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TAXONOMIC DESCRIPTION

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Mycetocola spongiae sp. nov., isolated from deep-sea sponge *Cacospongia mycofijiensis*

Yuling Chen, Jin Sang, Wei Sun, Qianqian Song and Zhiyong Li*

Abstract

A novel bacterial strain (MSC19^T) was isolated from a deep-sea sponge *Cacospongia mycofijiensis* collected in the Mariana Trench at a depth of 2681 m. The cells of the new isolate were Gram-stain-positive, non-motile, oxidase- and catalase-positive, rod-shaped and yellow-coloured. They could grow at 4–32 °C (optimum, 28 °C), pH 5.5–12 (optimum, pH 7.0) and with 0–12% (w/v) NaCl (optimum, 4%). The strain's 16S rRNA gene sequence showed 98.41% similarity to that of *Mycetocola saprophilus* CM-01^T. Phylogenetic analysis further suggested that strain MSC19^T represents a new species within the genus *Mycetocola*. The total genome of MSC19^T was approximately 3196754 bp in size with a G+C content of 66.43 mol%. The average nucleotide identity (ANI) and digital DNA–DNA hybridization (dDDH) values among MSC19^T and other *Mycetocola* type strains were 70.35–75.37% (ANIb), 83.79–84.73% (ANIm) and 20.3–21.7% (dDDH). The major fatty acids of MSC19^T were composed of anteiso-$C_{15:0}$, iso-$C_{16:0}$ and anteiso-$C_{17:0}$, and its predominant menaquinones were MK-10 and MK-9. The polar lipids of MSC19^T mainly consisted of diphosphatidylglycerol, phosphatidylglycerol and glycolipid. The diagnostic cell-wall diamino acid was lysine. Combined molecular, physiological, biochemical and chemotaxonomic analyses suggest that strain MSC19^T represents a novel species of the genus *Mycetocola*, for which the name *Mycetocola spongiae* sp. nov. is proposed. The type strain is MSC19^T (=MCCC 1K06265^T=KCTC 49701^T).

The genus *Mycetocola*, a member of the family *Microbacteraceae*, was first described by Tsukamoto *et al.* in 2001 [1]. At present, the genus *Mycetocola* includes eight species with validly published names (https://lpsn.dsmz.de/genus/Mycetocola) [2]. Members of the species have been isolated from a wide variety of habitats such as fungal fruiting bodies [1], cheese [3], desert sand [4], glacier [5], snow [6] and faeces of Tibetan antelopes [7]. Integrating the known reports, all species of the genus *Mycetocola* are described as obligately aerobic, Gram-stain-positive, non-sporulating, short-rod-shaped bacteria containing menaquinone-10 (MK-10) as the major respiratory quinone, anteiso-$C_{15:0}$ as the predominant fatty acid, lysine as the diagnostic diamino acid, and the G+C content ranges from 63.6 to 70.5 mol% [7]. In the present study, we report for the first time a halotolerant strain of *Mycetocola* isolated from deep-sea organisms, indicating the physiological diversity of the genus *Mycetocola* and highlighting the novel microbial resources in deep-sea sponges.

During an investigation of the bacterial diversity associated with deep-sea marine sponges, bacterial strain MSC19^T was isolated from a deep-sea sponge *Cacospongia mycofijiensis* (GenBank accession: OK135747) collected at a depth of 2681 m at the junction of the Mariana Trench and the Yap Trench (11.439670° N, 139.406620° E) in October 2019. A small part of the *C. mycofijiensis* sample was ground, suspended and diluted in sterile water. Then, 100 µl of each dilution was spread onto International *Streptomyces* Project (ISP) 2 agar plates (4.0 g yeast extract, 10.0 g malt extract, 4.0 g glucose, 1000 ml sterile seawater, 15.0 g agar, pH 7.0–7.2) and cultured at 28 °C. Once colonies emerged, they were selectively picked, and purified by serial streaking onto new plates. One of the purified strains was marked as MSC19^T. Strain MSC19^T was routinely grown on ISP 2 at 28 °C for further tests, unless indicated otherwise.

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Keywords: deep-sea sponge; *Mycetocola spongiae*; polyphasic taxonomy.
Abbreviations: ANI, average nucleotide identity; dDDH, digital DNA–DNA hybridization; GBDP, genome BLAST distance phylogeny; GGDC, Genome-to-Genome Distance Calculator; ISP, International *Streptomyces* Project; MK, menaquinone.
The GenBank accession numbers for the 16S rRNA gene sequence and the whole-genome sequence of the strain MSC19^T are OK039348 and CP080203, respectively.
Two supplementary tables and six supplementary figures are available with the online version of this article.

