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ORIGINAL PAPER

Sphingomonas montana sp. nov., isolated from a soil sample from the Tanggula Mountain in the Qinghai Tibetan Plateau

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Abstract An orange pigmented, Gram-staining negative, aerobic, motile, rod-shaped bacterium isolated from a soil from the Tanggula Mountain, China was studied using a polyphasic approach. Based on 16S rRNA gene sequence similarity, strain W16RD^T was found to be closely related to *Sphingomonas prati* DSM 103336^T (99%), *Sphingomonas fennica* DSM 13665^T (97.21%), followed by *Sphingomonas laterariae* DSM 25432^T (96.44%), *Sphingomonas haloaromaticamans* CGMCC 1.10206 ^{T} (96.36%) and *Sphingomonas formosensis* DSM 24164^T (96.06%). The strain was found to be catalase and oxidase positive and was found to grow optimally at temperatures of 20–25 °C, pH 8 and tolerated NaCl concentration up to 1% (w/v). The major fatty acids identified were summed feature eight comprising $C_{18:1}$ ω 7*c* and/or $C_{18:1}$ ω 6*c* (39.2%), summed feature three comprising of $C_{16:1}$ ω7*c and*/or $C_{16:1}$ ω6*c* (36.7%) and $C_{16:0}$ (7.0%). The polar lipids detected were phosphatidylcholine, sphingoglycolipid, phosphatidylglycerol, phosphatidylethanolamine, diphosphatidylglycerol, phosphatidyldimethylethanolamine, phosphatidylmonomethylethanolamine, and three unidentified lipids. The strain possessed ubiquinone-10 (Q-10) as the predominant respiratory quinone. Along with other distinguishing characteristics, we also describe the draft genome of strain W16RD^T. The final assembled draft genome sequence is 3,722,743 bp with 3390 coding and 48 RNA (45 tRNA and 3 rRNA) genes. The DNA G+C content of the genomic DNA was determined to be 67%. The DNA–DNA relatedness value between the strain W16RD^T and its closest phylogenetic relatives *S. prati* DSM 103336^T, *S. fennica* DSM 13665^T, *S. laterariae* DSM 25432^T, and *S. haloaromaticamans* CGMCC 1.10206^T were 52.17, 47.60, 20.93 and

The Genbank accession number for the 16S rRNA gene sequence of strain W16RD^T is KU535674.
The Genbank accession number for the whole genome sequence of the strain W16RD^T is MOLY00000000.

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17.09% respectively. The strain W16RD^T could be distinguished genotypically and phenotypically from the recognized species belonging to the genus *Sphingomonas* and thus represents a novel species, for which the name *Sphingomonas montana* sp. nov. is proposed. The type strain is W16RD^T (=CGMCC 1.15646^T = DSM 103337^T).

Keywords *Sphingomonas montana* sp.nov · Soil · 16S rRNA gene · Whole genome

Introduction

The genus *Sphingomonas*, which belongs to Alphaproteobacteria (Anzai et al. 2000), was originally described by Yabuuchi et al. (1990) taking *Sphingomonas paucimobilis* as the type species. Later Takeuchi et al. (2001) reclassified the genus *Sphingomonas* into four separate genera, as *Sphingomonas*, *Sphingobium*, *Novosphingobium*, and *Sphingopyxis* on the basis of phylogenetic, chemotaxonomic and phenotypic characteristics. The genus has been subsequently emended by others (Busse et al. 2003; Chen et al. 2012). Members of the genus *Sphingomonas* are Gram-negative, strictly aerobic, chemoheterotrophic, non-spore forming, and either motile or non-motile rods which can develop into off white, yellow or orange colored colonies. Chemotaxonomically they are distinguished by the presence of ubiquinone 10 (Q-10) and 2-hydroxy fatty acids, as well as the absence of 3-hydroxy fatty acids (Zhang et al. 2005), and they contain sphingoglycolipids as major components of their outer membrane. Species of the genus *Sphingomonas* share a wide range of environmental habitats such as soils, air (Kim et al. 2014; Lee et al. 2015; Margesin et al. 2012), ice (Liu et al. 2015), plants (Huang et al. 2012), the phyllosphere (Talà et al. 2013), marine bivalves (Romanenko et al. 2007) and ground water (Wittich et al. 2007). The genus *Sphingomonas* has been exploited for many biotechnological applications, such as the bioremediation of environmental contaminants (Coppotelli et al. 2008), or the production of extracellular polymers (Pollock 1993). At the time of writing, the genus *Sphingomonas* comprises of 95 recognized species (http://www.bacterio.net/sphingomonas.html). Here, we report on the taxonomic characterization of a bacterial strain designated as W16RD^T which was isolated from a soil sample collected from the Tanggula Mountain in the Qinghai- Tibetan Plateau. Based on the phenotypic, phylogenetic, chemotaxonomic and genotypic results, strain W16RD^T represents a novel species of the genus *Sphingomonas.*

