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TAXONOMIC DESCRIPTION

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Sphingobacterium solani sp. nov., isolated from potato stems

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Abstract

A Gram-stain-negative, non-motile, non-spore-forming bacterium, designated MLS-26-JM13-11^T, was isolated from potato stems, collected in Guyuan County, Hebei Province, China. Strain MLS-26-JM13-11^T could grow at 10–39 °C (optimum, 30 °C), pH 6.0–9.0 (optimum, pH 7.2) and in the presence of 0–4.0 % (w/v) NaCl (optimum, 1.0 % w/v). Phylogenetic analysis, based on 16S rRNA gene sequences, revealed that strain MLS-26-JM13-11^T formed a stable clade with *Sphingobacterium bambusae* IBFC2009^T and *Sphingobacterium griseoflavum* SCU-B140^T, with the 16S rRNA gene sequence similarities ranging from 95.9 % to 97.0 %. The major cellular fatty acids comprised iso-$C_{15:0}$ (36.9 %), summed feature 3 ($C_{16:1}\omega7c$ and/or $C_{16:1}$ $\omega6c$, 34.0 %), $C_{16:0}$ (3.0 %) and iso-$C_{17:0}$ 3-OH (13.4 %). Strain MLS-26-JM13-11^T contained sphingoglycolipid, phosphatidyl ethanolamine, six unknown lipids, one unknown aminolipid, four unknown polarlipids and two unknown aminophospholipids. The isoprenoid quinone was MK-7. The DNA G+C content was 42.6 mol%. Furthermore, the average nucleotide identity and *in silico* estimated DNA–DNA reassociation values among MLS-26-JM13-11^T and *S. bambusae* KCTC 22814^T were in all cases below the respective threshold for species differentiation. On the basis of phenotypic, genotypic and phylogenetic evidence, strain MLS-26-JM13-11^T (=ACCC 60057^T=JCM 32274^T) represents a novel species within the genus *Sphingobacterium*, for which the name *Sphingobacterium solani* sp. nov. is proposed.

The genus *Sphingobacterium* comprises Gram-stain-negative, non-spore-forming, straight-rod-shaped bacteria containing high quantities of *sphingophospholipids* in their cells. This genus was first described by Yabuuchi *et al.* [1] with the description of *Sphingobacterium spiritivorum*, *Sphingobacterium multivorum* and *Sphingobacterium mizutae*. The descriptions of the genus and *Sphingobacterium mizutaii* were amended by Wauters *et al.* [2]. Members of the genus *Sphingobacterium* have been isolated from clinical material, raw milk, lichen, fresh leaves, water, soil, compost, activated sludge and soybean plants. At the time of writing, the genus *Sphingobacterium* comprises 45 species with validly published names, including: *Sphingobacterium bambusae, Sphingobacterium griseoflavum*, *Sphingobacterium populi, Sphingobacterium chuzhouense, Sphingobacterium zeae, Sphingobacterium jejuense, Sphingobacterium cibi* and *Sphingobacterium soli* [3–10].

We isolated eight strains and a novel strain, MLS-26-JM13-11^T, from potato stems collected from cropland in Guyuan County, Shijiazhuang City, Hebei Province, China (41° 45′22″ N, 115° 30′24″ E; altitude 1531 m) by using surface disinfection of the stems and the 10-fold dilution method on M13 (R2A) agar (M13: 0.5 g l^{-1} yeast extract, 0.5 g l^{-1} proteose peptone, 0.5 g l^{-1} casamino acid, 0.5 g l^{-1} glucose, 0.5 g l^{-1} soluble starch, 0.3 g l^{-1} Na-pyruvate, 0.3 g l^{-1} K_2HPO_4, 0.05 g l^{-1} $MgSO_4{\cdot}7H_2O$, 15 g l^{-1} agar, 1000 ml distilled water; pH 7.2) at 30 °C for 2–3 days in the dark. Single colonies on the plates were purified by transfer onto fresh plates and subsequent reincubation. Strain MLS-26-JM13-11^T was thus obtained, preserved in a glycerol suspension (30 %, v/v) and maintained at −80 °C.