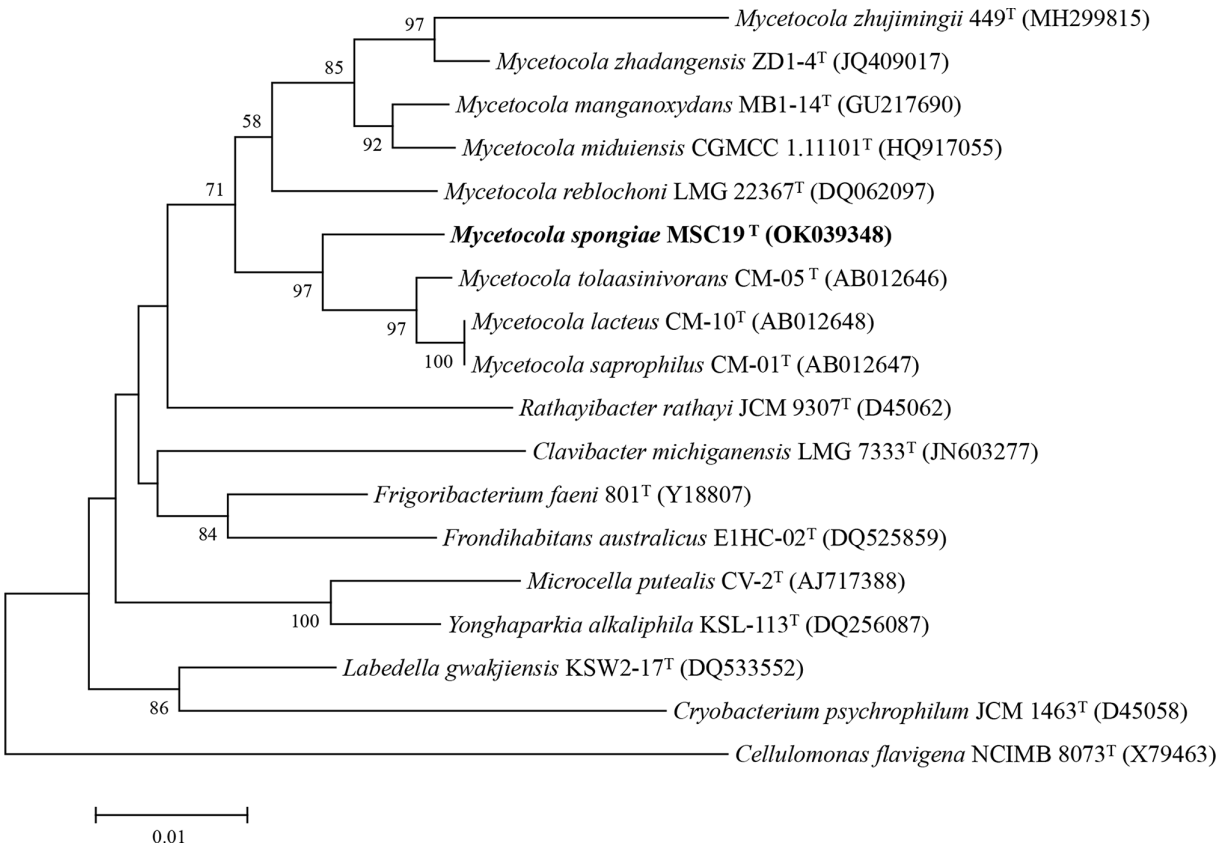

Fig. 1. Neighbour-joining tree showing the phylogenetic position of strain MSC19^T based on 16S rRNA gene sequences. The numerals (values >50% are noted) indicate percentages of bootstrap samplings as derived from 1000 replications. Bar, 0.01 substitutions per nucleotide position. GenBank accession numbers are given in parentheses. The sequence of *Cellulomonas flavigena* NCIMB 8073^T serves as an outgroup.

Genomic DNA was extracted using a TIANamp Bacteria DNA Kit (Tiangen Biotech) as described by the manufacturer. The 16S rRNA gene was amplified using reported methods [8]. The amplified gene fragments were recovered by agarose gel electrophoresis and then connected with plasmid pEASY -T5 Zero vector (TransGen Biotech). The connected plasmids were introduced into DH5*α* cells for blue–white screening. The white positive clones on the plate were selected for culture, and the plasmids were extracted and sequenced at Sangon Biotech (Shanghai, PR China) by the method of Sanger *et al*. [9] to obtain the complete 16S rRNA gene sequence of MSC19^T.