Materials and methods

Isolation of bacterium and culture conditions

In a study carried out to explore the diversity of culturable soil bacteria from different regions of the Tibetan Plateau, strain W16RD^T was isolated from a soil from the Tanggula Mountain, China. The soil sample was diluted with R2A broth and spread on R2A agar (Difco, USA) plates that were incubated at 10 °C for 4–7 days. The colonies developed were restreaked several times on R2A agar to obtain a pure culture. A single colony of the isolate W16RD^T was transferred to a fresh R2A agar plate and incubated at 25 °C, followed by routine subculturing. Purity was confirmed by microscopic examination. The strain was then stored at −80 °C in glycerol (15%, v/v). The strain has been deposited to the China General Microbiological Culture Collection Center (CGMCC 1.15646^T) and German Collection of Microorganisms and Cell cultures (DSM 103337^T). Reference strains, *Sphingomonas prati* DSM 103336^T, *Sphingomonas fennica* DSM 13665^T, *Sphingomonas laterariae* DSM 25432^T and *Sphingomonas formosensis* DSM 24164^T, were obtained from DSMZ (German Collection of Microorganisms and Cell cultures), and *Sphingomonas haloaromaticamans* CGMCC 1.10206^T was obtained from CGMCC (China General Microbiological Culture Collection Center). All reference strains were cultured in parallel with the strain W16RD^T under the same conditions throughout all experiments.

Phenotypic and biochemical characteristics

Cell morphology and the presence of flagella were determined by transmission electron microscopy (JEM-1400; JEOL) with fresh cells at their exponential phase of growth on R2A agar. The motility test

was carried out by stab inoculation of the bacterium on R2A medium with 0.5% agar (Gu et al. 2015). Gram staining was performed following the method of Smibert and Krieg (1994). Oxidase activity was examined by using an oxidase reagent (bioMerieux) and catalase activity was interpreted by the production of oxygen bubbles in 3% (v/v) aqueous hydrogen peroxide solution. Optimum conditions for the growth including temperature (0, 4, 10, 15, 20, 25, 30 and 35 °C), pH (4.0–11.0 at interval of 1.0 pH unit) and NaCl concentration [(0, 1, 2, 3, 4, 5 and 6% (w/v)] were determined on R2A broth over an incubation period of 7 days at 25 °C. The pH values were adjusted using the buffers 0.1 M citric acid/0.1 M sodium citrate (for the pH 4.0–5.0), 0.1 M KH_2PO_4/0.1 M NaOH (for the pH 6.0-8.0), 0.1 M $NaHCO_3$/0.1 M Na_2CO_3 (for the pH 9.0–10.0) and 0.05 M Na_2HPO_4/0.1 M NaOH (for the pH 11.0) (Zhu et al. 2015) and different concentrations of NaCl were obtained by supplementing the growth medium with 1–6% (w/v at interval of 1% unit) NaCl. Other physiological and biochemical properties were determined with API 20NE and API ZYM strips following the instructions provided by the manufacturer (bioMerieux,).

DNA extraction, PCR amplification, and whole genome sequencing

The genomic DNA was extracted according to the method described by Marmur (1961) and its purity was assessed using a Nano-Drop spectrophotometer (2000c; Thermo). The universal primers 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-CGGTTACCTTGTTACGACTT-3′) were used to amplify the 16S rRNA gene from strain W16RD^T (Embley 1991) and the purified PCR products were sequenced by Sangon Biotech (Beijing). The 16S rRNA gene sequence of the strain was then compared with the available sequences in the EzTaxon-e-server (http://eztaxon-e.ezbiocloud.net) (Kim et al. 2012) and GenBank using BLAST program (NCBI) in order to determine its phylogenetic affiliation.