Genomic DNA of MLS-26-JM13-11^T was prepared using a DNA extraction kit (Biotech) by following the manufacturer's instructions. PCR amplification of the 16S rRNA gene was performed with the primers 27F (5′-GAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-ACGGCTACCTTGTTACGACTT-3′). Purified PCR products (approximate 1.5 kb) were inserted into pGEM-T vector and sequenced by the Life Technologies Company (Shanghai, China). We obtained an almost-complete 16S rRNA gene sequence (1430 bp). We used NCBI's BLAST search (http://www.ncbi.nlm.nih.gov/blast) [11] and the EzTaxone server (www.ezbiocloud.net) [12] to identify phylogenetic neighbours and calculate pairwise sequence similarities. *S. bambusae*

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Abbreviations: ACCC, Agricultural Culture Collection of China; ANIb, average nucleotide identity blast; ANIm, average nucleotide identity mummer; DDH, DNA–DNA hybridization; JCM, *Journal of Clinical Microbiology*; NCBI, National Center of Biotechnology Information.
The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain MLS-26-JM13-11^T is MF614846.
Three supplementary figures are available with the online version of this article.

KCTC 22814^T and *S. griseoflavum* KCTC 42158^T exhibited the greatest similarity to MLS-26-JM13-11^T (96.7 and 95.9 % identity, respectively, both lower than 97.0 %). We used the software packages MEGA version 6.1 [13] to reconstruct phylogenetic trees based on maximum-likelihood [14], neighbour-joining [15] and minimum-evolution [16] models with bootstrap values under 1000 replications [17]. Calculation of evolutionary distances utilized Kimura's two-parameter model [18, 19]. The maximum-likelihood tree demonstrated that strain MLS-26-JM13-11^T belonged to the genus *Sphingobacterium* and forms a cluster with *S. bambusae* KCTC 22814^T with a high bootstrap value (96.7 %) (Fig. 1). Although strain *S. griseoflavum* KCTC 42158^T showed 95.9 % similarity to MLS-26-JM13-11^T, these strains were distributed in different clades in the maximum-likelihood trees. The neighbour-joining and minimum-evolution trees showed essentially the same topology. In conclusion, *S. bambusae* KCTC 22814^T and *S. griseoflavum* KCTC 42158^T were chosen as reference strains.

We then performed phenotypic and chemotaxonomic analyses of strain MLS-26-JM13-11^T. For this part of the study, cells were cultured in M13 (R2A) medium under aerobic conditions. We observed cell morphology and size under a scanning electron microscope (JSM-7500F, JEOL) using cells at the exponential growth phase (Fig. S1, available in the online version of this article). Gram staining was