Identification of phylogenetic neighbours and calculation of 16S rRNA gene sequence similarities were achieved using the BLAST program [10]. BLASTn showed that MSC19^T probably represented a novel species of the genus *Mycetocola*, having 98.41% similarity to *Mycetocola saprophilus* CM-01^T, 98.20% similarity to *Mycetocola lacteus* CM-10^T and 97.99% similarity to *Mycetocola tolaasinivorans* CM-05^T. Phylogenetic analysis based on 16S rRNA gene sequences was performed using the ClustalW algorithm of MEGA7 [11] with distance options according to the Kimura two-parameter model and clustering with the neighbour-joining, maximum-likelihood and minimum-evolution methods. The topologies of the three phylogenetic trees were evaluated with bootstrapping of 1000 replications. All of the phylogenic trees showed that strain MSC19^T formed a clade with *M. saprophilus* CM-01^T, *M. lacteus* CM-10^T and *M. tolaasinivorans* CM-05^T; still within the radiation of the genus *Mycetocola* but clearly distant from its five other species (Figs 1, S1 and S2, available in the online version of this article).

The whole genome of MSC19^T was sequenced using Illumina NovaSeq 6000 PE150 and Oxford Nanopore PromethION 48 apparatus at Guangdong Magigene Biotechnology Co., Ltd. (PR China) After quality control, reads were assembled using SMRT Link version 5.0.1 [12]. The genome size of strain MSC19^T was estimated to be 3196754 bp, and the average genome coverage was 800×. The total G+C content of strain MSC19^T was 66.43 mol%, within the range reported for the genus *Mycetocola* [7]. Analysis by CheckM indicated that the completeness of the strain MSC19^T genome was 99.49% with 0.57% contamination. The genome sequence was considered an excellent reference genome for the future analysis (≥95% completeness, ≤5% contamination) [13]. The genomic sequence of the strain was uploaded to NCBI GenBank to obtain the accession number CP080203, and genome annotation was carried out by the NCBI Prokaryotic Genome Annotation Pipeline. Genome annotation results showed that there

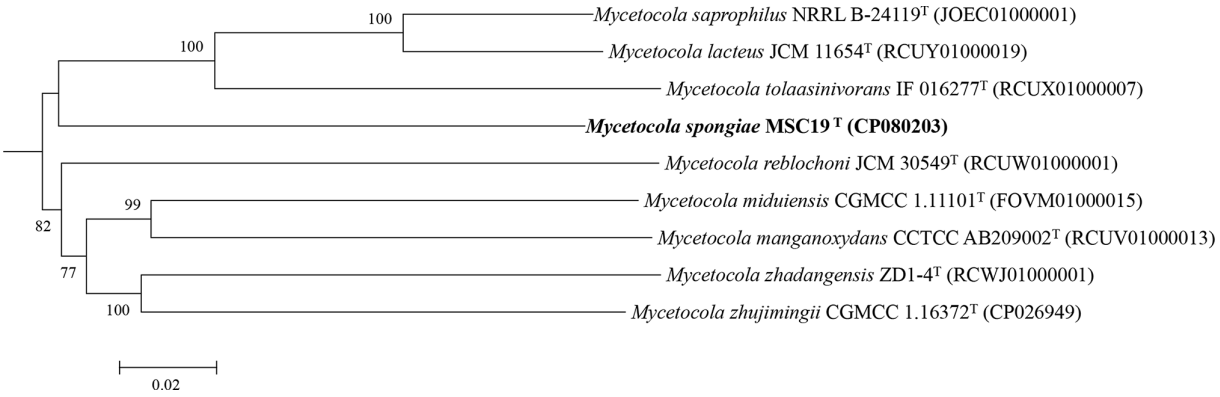

Fig. 2. Phylogenomic tree based on the genome sequences of strain MSC19^T and related type species using the genome BLAST distance phylogeny (GBDP) method. The numbers above branches are GBDP pseudo-bootstrap support values >70% from 100 replications, with an average branch support of 93.0%. GenBank accession numbers are included in parentheses.

were 2929 genes annotated in the genome, including 2856 coding genes, 13 rRNA genes, 57 tRNA genes, three ncRNA genes and 11 pseudo genes. As predicted by antiSMASH 6.0 [14], the genome of strain MSC19^T harboured four secondary metabolite biosynthetic gene clusters (Table S1). They were one T3PKS, one betalactone, one terpene and one ladderane gene cluster, all showing low similarity (≤50% of genes show similarity) to reported salinichelins, microansamycin and carotenoid. Compared to the identification of the secondary metabolism gene clusters of other type strains of genus *Mycetocola*, only strain MSC19^T had the potential to produce salinichelins, a new and unrelated group of peptidic siderophores [15].