The strain was further subjected to whole genome sequencing, for which genomic DNA of the bacterium was extracted using a TIANamp bacterial DNA kit (Tiangen Biotech (Beijing) Co. Ltd) according to the manufacturer's instructions. In brief, a single colony from the pure culture of the strain W16RD^T was cultured in R2A broth tubes at the temperature of 25 °C until abundant growth was observed and then subjected to DNA extraction. The extracted DNA was prepared for whole-genome sequencing, which was performed at the Microbial Genome Research Center, Institute of Microbiology, Chinese Academy of Sciences, Beijing. In brief, the genomic DNA was fragmented by ultrasonication, and the DNA fragments were subjected to the whole-genome sequencing workflow of the Illumina HiSeq 2000 system.

Genome assembly and annotation

Genome assembly was performed by SOAPdenovo (http://soap.genomics.org.cn) and the gaps were closed by using SOAP GapCloser (http://soap.genomics.org.cn). Glimer 3.02 (Delcher et al. 2007) served for the purpose of the prediction of open reading frame and tRNAscan-SE and RNAmmer (Lagesen et al. 2007) were used for the identification of tRNA and rRNA respectively. The annotation of the genome sequences was done with the help of the RAST program (Rapid Annotation using Subsystem Technology) (Aziz et al. 2008). The annotation results were then checked and compared with the database of NCBI-NR (http://www.ncbi.nlm.nih.gov/), COG (Tatusov et al. 2003).

This Whole Genome Shotgun project has been deposited at DDBJ/ENA/GenBank under the accession MOLY00000000. The version described in this paper is version MOLY01000000.

Comparative genomics

For the comparative analysis, genome sequences of 47 previously reported isolates (GenBank accession number NZ_MIPT01000001, NZ_FUYM01000001, NZ_LDTD01000060, NZ_CAVK010000039, NC_014006, NZ_LDTB01000010, NZ_LQQO01000001, NZ_CP010836, NZ_LT840185, NZ_CP018820, NZ_BBJS01000014, NZ_LDUA01000001, NZ_JFYY01000001, NZ_LYMJ01000001, FOXP01000001, NZ_CP009571, NZ_LDTF01000007, NC_008048, NZ_BCTR01000001, NZ_CP014168, NZ_JH584235, NZ_AGFU01000002, NZ_KK073876, NZ_AQUJ01000001, NZ_KB900605, NZ_ATTG01000001, NZ_ATTI01000001, NZ_KE386571, NZ_CP006644, NZ_AHKO01000024, NC_009511, NZ_BCWT01000001, NZ_BCYU010

00001, NZ_BBWU01000048, BCYW01000001, NZ_BCYX01000001, NZ_LQCK02000001, NZ_BBPI01000034, NZ_BCYY01000001, NZ_BCYZ01000002, NZ_BCTY01000001, NZ_LDTC01000066, NZ_LDTE01000010, BCTZ01000004, NZ_JXTP01000018, NZ_JQMC01000001, NZ_JONN01000001) were downloaded from the NCBI website and the average nucleotide identity between these genomes and the genome of the type strain W16RD^T was calculated based on fragmented alignments by using a Perl script.

Phylogenetic analysis

The 16S rRNA gene sequence (1483 bp) was extracted from the draft genome sequence of strain W16RD^T, and it was compared with the available sequences in EzTaxon-e-server (http://eztaxon-e.ezbiocloud.net) and GenBank using BLAST program (NCBI) to determine its phylogenetic affiliation. The 16S rRNA gene sequences were then analyzed with the software package MEGA 5.05 (Tamura et al. 2011). The phylogenetic tree was constructed using the neighbor-joining method with bootstrap values based on 1000 replications.