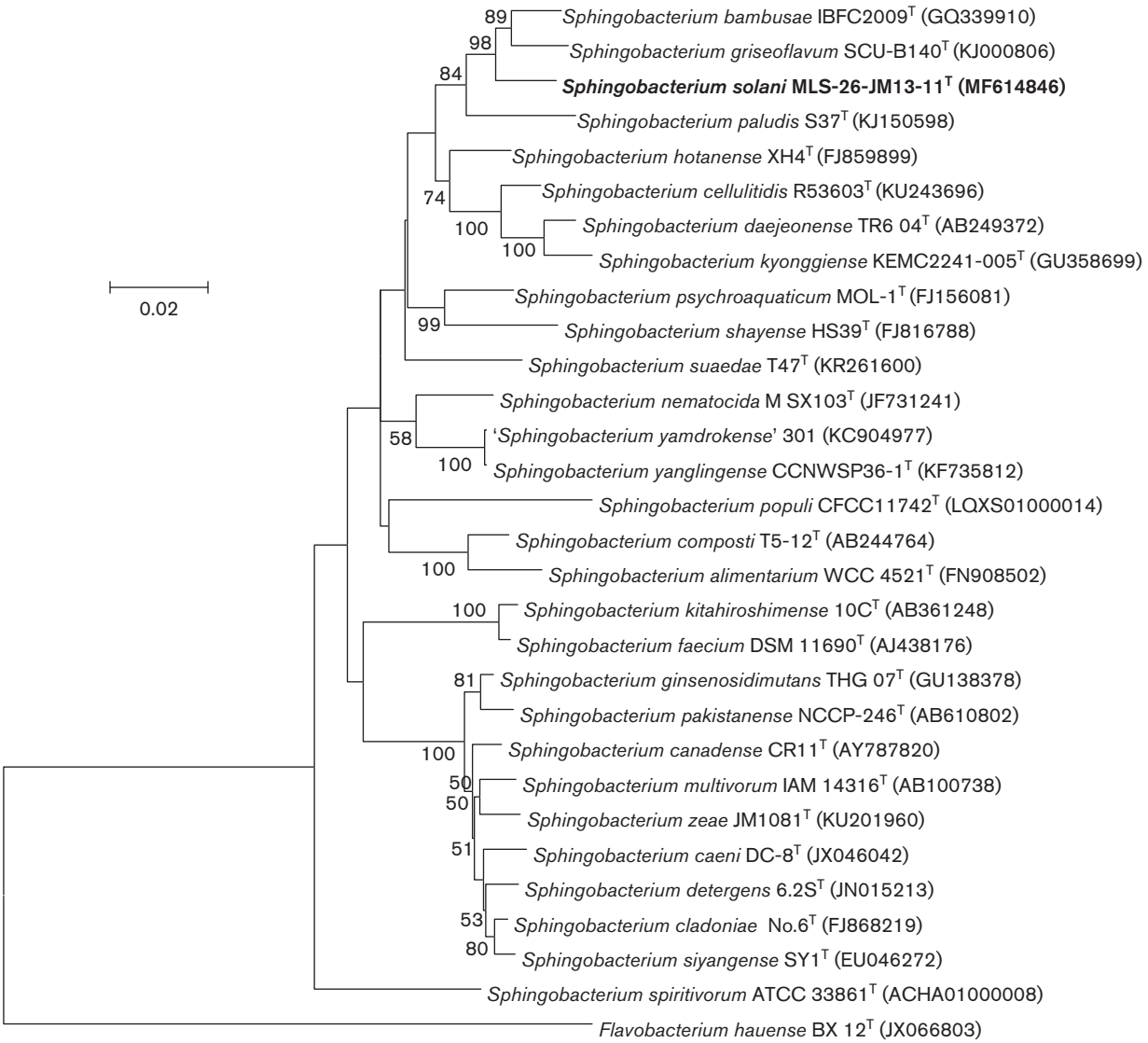

Fig. 1. Phylogenetic tree reconstructed by the neighbour-joining method based on the 16S rRNA gene sequences. *Flavobacterium hauense* BX12^T was used as an outgroup. Bootstrap values (numbers on branch nodes expressed as percentages of 1000 replications) of 50 % are shown at branching points. Bar, 0.02 substitutions per nucleotide position. Evolutionary analyses were conducted in MEGA version 6.1 [23].