The genome sequence data of strain MSC19^T and members of the genus *Mycetocola* were uploaded to the Type Strain Genome Server (https://tygs.dsmz.de/) [16] to reconstruct a phylogenomic tree. The phylogenetic tree (Fig. 2) shows that *M. saprophilus* CM-01^T, *M. lacteus* CM-10^T and *M. tolaasinivorans* CM-05^T form a stable cluster in the evolutionary tree with strain MSC19^T. The genomic similarity between the genomes of strain MSC19^T and those of closely related genera obtained from the GenBank database was estimated based on the average nucleotide identity (ANI) and digital DNA–DNA hybridization (dDDH) values, which were calculated by JSpecies WS (http://jspecies.ribohost.com/jspecieswsw/) [17] and the Genome-to-Genome Distance Calculator 2.1 web service with Formula 2 (http://ggdc.dsmz.de/distcalc2.php) [18]. As shown in Table 1, among the genomes of strain MSC19^T and eight related type species, the ANIb values were 70.35–75.37% and the ANIm values are 83.79–84.73%, which were lower than the recommended threshold values for species delineation [19]. The dDDH values between strain MSC19^T and its neighbouring type strains ranged from 20.3 to 21.7%, which are far below the 70% threshold for species delineation [18]. The

Table 1. The average nucleotide identity (ANI) and digital DNA–DNA hybridization (dDDH) values between MSC19^T and related type species of the genus *Mycetocola*

ANIb, average nucleotide identity based on BLAST; ANIm, average nucleotide identity based on MUMmer; GGDC, Genome-to-Genome Distance Calculator; C.I., confidence interval.

Strain MSC19^T vs	Accession no.	ANI (%)		GGDC	
		ANIb	ANIm	dDDH (%)*	Model C.I. (%)
M. saprophilus NRRL B-24119^T	JOEC01000001	75.37	84.73	21.7	19.4–24.1
M. zhujimingii CGMCC 1.16372^T	CP026949	70.89	84.1	21.5	19.3–24.0
M. reblochoni JCM 30549^T	RCUW01000001	70.45	83.93	20.5	18.3–23.0
M. manganoxydans CCTCC AB209002^T	RCUV01000013	71.06	84.59	20.8	18.6–23.2
M. tolaasinivorans IF 016277^T	RCUX01000007	74.42	84.3	20.8	18.6–23.3
M. lacteus JCM 11654^T	RCUY01000019	75.09	84.71	21.7	19.4–24.1
M. zhadangensis ZD1-4^T	RCWJ01000001	70.35	83.79	20.3	18.1–22.7
M. miduiensis CGMCC 1.11101^T	FOVM01000015	70.97	84.57	21.4	19.2–23.8

*Formula 2 based on a generalized linear model (identities/high-scoring segment pair) was used for dDDH.

results of the phylogenetic analyses of 16S rRNA genes and whole genomes provide strong evidence in favour of recognizing MSC19^T as representing a novel species in the genus *Mycetocola*.

Morphology of MSC19^T was examined by transmission electron microscopy (Talos L120C G2 Thermo Fisher) and the colonies on ISP 2 agar plate were observed after 2 days incubation at 28 °C. The Gram stain was determined using a Gram stain kit (Hangzhou Tianhe Microorganism Reagent Co. Ltd.) according to the manufacturer's instructions. Strain MSC19^T was incubated in an anaerobic jar in order to test oxygen requirement using the MGC AnaeroPack (Mitsubishi Gas Chemical Company, Inc.). The growth of MSC19^T at 4, 10, 15, 28, 30, 32, 35, 37, 42 °C was measured on ISP 2 agar plates with 0–12% NaCl (w/v) in increments of 1%, respectively. The pH range for MSC19^T growth was determined in an ISP 2 medium that was adjusted to various pH values (pH 5.5–13.0 at intervals of 0.5 pH units) by the addition of different buffers [20]. OD_{600} values of the cultures under various conditions were measured after 2 days of incubation at 28 °C.

Because *M. saprophilus* NRRL B-24119^T and *M. tolaasinivorans* NRRL B-24120^T had high similarity and form a stable cluster in the evolutionary tree with MSC19^T, these two strains were selected as reference strains for subsequent physiological and biochemical analyses under the same conditions. Catalase activity was determined by monitoring bubble production in 10% H_2O_2 solution. Oxidase activity was evaluated using oxidase reagents containing *N*,*N*,*N*,*N*-tetramethyl-1,4-phenylenediamine (bioMérieux). Acid production from 49 carbohydrates and enzyme activities were examined using API 50CH and API ZYM systems (bioMérieux), respectively. Other biochemical characteristics and utilization of carbon sources were determined using the API 20E, API 20NE systems (bioMérieux) and Biolog GENIII MicroPlates according to the manufacturers' instruction.