Cellular fatty acids, polyamines, isoprenoid quinones and polar lipid analysis

For the analysis of cellular fatty acids, strain W16RD^T and the five reference strains were grown on R2A agar at 25 °C for 2–4 days, and cells were collected at their exponential phase of growth. The extraction and analysis of fatty acid methyl esters were performed according to the standard protocol of the Microbial Identification System (MIDI, version 6.0). The standardization of the physiological age of strain W16RD^T and its reference strains was done following the protocol by MIDI (http://www.microbialid.com/PDF/TechNote_101.pdf). For the determination of isoprenoid quinone and polar lipids, cells were harvested after 72 h of growth at 25 °C. Isoprenoid quinones were analyzed as per the method described by Hiraishi et al. (1998) by using a Waters Acquity Ultra Performance LC (UPLC)-Q-TOF–MS spectrometer in electrospraying ionization mode (Romano et al. 2006). The extraction and analysis of polar lipids were performed by two-dimensional TLC (Altenburgera et al. 1996; Tindall 1990). Total lipid material and specific functional groups were detected by using molybdatophosphoric acid (total lipids), molybdenum blue (phosphor group), ninhydrin (amino group), and α-naphthol (glycolipid). To determine the polyamine content of the strain W16RD^T, the draft genome of the strain was compared with the spermidine synthase gene [key enzyme responsible for spermidine biosynthesis (Lee et al. 2009)] of *Sphingomonas wittichii* RW1 using BLAST (Altschul et al. 1990). All the proteins of the strain W16RD^T and homospermidine synthase proteins of *S. wittichii* RW1 were compared against the Pfam-A database version 27.0 (Finn et al. 2014) using the HMMER 3.0 software (Eddy 2008).

DNA–DNA hybridisation and determination of DNA G+C content

DNA–DNA hybridisation experiments were carried out between strain W16RD^T and four reference strains, *S. prati* DSM 103336^T, *S. fennica* DSM 13665^T, *S. laterariae* DSM 25432^T and *S. haloaromaticamans* CGMCC 1.10206 T. The hybridisation experiment was performed as described by Ley et al. (1970) and Huss et al. (1983). The mean (±SD) DNA reassociation rate between the strains was calculated taking data from three independent experiments.

The DNA G+C content percentage of the strain W16RD^T was calculated from the draft genome sequences as (G+C)/(A+T+C+G) by using a perl script.

Results and discussion

Morphological and phenotypic characteristics

Colonies of strain W16RD^T were seen to be orange, circular, smooth, flat and transparent on R2A agar plates after incubation for 3–4 days at 25 °C. The cells were found to be rod-shaped, motile by single polar flagellum and measured 1.2 µm in length and 0.91 µm in diameter (Figure S1). The strain was found to grow in the temperature range 4–30 °C (optimum, 20–25 °C) and pH range 5–9 (optimum, 8) and to be tolerant of NaCl concentration up to 1% (w/v) and catalase and oxidase positive. In the APIZYM system, the strain was positive for esterase lipase (C8), β-galactosidase, β-glucosidase, alkaline phosphatase, esterase (C4), leucine arylamidase, acid phosphatase

and weakly positive for valine arylamidase, naphthol-AS-BI-phosphohydrolase, α–galactosidase and in the API 20NE system it was positive for para-nitrophenyl-βD-galactopyranosidase, esculin hydrolysis and assimilation of L-arabinose. A number of differentiating characteristics that can be used to distinguish the strain W16RD^T from other recognized species of the genus *Sphingomonas* are displayed in Table 1.

Genome properties and annotation

The genome of strain W16RD^T produced a total of 6,617,100 raw reads with a mean read length of 125 bp. After assembling, a total of 35 scaffolds with N50 length of 20,22,37 bp and maximum length of 425,724 bp were obtained. The completed assembled draft genome is 37,22,743 bp. Of the 3438 predicted genes, 3390 were protein coding genes and 48 RNA (45 tRNA genes and 3 rRNA) genes. Rast annotation of the whole genome showed the presence of 2654 COG categories. Basic sequence statistics of the draft genome of the strain W16RD^T are shown in Table 3.

Comparative genomics

The ANI comparison between the genome of the strain W16RD^T with that of the published genomes of related species (Figure S3) showed results less than 95% (the species ANI cut off value), clearly indicating that the strain W61RD^T belongs to a new species.