performed as described by Smibert and Krieg [20]. Motility was examined by stab-culture in semi-solid medium as described by Gerhardt *et al.* [21]. Anaerobic growth was assessed in an anaerobic chamber (Oxoid) on R2A agar supplemented with 0.1 % KNO_3 for 1 month. Salt tolerance tests were performed in basal medium (0.5 g l^{-1} yeast extract, 0.5 g l^{-1} proteose peptone, 0.5 g l^{-1} casamino acid, 0.5 g l^{-1} glucose, 0.5 g l^{-1} soluble starch, 0.3 g l^{-1}Na-pyruvate, 0.3 g l^{-1} K_2HPO_4, 0.05 g l^{-1} $MgSO_4{\cdot}7H_2O$, 15 g l^{-1} agar, distilled water 1000 ml; pH adjusted to 7.2 by HCl/NaOH) using different concentrations of NaCl (0, 0.1, 0.5, 1, 2, 4, 6, 8 and 10 %, w/v). The optimal pH and temperature for growth of MLS-26-JM13-11^T were determined by incubating the strain in M13 (R2A) medium at different pH levels (pH 4.0, 6.0, 7.0, 7.2, 7.5, 8.0, 9.0 and 10.0; acetate buffer was used for pH 4.0–7.0 and phosphate buffer was used for pH 7.0–10.0.) and temperatures (0, 5, 10, 15, 25, 30, 35, 39 and 40 °C). Bacterial concentration was measured at 600 nm using an UV-vis spectrophotometer. Tests for the ability to reduce nitrate and to hydrolyse gelatin, starch, Tween 20, Tween 80, cellulose and urea were carried out according to methods previously described by Cappuccino and Sherman [22]. Catalase ability was tested with 3 % H_2O_2 and oxidase ability was determined using tetra-methyl-p-phenylenediamine dihydrochloride according to methods described by Barrow and Feltham [23]. Other enzyme activities were assayed using the API ZYM and API 20NE systems. Other biochemical tests were determined using the API 50CH system and the Biolog GN2 microplate according to the manufacturers' instructions. Although strain MLS-26-JM13-11^T shared many phenotypic features with the closely related taxa, *S. bambusae* KCTC 22814^T and *S. griseoflavum* KCTC 42158^T, there were some differences between them. The Biolog GN2 results showed positive results for glycerol, D-galactose, maltose, raffinose and L-arabinose for strain MLS-26-JM13-11^T and SCU-B140^T, but negative results for strain *S. bambusae* KCTC 22814^T. Biolog GN2 results also showed positive results for L-rhamnose for strain *S. bambusae* KCTC 22814^T, but negative results for MLS-26-JM13-11^T and SCU-B140^T. API 50CH results showed positive results for D-xylose, galactose and L-fucose for strain MLS-26-JM13-11^T and its two relatives, but negative results for glycogen. Acid was produced from melibiose and L-arabinose for strain MLS-26-JM13-11^T, but not from L-rhamnose. Phenotypic characteristics are summarized in the species description and a comparison of strain MLS-26-JM13-11^T and related type strains is given in Table 1.

The High Pure PCR Template Preparation kit (Roche) was employed for isolation of genomic DNA for whole-genome sequencing and DNA–DNA hybridization experiments. The genomes of strains MLS-26-JM13-11^T and *S. bambusae* KCTC 22814^T were sequenced at Sistemas Genómicos (Valencia, Spain) using Illumina paired-end sequencing technology. The reads were trimmed using Trimmomatic 0.32 [24]. Genome assembly was performed using SPAdes 3.6.1 [25]. The G+C content of the chromosomal DNA was calculated on the basis of its whole-genome sequence. The average nucleotide identity blast (ANIb) and the average nucleotide identity mummer (ANIm) values were calculated as described by Richter and Rosselló-Móra using JSpecies (version 1.2.1) [26], and Lee *et al.* [27]. The estimated DNA–DNA hybridization value was determined between these two strains using the Genome-to-Genome Distance Calculator (version 2.1) [28, 29]. The DNA G+C content was estimated according to the draft genome of strains MLS-26-JM13-11^T and *S. bambusae* KCTC 22814^T, and was found to be 42.6 and 43.5 %, respectively, which are in the range of the genus *Sphingobacterium*: 35–44 mol% [30, 31]. The DNA–DNA hybridization values for strain MLS-26-JM13-11^T with *S. bambusae* KCTC 22814^T were 4.3 %, which were all below the threshold value (70 %) recommended for defining a novel species [32]. The average nucleotide identity (ANIb=73.5 %) and *in silico* estimated DNA–DNA reassociation values among MLS-26-JM13-11^T and *S. bambusae* KCTC 22814^T were in all cases below the respective threshold for species differentiation (95–96 %) [27].

Table 1. Differential phenotypic and chemotaxonomic characteristics of MLS-26-JM13-11^T and related strains

Strains: 1, MLS-26-JM13-11^T (data from this study); 2, *S. bambusae* KCTC 22814^T (data from this study); 3, *S. griseoflavum* SCU-B140^T (data from this study except for the DNA G+C content, which was taken from Long *et al.* [4]); All strains were positive for oxidase and catalase activities, and assimilation of D-glucose. All strains were negative for Gram-staining, motility, sporulation and assimilation of D-mannitol. +, Positive; –, negative; W, weakly positive.