Consistent with the characteristics of the genus *Mycetocola*, cells of strain MSC19^T were aerobic, Gram-stain-positive, rod-like (0.3–0.5×0.8–1.8 µm; Fig. S3), non-spore-producing and without flagella. Colonies on ISP 2 plates were circular, slightly yellow, opaque, smooth surface, had regular edges, raised in the centre, with a diameter of 2.0–2.5 mm after growing at 28 °C for 2 days. Growth of strain MSC19^T was observed at 4–32 °C (optimum, 28 °C) and at pH 5.5–12 (optimum, pH 7.0). Strain MSC19^T grew in medium with 0–12% (w/v) NaCl, with optimal growth at 4% (w/v) NaCl. As summarized in Table 2, the ability to grow at 4 °C is consistent with the psychrotolerant nature of most *Mycetocola* species. The ability to grow in high-salt environment and the inability to produce acid from all 49 carbohydrates (API 50CH) separate strain MSC19^T from other *Mycetocola* type strains. Other biochemical differences were observed with respect to the reference strains *M. saprophilus* NRRL B-24119^T and *M. tolaasinivorans* NRRL B-24120^T (Table S2). The maltose, cellobiose, turanose, D-galactose, D-serine, D-mannitol, D-arabitol, L-histidine, D-gluconic acid, L-lactic acid, acetoacetic acid and acetic acid utilization of MSC19^T was significantly different from that of the reference strains. Additionally, no mannitol reaction was noted, but potassium gluconate and trisodium citrate were found.

For analysis of fatty acids, strain MSC19^T, *M. saprophilus* NRRL B-24119^T and *M. tolaasinivorans* NRRL B-24120^T were grown aerobically in ISP 2 medium at 28 °C for 2 days. The extraction, saponification and esterification of the fatty acids were performed according to the standard MIDI (Sherlock Microbial Identification System, version 6.0B) protocol [21]. The fatty acids were analysed by gas chromatography (Agilent Technologies 6850) and identified using the TSBA6.0 database of the Microbial Identification System. Isoprenoid quinones were extracted from freeze-dried cells (100 mg) with chloroform–methanol (2:1, v/v) and analysed using previously described methods [22, 23]. The polar lipids were extracted using a chloroform–methanol system and analysed with one- and two-dimensional thin-layer chromatography (TLC), as described previously [24]. Merck silica gel 60 F254 aluminum-backed thin-layer plates were used in this TLC analysis. The plate dotted with samples was subjected to two-dimensional development, with the first solvent of chloroform–methanol–water (65:25:4, v/v/v) and a second solvent of chloroform–acetic acid–methanol–water (85:15:12:4, v/v/v). The total lipids were detected using molybdatophosphoric acid, and specific functional groups were detected with spray reagents special for defined functional groups. The amino acids of the cell wall were determined as described previously [25] with TLC cellulose 50 glass plates (Merck).

As summarized in Table 3, The major fatty acids of strain MSC19^T were found to be anteiso-$C_{15:0}$ (43.1%), iso-$C_{16:0}$ (18.3%), anteiso-$C_{17:0}$(14.4%), $C_{16:0}$ (6.0%) and $C_{18:0}$ (3.1%). The presence of large amounts of anteiso-$C_{15:0}$, anteiso-$C_{17:0}$ and iso-$C_{16:0}$ was typical of most members of the genus *Mycetocola* [7], but strain MSC19^T had more iso-$C_{16:0}$ content (18.4%) than *M. saprophilus* NRRL B-24119^T (12.4%), *M. tolaasinivorans* NRRL B-24120^T (12.1%) and other type strains. The predominant menaquinones of strain MSC19^T were MK-10 and MK-9 (30.2 and 26.3%, respectively), which are basically the same as other type strains of the genus *Mycetocola*, but there were some differences in the proportion of each component. Diphosphatidylglycerol, phosphatidylglycerol and glycolipid were the major polar lipids in strain MSC19^T (Fig. S4). Apart from these, two unidentified polar lipids in strain MSC19^T make its lipid composition significantly different from those of the reference strains (Fig. S5). The primary cell-wall amino acids were lysine, alanine, glutamic acids and glycine, and the diagnostic cell-wall diamino acid was lysine (Fig. S6).

In conclusion, the molecular, physiological, biochemical and chemotaxonomic data obtained in this study indicate that strain MSC19^T represents a novel species of the genus *Mycetocola*, for which the name *Mycetocola spongiae* sp. nov. is proposed.

Table 2. Differential characteristics of strain MCS19^T with respect to the known type strains of the genus *Mycetocola*