Phylogenetic analysis

The 16S rRNA gene sequence of the strain W16RD^T obtained in this study was a continuous stretch of 1483 bp and has been deposited in the GenBank database (accession number KU535674). Comparison of the 16S rRNA gene sequence of strain W16RD^T with the available sequences in EzTaxon-e-server (http://eztaxon-e.ezbiocloud.net) and GenBank (http://www.ncbi.nlm.nih.gov) revealed the strain to be closely related to *S. prati* DSM 103336^T (99%) followed by *S. fennica* DSM 13665^T (97.21%), *S. laterariae* DSM 25432^T (96.44%), *S. haloaromaticamans* CGMCC 1.10206 ^{T} (96.36%) and *Sphingomonas formosensis* DSM 24164^T (96.06%). The relationship between the stain W16RD^T and members of the genus *Sphingomonas* was further evident in a neighbor joining phylogenetic tree based on 16S rRNA gene sequences, which positioned the strain W16RD^T within a clade comprising species of the genus *Sphingomonas* (Fig. 1). Similar topologies were recovered using the maximum-parsimony and maximum- likelihood algorithms (data not shown) supporting the phylogenetic position of strain W16RD^T within the genus *Sphingomonas*.

Cellular fatty acids, isoprenoid quinones, polar lipid and polyamines analysis

The predominant fatty acids in the strain W16RD^T were found to be summed feature eight comprising $C_{18:1}$ ω 7*c* and/or $C_{18:1}$ ω 6*c* (39.2%), summed feature three comprising of $C_{16:1}$ ω7*c and*/or $C_{16:1}$ ω 6*c* (36.7%) and $C_{16:0}$ (7.0%).The relative differences in the respective proportions of $C_{16:1}$ 2-OH, $C_{18:1}$ 2-OH, $C_{16:1}$ ω5*C*, $C_{17:1}$ ω6*c* and a high percentage of $C_{16:1}$ ω 7*c* and/or $C_{16:1}$ω 6*c* distinguish the strain W16RD^T from its close relatives considered in this study (Table 2). Ubiquinone 10 (Q-10) was determined to be the major respiratory quinone and the polar lipids detected were phosphatidylcholine (PC), sphingoglycolipid (SGL), phosphatidylglycerol (PG), phosphatidylethanolamine (PE), diphosphatidylglycerol (DPG), phosphatidyldimethylethanolamine (PDE), phosphatidylmonomethylethanolamine (PME) and three unidentified lipids, L1-L3 (Figure S2.a, A1). The presence of phosphatidyldimethylethanolamine (PDE) in strain W16RD^T made it distinct from its close relative *S. prati*. Also, the strain W16RD^T showed the presence of three unidentified lipids (L1-L3) in contrast to the *S. prati* type strain which showed the presence of only two unidentified lipids, L1-L2 (Fig S2.a, A1/B1). However, the presence of PDE in the strain W16RD^T was unique amongst its closely related reference strains considered in this study (Figure S2.a, S2.d). The polar lipid profile of strain W16RD^T and the reference strains is presented in Table S1. One protein of strain W16RD^T showed 56% amino acid identity to spermidine synthase protein in *S. wittichii* RW1 but no protein showed any match to homospermidine synthase. We also compared all proteins of the strain to the Pfam database, but did not find any protein containing the domain of homospemidine synthase protein. Thus

Table 1 Differentiating characteristics of strain W16RD^T and type strains of phylogenetically related species of the genus *Sphingomonas*. DNA–DNA relatedness values are shown relative to strain W16RD^T