Characteristic	1	2	3
Colour of colony	Light yellow	Yellow	Greyish yellow
Growth temperature (°C)	10–39	11–39	10–40
NaCl range (w/v)	0–4 %	0–5 %	0–4 %
Biolog GN2 results:			
Glycerol	+	–	+
D-Galactose	+	–	+
L-Rhamnose	–	–	+
Melibiose	+	W*	+
L-Arabinose	+	–	+
Maltose	+	–	+
Raffinose	+	–	+
Utilization of (API 50CH):			
Glycogen	–	–	–
D-Xylose	+	+	+
Galactose	+	+	+
L-Fucose	+	+	+
Acid production from(API 20E):			
L-Arabinose	+	W*	+
Melibiose	+	+	–
L-Rhamnose	–	+	–
DNA G+C content (mol%)	42.6	43.6*	41.2

*Some differences were observed between the literature and this study.

When determining the composition of fatty acids, strain MLS-26-JM13-11^T, *S. bambusae* KCTC 22814^T and *S. griseoflavum* KCTC 42158^T were all assayed in order to examine differences between the strain MLS-26-JM13-11^T and the most closely related species. The isolates were cultured under aerobic conditions on TSA medium at 30 °C until the exponential growth phase according to the cell growth curve. Fatty acid methyl esters were prepared and identified with a MIDI Sherlock Microbial Identification System (Sherlock version 6.1). Whole cell fatty acid analysis revealed that the predominant fatty acids in MLS-26-JM13-11^T were iso-$C_{15:0}$, summed feature 3 ($C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$) and iso-$C_{17:0}$ 3-OH. These results were in line with other members of the genus *Sphingobacterium*. However, there were several differences in the proportions of some fatty acids (Table 2).

The polar lipids were extracted from 1 g freeze-dried cells using methanol/chloroform/saline extraction (2 : 1 : 0.8 ratio by vol.), as described by Kates *et al.* [33]. We separated and identified the polar lipids using two-dimensional chromatography on a silica gel thin-layer chromatography (TLC) plate (10×10 cm), as previously described by Raj *et al.* [34]. For the presence of lipids, the following spraying reagents were used: molybdatophosphoric acid, ninhydrin, molybdenum blue and α-naphthol. Strain MLS-26-JM13-11^T had sphingoglycolipid, phosphatidyl ethanolamine, six unknown lipids, one unknown aminolipid, four unknown polarlipids and two unknown aminophospholipids (Fig. S2). The quinones of MLS-26-JM13-11^T were extracted from 0.1 g freeze-dried cells with chloroform/methanol extraction (2 : 1, v/v), purified by TLC, and analysed by high-performance liquid chromatography using the quinones of the reference type strains as standards [35]. MK-7 was found to be the major quinone, in agreement with other members of the genus *Sphingobacterium* (Fig. S3).

Table 2. Cellular fatty acid composition comparison of strain MLS-26-JM13-11^T and related members of the genus *Sphingobacterium*

Strains: 1, MLS-26-JM13-11^T (data from this study); 2, *S. bambusae* KCTC 22814^T (data from this study); 3, *S. griseoflavum* SCU-B140^T. All data were from this study and represent percentages of total fatty acids. TR, Trace amount (<0.1 %).

Fatty acid	1	2	3
Saturated straight-chain			
$C_{14:0}$	1.1	1.5	0.5
$C_{16:0}$	3.0	2.5	2.0
$C_{18:0}$	0.2	0.2	TR
Saturated branched			
anteiso-$C_{13:0}$	1.1	0.2	0.1
iso-$C_{15:0}$	36.9	27.4	30.2
anteiso-$C_{15:0}$	0.6	0.5	0.8
iso-$C_{15:0}$ 3-OH	2.1	3.3	2.5
iso-$C_{17:0}$ 3-OH	13.4	15.9	27.1
Hydroxy			
$C_{16:0}$ 3-OH	3.0	TR	3.8
Summed feature*			
1	0.4	0.3	0.2
3	34.0	42.0	25.4
9	0.8	0.6	0.6

*Summed feature 1 contained iso $C_{15:1}$ H and/or $C_{13:0}$ 3OH; summed feature 3 contained $C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$; summed feature 9 contained $C_{17:1}\omega9c$ and/or 10-methyl C16 : 0.