Strains: 1, MSC19^T; 2, *M. saprophilus* NRRL B-24119^T; 3, *M. tolaasinivorans* NRRL B-24120^T; 4, *M. lacteus* JCM 11654^T; 5, *M. reblochoni* JCM 30549^T; 6, *M. manganoxydans* CCTCC AB209002^T; 7, *M. miduiensis* CGMCC 1.11101^T; 8, *M. zhadangensis* ZD1-4^T; 9, *M. zhujimingii* 449^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	8	9
Isolation source	Sponge *C. mycofijiensis*	Fungal fruiting bodies	Fungal fruiting bodies	Fungal fruiting bodies	Cheese	Desert sand	Glacier	Snow	Faeces
Temperature range for growth (°C)	4–32	4–33[a]	4–33[a]	4–33[a]	20–30[b]	20–35[c]	0–34[d]	0–40[e]	4–30[f]
NaCl (%) range for growth	0–12	No growth with 10% (w/v) NaCl[b]	No growth with 10% (w/v) NaCl[b]	No growth with 10% (w/v) NaCl[b]	No growth with 10 % (w/v) NaCl[b]	0–5[c]	0–7[d]	NA	0.5–3.0[f]
G+C content (mol%)	66.43	66.1[f]	66.1[f]	65.5[f]	70.5[b]	65.3[f]	65.9[d]	63.6[f]	64.9[f]
Acid production from (API 50CH):									
D-Glucose	–	+	+	+[f]	+[f]	+[f]	+[f]	–[f]	–[f]
Maltose	–	+	+	+[f]	+[f]	+[f]	+[f]	+[f]	–[f]
D-Gentiobiose	–	+	+	+[f]	+[f]	–[f]	–[f]	+[f]	–[f]
D-Tagatose	–	–	–	–[f]	–[f]	–[f]	+[f]	–[f]	+[f]
Melibiose	–	–	w	+[d]	–[d]	–[d]	–[d]	NA	NA
Sorbose	–	–	w	+[d]	–[d]	–[d]	–[d]	NA	NA
Sorbitol	–	–	–	+[d]	+[d]	–[d]	–[d]	NA	NA
Enzyme activity (API ZYM):									
Cystine arylamidase	+	+	+	–[f]	–[f]	w[f]	–[f]	+[f]	+[f]
β-Galactosidase	–	+	w	+[f]	–[f]	–[f]	–[f]	–[f]	–[f]
α-Glucosidase	–	+	+	+[f]	+[f]	–[f]	–[f]	+[f]	–[f]
β-Glucosidase	+	+	+	+[f]	+[f]	+[f]	–[f]	NA	NA
α-Mannosidase	–	–	–	–[f]	+[f]	–[f]	–[f]	–[f]	+[f]
Alkaline phosphatase	w	+	+	–[f]	–[f]	+[f]	–[f]	NA	NA
Esterase lipase (C4)	+	+	+	–[f]	+[f]	+[f]	+[f]	NA	NA
Esterase lipase (C8)	+	+	+	+[f]	+[f]	+[f]	+[f]	NA	NA
Valine arylamidase	+	+	+	–[f]	–[f]	w[f]	+[f]	NA	NA
Acid phosphatase	+	+	+	–[f]	+[f]	+[f]	+[f]	NA	NA
N-Acetyl-β-glucosaminidase	w	–	–	–[e]	–[e]	+[e]	NA	–[e]	NA

*Data from: *a*, Tsukamoto *et al.* [1]; *b*, Bora *et al.* [3]; *c*, Luo *et al.* [4]; *d*, Zhu *et al.* [5]; *e*, Shen *et al.* [6]; *f*, Li *et al.* [7].

Table 3. Differential chemotaxonomic characteristics of strain MSC19^T and closely related species of the genus *Mycetocola*

Strains: 1, MSC19^T; 2, *M. saprophilus* NRRL B-24119^T; 3, *M. tolaasinivorans* NRRL B-24120^T; 4, *M. lacteus* JCM 11654^T; 5, *M. reblochoni* JCM 30549^T; 6, *M. manganoxydans* CCTCC AB209002^T; 7, *M. miduiensis* CGMCC 1.11101^T; 8, *M. zhadangensis* ZD1-4^T; 9, *M. zhujimingii* 449^T. Data are from this study, except where indicated. TR, Trace amount (<1%); –, not detected; NA, no data available. DPG, diphosphatidylglycerol; PG, phosphatidylglycerol; GL, unidentified glycolipid.

Component (%)*	1	2	3	4	5	6	7	8	9
Menaquinones:									
MK-10	30.2	53.0[a]	58.0[a]	54.0[a]	68.4[c]	70.9[c]	61.0[d]	63.0[e]	61.4[f]
MK-9	26.3	23.0[a]	21.0[a]	21.0[a]	20.7[c]	8.3[c]	NA	19.3[e]	18.4[f]
MK-11	21.8	12.0[a]	14.0[a]	14.0[a]	5.4[c]	19.8[c]	38.0[d]	17.3[e]	20.2[f]
MK-8	21.7	8.0[a]	4.0[a]	4.0[a]	5.6[c]	TR[c]	NA	NA	NA
Major fatty acids:									
anteiso-$C_{15:0}$	43.1	36.7	44.9	55.0[e]	30.0[e]	46.9[e]	51.0[d]	28.9[e]	52.5[f]
iso-$C_{16:0}$	18.3	12.4	12.1	12.8[e]	11.0[e]	4.6[e]	11.5[d]	7.4[e]	4.2[f]
anteiso-$C_{17:0}$	14.4	22.3	18.6	26.5[e]	20.2[e]	12.4[e]	20.7[d]	9.1[e]	24.8[f]
$C_{16:0}$	6.0	8.4	6.0	2.6[e]	9.0[e]	8.7[e]	10.0[d]	21.2[e]	NA
$C_{18:0}$	3.1	6.2	3.6	2.0[e]	6.3[e]	6.3[e]	NA	17.5[e]	NA
Polar lipids:	DPG, PG, GL	DPG, PG, GL	DPG, PG, GL	DPG, PG[b]	DPG, PG[b]	DPG, PG, GL[c]	DPG, PG, GL[d]	DPG, GL[e]	DPG, PG[f]