Characteristics	1	2	3	4	5	6
Isolation source	Soil	Soil[#]	ground water[‡]	Dump site[†]	Water and soil‡	Agricultural Soil[*]
Colony colour	Or	Or	LY	Cr	Cr	LY
Optimum temperature °C	20–25	25	25–30	25	30	25
Optimum pH	8	8	7	9	6-May	7
NaCl tolerance (%, w/v)	0–1	0–1	0	0–2	0–2	0–1
Oxidase	+	W	+	+	+	W
API 20 NE tests						
Nitrate reduction	−	−	−	+	−	−
Indole production	−	W	W	−	−	−
Hydrolysis of esculin	+	+	−	−	+	+
Assimilation of						
D-glucose	−	−	−	−	+	+
L-arabinose	+	−	−	+	+	W
N-acetyl-glucosamine	−	−	−	+	+	−
Para-Nitrophenyl-*βD*-galactopyranosidase	+	W	−	−	−	−
API ZYM results						
Esterase lipase (C8)	+	+	+	W	+	−
Valine arylamidase	W	W	−	+	+	−
Cystine arylamidase	−	+	−	+	+	−
Trypsin	−	+	−	−	+	+
α-chymotrypsin	−	W	−	−	−	−
Naphthol-AS-BI-phosphohydrolase	W	W	+	+	+	+
α-galactosidase	W	W	−	−	+	−
β-galactosidase	+	W	−	−	−	−
β-glucuronidase	−	−	−	−	−	+
β-glucosidase	+	+	−	−	+	+
DNA–DNA hybridisation (%)		52.17	47.60	20.93	17.09	

All data are from the present study except where indicated. All strains are positive for the activities of alkaline phosphatase, esterase (C4), leucine arylamidase and acid phosphatase and negative for the activities of lipase (C14), α-glucosidase, *N*-acetyl-*β*-glucosaminidase, α-mannosidase, α-fucosidase, urease, arginine dihydrolase, hydrolysis of gelatin, fermentation of glucose, assimilation of D-mannose, D-mannitol, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid

Strains: 1 W16RD^T, 2 *Sphingomonas prati* DSM 103336^T, 3 *Sphingomonas fennica* DSM 13665^T, 4 *Sphingomonas laterariae* DSM 25432^T, 5 *Sphingomonas haloaromaticamans* CGMCC 1.10206^T, 6 *Sphingomonas formosensis* DSM 24164^T

+ Positive, − negative, *w* weakly positive, *LY* light yellow, *Or* orange, *Cr* cream

[#] Data from Manandhar et al. (2016)

[‡] Data from Wittich et al. (2007)

[*] Data from Lin et al. (2012)

[†] Data from Kaur et al.(2012)

strain W16RD^T is predicted to possess spermidine as its polyamine, as has been reported in many species of the genus *Sphingomonas*, such as *S. lacus, S. aquatilis, S. koreensis, S. cloacae, S. wittichii* and *S. abikonensis* (Hamana et al. 2003; Kim et al. 2015; Yabuuchi and Kosako 2005).