In conclusion, the characteristics of the novel species are consistent with the description of the genus *Sphingobacterium* according to morphological, biochemical and chemotaxonomic properties; however, there are several differences between MLS-26-JM13-11^T and other members of the genus *Sphingobacterium*. Phylogenetic and chemotaxonomic analyses demonstrate that strain MLS-26-JM13-11^T represents a novel species within the genus *Sphingobacterium*.

DESCRIPTION OF *SPHINGOBACTERIUM SOLANI* SP. NOV.

Sphingobacterium solani (so.la′ni. L. gen. n. solani of a nightshade, of the genus *Solanum* to which the potato plant belongs).

Cells are Gram-stain-negative, aerobic, non-motile, rod-shaped, 0.4–0.5 µm in width and 2.5–3.2 µm in length. Colonies grown on M13 medium are smooth, round and light yellow in colour. Growth occurs at 10–39 °C (optimum 30 °C) and at pH 6.0–9.0 (optimum pH 7.2). The salt tolerance range for growth is 0–4 % (w/v) NaCl (optimum 1.0 %). Catalase and oxidase reactions are positive. Nitrate reductase and hydrolysis of Tweens 20 and 80 are present. Cells are positive for urease, Tween 40, Tween 80, D-galactose, D-sorbitol, citric acid, glycerol, L-arabinose, cellobiose, D-fructose, D-galactose, gentiobiose, α-D-glucose, lactose, lactulose, maltose, D-mannose, melibiose, β-methyl-D-glucoside, raffinose, sucrose, L-asparagine, methyl pyruvate, mono-methyl succinate, trehalose, D-gluconic acid, turanose, α-ketovaleric acid, L-alaninamide, acetic acid, L-alanyl-glycine, L-serine and L-threonine, but not indole production, methyl red, Voges–Proskauer, H_2S production test, hydrolysis of gelatin and starch, assimilates dextrin, α-cyclodextrin, i-erythritol, L-fucose, D-mannitol, xylitol, cis-aconitic acid, D-galactonic acid lactone, D-glucosaminic acid, α-hydroxybutyric acid, β-hydroxybutyric acid, γ-hydroxybutyric acid, itaconic acid, α-ketobutyric acid, α-ketoglutaric acid, D, L-lactic acid, malonic acid, D-saccharic acid, sebacic acid, succinic acid, bromosuccinic acid, succinamic acid, glucuronamide, D-alanine, L-aspartic acid, L-glutamic acid, glycyl-L-aspartic acid, L-histidine, hydroxy L-proline, L-leucine, L-ornithine, L-phenylalanine, L-proline, L-pyroglutamic acid, D-serine, D, L-camitine, γ-amino butyric acid, urocanic acid, inosine, thymidine, phenyl ethylamine, putrescine, 2-aminoethanol, 2,3-butanediol, D,L-α-glycerol phosphate, glucose 1-phosphate, glucose 6-phosphate, adonitol, uridine, *N*-acetyl-D-galactosamine, D-mannitol, L-rhamnose and glycyl-L-glutamic acid. Acids are produced from D-fructose, D-galactose, D-glucose,

lactose, maltose, D-mannose, raffinose, sucrose, trehalose, glycerol and L-arabinose, but not from L-rhamnose, D-mannitol, D-sorbitol, glycogen, inositol, L-sorbose and starch. The major cellular fatty acids are iso-$C_{15:0}$, summed feature 3 (iso-$C_{15:0}$ 2-OH and/or $C_{16:1}\omega7c$), $C_{16:0}$, $C_{16:0}$ 3-OH, $C_{18:0}$ and $C_{14:0}$. Strain MLS-26-JM13-11^T contains sphingoglycolipid, phosphatidyl ethanolamine, six unknown lipids, one unknown aminolipid, four unknown polarlipids and two unknown aminophospholipids. The isoprenoid quinone is MK-7. The isoprenoid quinone of strain MLS-26-JM13-11^T is MK-7.