*Data from: *a*, Tsukamoto *et al.* [1]; *b*, Bora *et al.* [3]; *c*, Luo *et al.* [4]; *d*, Zhu *et al.* [5]; *e*, Shen *et al.* [6]; *f*, Li *et al.* [7].

DESCRIPTION OF *MYCETOCOLA SPONGIAE* SP. NOV.

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

Cells are Gram-stain-positive, strictly aerobic, non-spore-forming and non-motile rods (0.3–0.5 µm wide and 0.8–1.8 µm long). After growth on ISP 2 agar at 28 °C for 2 days, colonies are circular, slightly yellow, opaque and shiny with regular edges. Growth occurs at 4–32 °C (optimum, 28 °C), at pH 5.5–12 (optimum, pH 7.0) and in the presence of 0–12% (w/v) NaCl (optimum, 4%). Positive for catalase and oxidase. In API 20CH tests, strain MSC19^T does not produce acid from all 49 carbohydrates, differentiating it from other species of the genus. In the API ZYM tests, positive for production of alkaline phosphatase, esterase (C4), esterase lipase (C8), lipase (C14), leucine arylamidase, valine arylamidase, cystine arylamidase, *α*-chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, *β*-glucosidase and *N*-acetyl-*β*-glucosaminidase, but negative for production of trypsin, *α*-galactosidase, *β*-galactosidase, *β*-glucuronidase, *α*-glucosidase, *α*-mannosidase and *α*-fucosidase. In the Biolog GEN III MicroPlate, strain MSC19^T can oxidize dextrin, gentiobiose, *α*-D-glucose, D-mannose, D-fructose, inosine, glycerol, glycyl-L-proline, L-aspartic acid, L-glutamic acid, L-pyroglutamic acid, D-gluconic acid, D-glucuronic acid, glucuronamide, L-lactic acid, citric acid, D-malic acid, L-malic acid, bromo-succinic acid, Tween 40, acetoacetic acid and acetic acid. In API 20E tests, positive for *β*-galactosidase, Voges–Proskauer activities, and acid production from D-glucose and amygdalin; negative for urease, gelatinase, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, citrate utilization, H_2S and indole production, tryptophane deaminase, and acid production from mannitol, inositol, sorbitol, rhamnose, melibiose and arabinose. In API 20NE tests, positive for *β*-galactosidase and *β*-glucosidase activity, and assimilation of D-glucose, D-mannose, potassium gluconate, malic acid and trisodium citrate; but negative for reduction of nitrate, denitrification, indole production, D-glucose fermentation, urease, arginine dihydrolase, aesculin hydrolysis, gelatin hydrolysis and the assimilation of L-arabinose, D-mannitol, *N*-acetyl-*β*-glucosamine, maltose, capric acid, adipic acid and phenylacetic acid. The fatty acid profile consists predominantly of anteiso-$C_{15:0}$, iso-$C_{16:0}$ and anteiso-$C_{17:0}$. The polar lipids are diphosphatidylglycerol, phosphatidylglycerol, glycolipid and two unknown polar lipids. The main menaquinones are MK-10 and MK-9. The diagnostic cell-wall diamino acid is lysine.

The type strain, MSC19^T (=MCCC 1K06265^T=KCTC 49701^T), was isolated from a deep-sea sponge specimen (*Cacospongia mycofijiensis*) collected from the junction of the Mariana Trench and the Yap Trench (11.439670° N, 139.406620° E) at a water depth of 2681 m. The genomic DNA G+C content of MSC19^T is 66.43mol%.

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Author contributions
Z.L. designed the research and project outline. Q.S. identified the sponge sample. Y.C., J.S. and W.S. performed isolation and polyphasic taxonomy. Y.C. performed genome analysis. Y.C. 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.

