquatilis* KCTC 2881 ^{T}; 3, *Sphingomonas phyllosphaerae* FA2^T

*Data from Huang et al. (2012) and Rivas et al. (2004)

[a]*DPG* diphosphatidylglycerol, *GL* unidentified glycolipid, *L* unidentified polar lipid, *PC* phosphatidylcholine, *PE* phosphatidylethanolamine, *PG* phosphatidylglycerol, *PL* unidentified phospholipid, *PIM* phosphatidylinositol mannosides, *SGL* sphingoglycolipid. Components in parentheses were detected in small amounts. + , Positive; −, negative; n.d., no data available

methyl, summed feature 3 ($C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$), and summed feature 8 ($C_{18:1}\omega7c$ and/or $C_{18:1}\omega6c$). The predominant isoprenoid quinone is ubiquinone-10. The polar lipids are diphosphatidylglycerol (DPG), phosphatidylethanolamine (PE), phosphatidylglycerol (PG), sphingoglycolipid (SGL), phosphatidylinositol mannosides (PIM), and an unidentified glycolipid (GL).

The type strain, HHU CXW^T (= KCTC 72946^T = CGMCC 1.17504^T = MCCC 1K04223^T), was isolated from the hair of a 21-year old female student of Hohai University, Nanjing, China. The GenBank/EMBL/DDBJ accession numbers for the genome sequence is JABULH000000000. The DNA G + C content is 67.1%.

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Author contributions DFZ and ZZ designed research and project outline. XWC, DFZ, and AHZ performed isolation, deposition and polyphasic taxonomy. DFZ, and JH performed genome analysis. DFZ and AHZ drafted the manuscript. WJL revised the manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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

References

Altschul SF, Madden TL, Schaffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res 25:3389–3402

Asaf S, Numan M, Khan AL, Al-Harrasi A (2020) *Sphingomonas*: from diversity and genomics to functional role in environmental remediation and plant growth. Crit Rev Biotechnol 40:138–152

Aylward FO, McDonald BR, Adams SM et al (2013) Comparison of 26 sphingomonad genomes reveals diverse

environmental adaptations and biodegradative capabilities. Appl Environm Microbiol 79:3724–3733

Collins MD, Jones D (1980) Lipids in the classification and identification of coryneform bacteria containing peptidoglycans based on 2, 4-diaminobutyric acid. J Appl Microbiol 48:459–470

Collins MD, Pirouz T, Goodfellow M, Minnikin DE (1977) Distribution of menaquinones in actinomycetes and corynebacteria. J Gen Microbiol 100:221–230

Cowan ST, Steel KJ (1996) Manual for the identification of medical bacteria. Cambridge University Press, London, p 232

Felsenstein J (1981) Evolutionary trees from DNA sequences: a maximum likelihood approach. J Mol Evol 17:368–376

Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39:783–791

Feng GD, Yang SZ, Zhu HH, Li HP (2018) Emended descriptions of the species *Sphingomonas adhaesiva* Yabuuchi et al. 1990 and *Sphingomonas ginsenosidimutans* Choi et al. 2011. Int J Syst Evol Microbiol 68:970–973

Fitch WM (1971) Toward defining the course of evolution: minimum change for a specific tree topology. Syst Biol 20:406–416

Galperin MY, Makarova KS, Wolf YI, Koonin EV (2015) Expanded microbial genome coverage and improved protein family annotation in the COG database. Nucleic Acids Res 43:D261–269

Goris J, Konstantinidis KT, Klappenbach JA, Coenye T, Vandamme P, Tiedje JM (2007) DNA–DNA hybridization values and their relationship to whole-genome sequence similarities. Int J Syst Evol Microbiol 57:81–91

Huang HY, Li J, Zhao GZ et al (2012) *Sphingomonas endophytica* sp. nov., isolated from *Artemisia annua* L. Int J Syst Evol Microbiol 62:1576–1580

Huerta-Cepas J, Forslund K, Coelho LP, Szklarczyk D, Jensen LJ, von Mering C, Bork P (2017) Fast genome-wide functional annotation through orthology assignment by eggNOG-mapper. Mol Biol Evol 34:2115–2122