The type strain, MLS-26-JM13-11^T (=ACCC 60057^T =JCM 32274^T), was isolated from potato stems in a field in Guyuan county, China. The DNA G+C content is 42.6 mol%.

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

References

1. Yabuuchi E, Kaneko T, Yano I, Moss CW, Miyoshi N. *Sphingobacterium* gen. nov., *Sphingobacterium spiritivorum* comb. nov., *Sphingobacterium multivorum* comb. nov., *Sphingobacterium mizutae* sp. nov., and *Flavobacterium indologenes* sp. nov.: glucose-nonfermenting Gram-negative rods in CDC groups IIK-2 and IIb. *Int J Syst Bacteriol* 1983;33:580–598.
2. Wauters G, Janssens M, de Baere T, Vaneechoutte M, Deschaght P. Isolates belonging to CDC group II-i belong predominantly to *Sphingobacterium mizutaii* Yabuuchi *et al.* 1983: emended descriptions of *S. mizutaii* and of the genus *Sphingobacterium*. *Int J Syst Evol Microbiol* 2012;62:2598–2601.
3. Duan S, Liu Z, Feng X, Zheng K, Cheng L. *Sphingobacterium bambusae* sp. nov., isolated from soil of bamboo plantation. *J Microbiol* 2009;47:693–698.
4. Long X, Liu B, Zhang S, Zhang Y, Zeng Z *et al.* *Sphingobacterium griseoflavum* sp. nov., isolated from the insect Teleogryllus occipitalis living in deserted cropland. *Int J Syst Evol Microbiol* 2016;66:1956–1961.
5. Li Y, Song LM, Guo MW, Wang LF, Liang WX. *Sphingobacterium populi* sp. nov., isolated from bark of *Populus* × *euramericana*. *Int J Syst Evol Microbiol* 2016;66:3456–3462.
6. Wang X, Zhang CF, Yu X, Hu G, Yang HX *et al.* *Sphingobacterium chuzhouense* sp. nov., isolated from farmland soil. *Int J Syst Evol Microbiol* 2016;66:4968–4974.
7. Kämpfer P, Busse HJ, Kleinhagauer T, McInroy JA, Glaeser SP. *Sphingobacterium zeae* sp. nov., an endophyte of maize. *Int J Syst Evol Microbiol* 2016;66:2643–2649.
8. Siddiqi MZ, Muhammad Shafi S, Choi KD, Im WT, Aslam Z. *Sphingobacterium jejuense* sp. nov., with ginsenoside-converting activity, isolated from compost. *Int J Syst Evol Microbiol* 2016;66:4433–4439.
9. Lai WA, Hameed A, Liu YC, Hsu YH, Lin SY *et al.* *Sphingobacterium cibi* sp. nov., isolated from the food-waste compost and emended descriptions of *Sphingobacterium spiritivorum* (Holmes *et al.* 1982) Yabuuchi *et al.* 1983 and *Sphingobacterium thermophilum* Yabe *et al.* 2013. *Int J Syst Evol Microbiol* 2016;66:5336–5344.
10. Fu YS, Hussain F, Habib N, Khan IU, Chu X *et al.* *Sphingobacterium soli* sp. nov., isolated from soil. *Int J Syst Evol Microbiol* 2017;67:2284–2288.
11. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search tool. *J Mol Biol* 1990;215:403–410.
12. Yoon SH, Ha SM, Kwon S, Lim J, Kim Y *et al.* Introducing EzBioCloud: a taxonomically united database of 16S rRNA gene sequences and whole-genome assemblies. *Int J Syst Evol Microbiol* 2017;67:1613–1617.
13. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA6: molecular evolutionary genetics analysis version 6.0. *Mol Biol Evol* 2013;30:2725–2729.
14. Felsenstein J. Evolutionary trees from DNA sequences: a maximum likelihood approach. *J Mol Evol* 1981;17:368–376.
15. Saitou N, Nei M. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* 1987;4:406–425.