References

1. Tsukamoto T, Takeuchi M, Shida O, Murata H, Shirata A. Proposal of *Mycetocola* gen. nov. in the family *Microbacteriaceae* and three new species, *Mycetocola saprophilus* sp. nov., *Mycetocola tolaasinivorans* sp. nov. and *Mycetocola lacteus* sp. nov., isolated from cultivated mushroom, *Pleurotus ostreatus*. *Int J Syst Evol Microbiol* 2001;51:937–944.
2. Parte AC. LPSN - List of Prokaryotic names with Standing in Nomenclature (bacterio.net), 20 years on. *Int J Syst Evol Microbiol* 2018;68:1825–1829.
3. Bora N, Vancanneyt M, Gelsomino R, Snauwaert C, Swings J, *et al*. *Mycetocola reblochoni* sp. nov., isolated from the surface microbial flora of Reblochon cheese. *Int J Syst Evol Microbiol* 2008;58:2687–2693.
4. Luo X, Wang J, Zeng X-C, Wang Y, Zhou L, *et al*. *Mycetocola manganoxydans* sp. nov., an actinobacterium isolated from the Taklamakan desert. *Int J Syst Evol Microbiol* 2012;62:2967–2970.
5. Zhu L, Liu Q, Liu H, Zhou Y, Xin Y, *et al*. *Mycetocola miduiensis* sp. nov., a psychrotolerant bacterium isolated from Midui glacier. *Int J Syst Evol Microbiol* 2013;63:2661–2665.
6. Shen L, Liu Y, Yao T, Kang S, Wang Y, *et al*. *Mycetocola zhadangensis* sp. nov., isolated from snow. *Int J Syst Evol Microbiol* 2013;63:3375–3378.
7. Li J, Yang J, Lu S, Jin D, Lai X-H, *et al*. *Mycetocola zhujimingii* sp. nov., isolated from faeces of Tibetan antelopes (*Pantholops hodgsonii*). *Int J Syst Evol Microbiol* 2019;69:1117–1122.
8. Weisburg WG, Barns SM, Pelletier DA, Lane DJ. 16S ribosomal DNA amplification for phylogenetic study. *J Bacteriol* 1991;173:697–703.
9. Sanger F, Nicklen S, Coulson AR. DNA sequencing with chain-terminating inhibitors. *Proc Natl Acad Sci USA* 1977;74:5463–5467.
10. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic Local Alignment Search Tool. *J Mol Biol* 1990;215:403–410.
11. Kumar S, Stecher G, Tamura K. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Mol Biol Evol* 2016;33:1870–1874.
12. Ardui S, Ameur A, Vermeesch JR, Hestand MS. Single molecule real-time (SMRT) sequencing comes of age: applications and utilities for medical diagnostics. *Nucleic Acids Res* 2018;46:2159–2168.
13. Parks DH, Imelfort M, Skennerton CT, Hugenholtz P, Tyson GW. CheckM: assessing the quality of microbial genomes recovered from isolates, single cells, and metagenomes. *Genome Res* 2015;25:1043–1055.
14. Blin K, Shaw S, Kloosterman AM, Charlop-Powers Z, van Wezel GP, *et al*. antiSMASH 6.0: improving cluster detection and comparison capabilities. *Nucleic Acids Res* 2021;49:W29–W35.
15. Bruns H, Crüsemann M, Letzel A-C, Alanjary M, McInerney JO, *et al*. Function-related replacement of bacterial siderophore pathways. *ISME J* 2018;12:320–329.
16. Meier-Kolthoff JP, Göker M. TYGS is an automated high-throughput platform for state-of-the-art genome-based taxonomy. *Nat Commun* 2019;10:2182.
17. Richter M, Rosselló-Móra R, Oliver Glöckner F, Peplies J. JSpeciesWS: a web server for prokaryotic species circumscription based on pairwise genome comparison. *Bioinformatics* 2016;32:929–931.
18. 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.
19. Richter M, Rosselló-Móra R. Shifting the genomic gold standard for the prokaryotic species definition. *Proc Natl Acad Sci USA* 2009;106:19126–19131.
20. Jin S, Xia J, Dunlap CA, Rooney AP, Du ZJ. *Psychroflexus saliphilus* sp. nov., isolated from a marine solar saltern. *Int J Syst Evol Microbiol* 2016;66:5124–5128.
21. Sasser M. Identification of bacteria by gas chromatography of cellular fatty acids. *USFCC Newsl* 1990;20:1–6.
22. Komagata K, Suzuki K. Lipid and cell wall analysis in bacterial systematics. *Methods Microbiol* 1987;19:161–206.
23. Collins MD, Jones D. Distribution of isoprenoid quinone structural types in bacteria and their taxonomic implication. *Microbiol Rev* 1981;45:316–354.
24. Kates M. Lipid extraction procedures. In: Kates M (eds). *Techniques of Lipidology Isolation, Analysis, and Identification of Lipids*. Amsterdam: Elsevier Science Publisher; 1986. pp. 100–111.
25. Komagata K, Suzuki K. Lipid and cell-wall analysis in bacterial systematics. *Methods Microbiol* 1987;19:161–207.