Khan AL, Waqas M, Kang S-M et al (2014) Bacterial endophyte *Sphingomonas* sp. LK11 produces gibberellins and IAA and promotes tomato plant growth. J Microbiol 52:689–695

Kimura M (1980) A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J Mol Evol 16:111–120

Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: molecular evolutionary genetics analysis across computing platforms. Mol Biol Evol 35:1547–1549

Lane DJ (1991) 16S/23S rRNA sequencing. In: Stackebrandt E, Goodfellow M (eds) Nucleic acid techniques in bacterial systematics. Wiley, Chichester, pp 115–175

Larkin MA, Blackshields G, Brown NP et al (2007) Clustal W and Clustal X version 2.0. Bioinformatics 23:2947–2948

Li YQ, Rao MPN, Zhang H et al (2019) Description of *Sphingomonas mesophila* sp. nov., isolated from *Gastrodia elata* Blume. Int J Syst Evol Microbiol 69:1030–1034

Meier-Kolthoff JP, Auch AF, Klenk HP, Goker M (2013) Genome sequence-based species delimitation with confidence intervals and improved distance functions. BMC Bioinform 14:60

Minnikin DE, Collins MD, Goodfellow M (1979) Fatty acid and polar lipid composition in the classification of *Cellulomonas*, *Oerskovia* and related taxa. J Appl Microbiol 47:87–95

Nurk S, Bankevich A, Antipov D et al (2013) Assembling single-cell genomes and mini-metagenomes from chimeric MDA products. J Comput Biol 20:714–737

Okonechnikov K, Golosova O, Fursov M, Team U (2012) Unipro UGENE: a unified bioinformatics toolkit. Bioinformatics 28:1166–1167

Parks DH, Chuvochina M, Waite DW, Rinke C, Skarshewski A, Chaumeil PA, Hugenholtz P (2018) A standardized bacterial taxonomy based on genome phylogeny substantially revises the tree of life. Nat Biotechnol 36:996–1004

Parte AC (2018) LPSN - List of Prokaryotic names with Standing in Nomenclature (bacterio.net), 20 years on. Int J Syst Evol Microbiol 68:1825–1829

Price MN, Dehal PS, Arkin AP (2009) FastTree: computing large minimum evolution trees with profiles instead of a distance matrix. Mol Biol Evol 26:1641–1650

Richter M, Rossello-Mora R (2009) Shifting the genomic gold standard for the prokaryotic species definition. P Natl Acad Sci USA 106:19126–19131

Rivas R, Abril A, Trujillo ME, Velazquez E (2004) *Sphingomonas phyllosphaerae* sp. nov., from the phyllosphere of Acacia caven in Argentina. Int J Syst Evol Microbiol 54:2147–2150

Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4:406–425

Sasser M (1990) Identification of bacteria by gas chromatography of cellular fatty acids, MIDI Technical Note 101. Microbial ID, Inc, Newark

Siddaramappa S, Viswanathan V, Thiyagarajan S, Narjala A (2018) Genomewide characterisation of the genetic diversity of carotenogenesis in bacteria of the order *Sphingomonadales*. Microb Genom 4:e000172

Sievers F, Wilm A, Dineen D et al (2011) Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. Mol Syst Biol 7:539

Tamaoka J (1986) Analysis of bacterial menaquinone mixtures by reverse-phase high-performance liquid chromatography. Methods Enzymol 123:251–256

Tatusova T, DiCuccio M, Badretdin A et al (2016) NCBI prokaryotic genome annotation pipeline. Nucleic Acids Res 44:6614–6624

The Gene Ontology Consortium (2019) The gene ontology resource: 20 years and still GOing strong. Nucleic Acids Res 47:D330–D338

Yang S, Zhang X, Cao Z et al (2014) Growth-promoting *Sphingomonas paucimobilis* ZJSH1 associated with *Dendrobium officinale* through phytohormone production and nitrogen fixation. Microbial Biotechnol 7:611–620

Yoon SH, Ha SM, Kwon S, Lim J, Kim Y, Seo H, Chun J (2017) Introducing EzBioCloud: a taxonomically united database of 16S rRNA gene sequences and whole-genome assemblies. Int J Syst Evol Microbiol 67:1613–1617