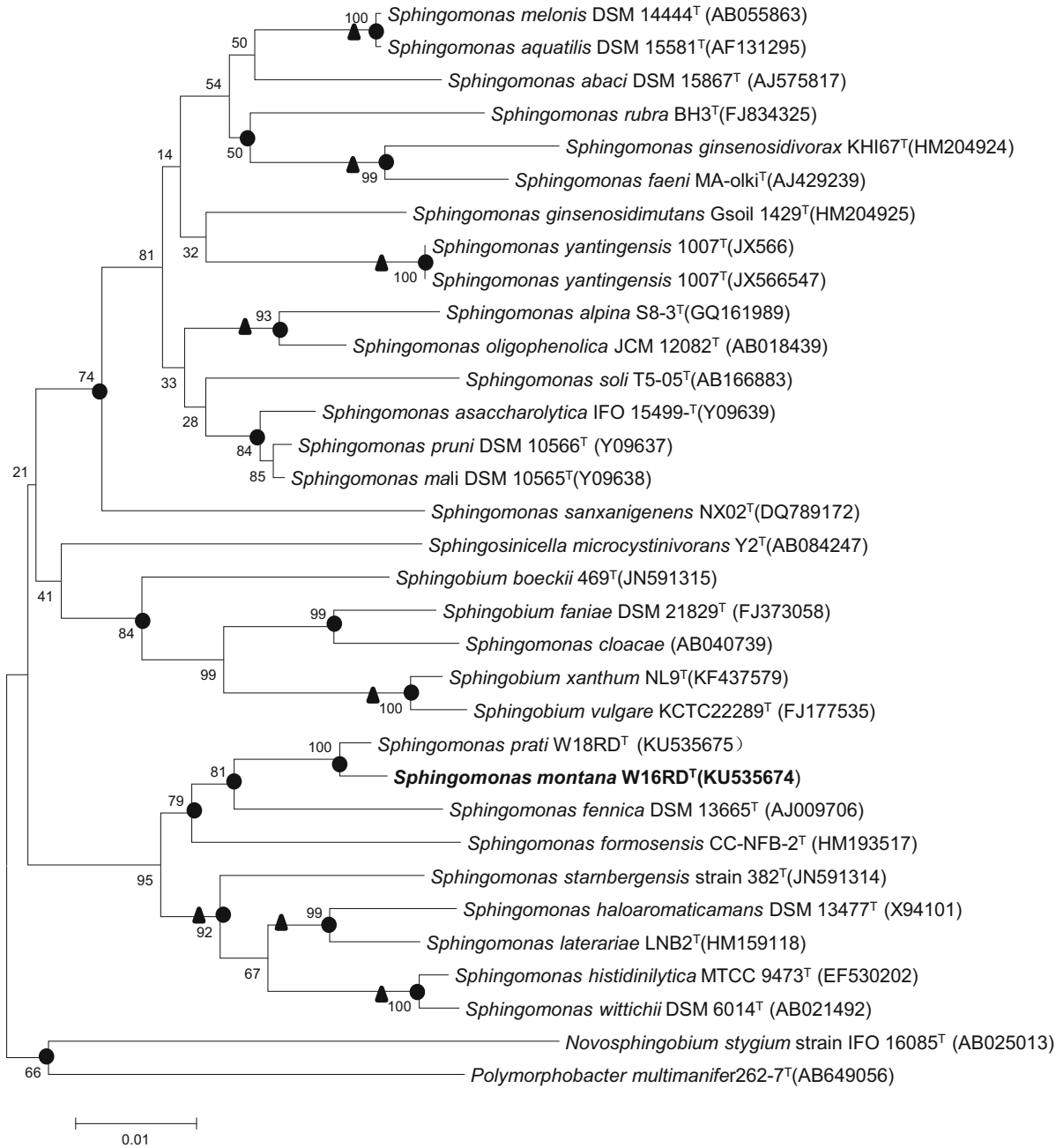

Fig. 1 Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the position of strains W16RD^T in relation to the type strains of *Sphingomonas* species and *Novosphingobium stygium* strain IFO 16085^T and *Polymorphobacter multimanifer* 262-7^T was used as an outgroup. *Filled circles* and *filled triangles* indicate that the corresponding nodes were also recovered in the trees generated with the maximum- likelihood and maximum-parsimony algorithms respectively. Bootstap percentages were based on 1000 replications. *Bar* 0.01 substitutions per nucleotide position

DNA–DNA hybridisation and DNA G+C content

The mean (± SD) DNA reassociation rate between the strain W16RD^T and *S. prati* DSM 103336^T, *S. fennica* DSM 13665^T, *S. laterariae* DSM 25432^T, and *S. haloaromaticamans* CGMCC^T were 52.17, 47.60, 20.93 and 17.09% respectively (Table 1) and the

Table 2 Fatty acid composition of strain W16RD^T and type strains of phylogenetically related species of the genus *Sphingomonas*

Fatty acid	1	2	3	4	5	6
$C_{14:0}$	0.2	0.3	1.0	0.4	0.2	0.2
$C_{16:0}$	7.0	7.9	20.2	16.5	10.4	10.5
$C_{18:0}$	0.1	–	0.4	1.9	0.4	6.1
$C_{14:0}$ 2-OH	4.5	6.3	7.3	8.6	9.0	4.9
$C_{16:1}$ 2-OH	1.0	0.8	–	–	–	–
$C_{18:1}$ 2-OH	1.3	0.7	–	–	–	–
iso-$C_{16:0}$ 3-OH	1.4	1.1	0.2	–	–	–
$C_{16:1}$ ω5*c*	1.3	1.5	0.9	0.7	1.9	7.9
$C_{17:1}$ ω 6*c*	0.8	–	1.4	3.1	1.8	–
$C_{17:1}$ ω8*C*	–	–	–	1.0	0.4	–
$C_{18:1}$ ω5c	1.3	0.6	1.7	–	–	1.51
$C_{18:1}$ ω7*c* 11-methyl	3.1	3.2	3.2	5.0	23.8	4.8
$C_{19:0}$ cyclo ω8c	0.3	0.7	7.1	–	6.9	0.1
Summed features						
Sum in feature 3	36.7	32.2	14.0	14.8	12.3	3.5
Sum in feature 8	39.2	44.1	41.7	46.6	32.1	58.6