16. Rzhetsky A, Nei M. A simple method for estimating and testing minimum-evolution trees. *Mol Biol Evol* 1992;9:945–967.
17. Felsenstein J. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 1985;39:783–791.
18. Kimura M. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* 1980;16:111–120.
19. Kimura M. *The Neutral Theory of Molecular Evolution*. New York: Cambridge University Press; 1983.
20. Smibert RM, Krieg NR. Phenotypic characteristics. In: Gerhardt P, Murray RGE, Wood WA and Krieg NR (editors). *Manual of Methods for General and Molecular Bacteriology*. Washington, DC: American Society for Microbiology; 1994. pp. 607–654.
21. Gerhardt P, Murray RGE, Costilow RN, Nester EW, Wood WA *et al.* (editors). *Manual of Methods for General Bacteriology*. Washington, DC: American Society for Microbiology; 1981.
22. Cappuccino JG, Sherman N. *Microbiology: A Laboratory Manual*, 8th ed. San Francisco, CA: Pearson/Benjamin Cummings; 2008.
23. Barrow GI, Feltham RKA. (editors). *Cowan and Steel's Manual for the Identification of Medical Bacteria*. Cambridge: Cambridge University Press; 2004.
24. Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* 2014;30:2114–2120.
25. Nurk S, Bankevich A, Antipov D, Gurevich A, Korobeynikov A *et al.* Assembling genomes and Mmni-metagenomes from highly chime-ric reads. *Lect N Bioinformat* 2013;7821:158–170.
26. Richter M, Rosselló-Móra R. Shifting the genomic gold standard for the prokaryotic species definition. *Proc Natl Acad Sci USA* 2009;106:19126–19131.
27. Lee I, Ouk Kim Y, Park SC, Chun J. OrthoANI: an improved algorithm and software for calculating average nucleotide identity. *Int J Syst Evol Microbiol* 2016;66:1100–1103.
28. Auch AF, von Jan M, Klenk HP, Göker M. Digital DNA-DNA hybridization for microbial species delineation by means of genome-to-genome sequence comparison. *Stand Genomic Sci* 2010;2:117–134.
29. Meier-Kolthoff JP, Auch AF, Klenk HP, Göker M. Genome sequence-based species delimitation with confidence intervals and improved distance functions. *BMC Bioinformatics* 2013;14:60.
30. Ahmed I, Ehsan M, Sin Y, Paek J, Khalid N *et al.* *Sphingobacterium pakistanensis* sp. nov., a novel plant growth promoting rhizobacteria isolated from rhizosphere of Vigna mungo. *Antonie van Leeuwenhoek* 2014;105:325–333.
31. Lee DH, Hur JS, Kahng HY. *Sphingobacterium cladoniae* sp. nov., isolated from lichen, *Cladonia* sp., and emended description of *Sphingobacterium siyangense*. *Int J Syst Evol Microbiol* 2013;63:755–760.
32. Stackebrandt E, Goebel BM. Taxonomic Note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *Int J Syst Evol Microbiol* 1994;44:846–849.
33. Kates M. Techniques of lipidology: isolation, analysis and identification of lipids. In: Work TS and Work E (editors). *Laboratory Techniques in Biochemistry and Molecular Biology*, vol. 3. Amsterdam: Elsevier; 1972. pp. 269–610.

34. **Raj PS, Ramaprasad EV, Vaseef S, Sasikala C, Ramana C.** *Rhodobacter viridis* sp. nov., a phototrophic bacterium isolated from mud of a stream. *Int J Syst Evol Microbiol* 2013;63:181–186.

35. **Hiraishi A, Hoshino Y.** Distribution of rhodoquinone in *Rhodospirillaceae* and its taxonomic implications. *J Gen Appl Microbiol* 1984; 30:435–448.