Strains: 1 W16RD^T, 2 *Sphingomonas prati* DSM 103336^T, 3 *Sphingomonas fennica* DSM 13665^T, 4 *Sphingomonas laterareae* DSM 25432^T, 5 *Sphingomonas haloaromaticamans* CGMCC 1.10206^T, 6 *Sphngomonas formosensis* DSM 24164^T

All data are from this study. Values are percentage of the total fatty acids. Fatty acids amounting to <1% in all the strains are not shown

Summed features represents two or more fatty acids that cannot be separated by the MIDI system. Summed feature consists of: 3 $C_{16:1}$ ω 7*c* and/or C16:1 ω 6*c*; 8 $C_{18:1}$ ω 7*c* and/or $C_{18:1}$ ω 6*c*

– not detected

Table 3 Sequence statistic for the whole genome sequence of strain W16RD^T

Strain	W16RD^T
Raw data (Mb)	827.1375
Mean length	106,364
Maximum length	425,724
Total sequence length (bp)	3,722,743
GC	67.0%
Total assembly gap length	13
Gaps between scaffolds	0
Number of scaffolds	35
Scaffold N50	202,237
Scaffold L50	6
Number of contigs	48
Contig N50	163,730
Contig L50	7
tRNA genes	
rRNA genes	45
	3
Predicted genes	3438
CDS	3390
Genes assigned to COG	2654
Total number of chromosomes and plasmids	0

DNA G + C content of the genomic DNA was determined to be 67%.

Taxonomic conclusion

On the basis of 16S rRNA gene sequence analysis, strain W16RD^T showed high sequence similarity with *S. prati* DSM 103336^T (99%) followed by *S. fennica* DSM 13665^T (97.21%), *S. laterariae* DSM 25432^T (96.44%), *S. haloaromaticamans* CGMCC 1.10206^T (96.36%) and *S. formosensis* DSM 24164^T (96.06%). The phylogenetic affiliation of the strain with the genus *Sphingomonas* is supported by its position in the neighbor-joining tree where it clustered with the species of the genus *Sphingomonas* (Fig. 1). Distinct phenotypic characteristics, a low level of DNA–DNA hybridisation values (Table 1) and a low ANI value of <95%, (Figure S3) justify assignment of strain W16RD^T to the genus *Sphingomonas* as the type strain of a novel species, for which the name *Sphingomonas montana* sp. nov. is proposed.

Description of *Sphingomonas montana* sp. nov.

Sphingomonas montana (mon.ta'na. L. fem. adj. montana belonging to a mountain).

Colonies on R2A agar are round, smooth, opaque, flat and orange colored. Cells are Gram-staining negative, rod-shaped, motile by single polar flagellum and 1.2 μm in length and 0.9 μm in diameter. Growth in R2A broth occurs at the temperature range 4–30 °C (optimum, 20–25 °C), pH 5–9 (optimum, 8) and with 0–1% (w/v) NaCl (optimum 0%). The major fatty acids are summed feature eight comprising $C_{18:1}$ ω 7*c* and/or $C_{18:1}$ ω 6*c*, summed feature three comprising of $C_{16:1}$ ω 7*c and*/or $C_{16:1}$ ω 6*c* and $C_{16:0}$ and Q-10 is the major respiratory quinone. The G + C content of the genomic DNA of the type strain is 67% and the final assembled draft genome sequence of the type

strain is 3,722,743 bp. The polar lipids are phosphatidylcholine, sphingoglycolipid, phosphatidylglycerol, phosphatidylethanolamine, diphosphatidylglycerol, phosphatidyldimethylethanolamine, phosphatidylmonomethylethanolamine and three unidentified lipids. The type strain is W16RD^T (=CGMCC 1.15646^T=DSM 103337^T) isolated from soil from the Tangulla Mountain, China. The Genbank accession number for the 16S rRNA gene sequence and genome of the strain is KU535674 and MOLY00000000 respectively. The DPD TaxonNumber is TA00189.

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

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