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TAXONOMIC DESCRIPTION
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Mesorhizobium oceanicum sp. nov., isolated from deep seawater

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Abstract

A novel Gram-staining-negative, oval-shaped (0.4–0.6×0.8–1.0 µm), non-motile strain without flagella, designated B7^T, was isolated from deep seawater in the South China Sea. Strain B7^T was able to grow at 25–40 °C (optimum 35 °C), at pH 5.5–9.0 (optimum pH 7.0) and with 0–8 % (w/v) NaCl (optimum 3 %). Chemotaxonomic analysis showed that the predominant isoprenoid quinone was Q-10 and the dominant fatty acids were $C_{19:0}$ cyclo 8*c* and summed feature 8 ($C_{18:1}\omega7c/C_{18:1}\omega6c$). The polar lipids of strain B7^T were diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylcholine, one unknown aminophospholipid, one unknown glycolipid and three unknown lipids. The DNA G+C content of the genomic DNA was 65.1 mol%. Phylogenetic analysis of 16S rRNA gene sequences showed that strain B7^T belongs to the genus *Mesorhizobium* with similarities ranging from 96.2 to 97.5 %. Phylogenetic analyses of housekeeping genes *recA*, *atpD* and *glnII* indicated that strain B7^T represented a distinct evolutionary lineage with the genus *Mesorhizobium*. OrthoANI values between strain B7^T and related strains of the genus *Mesorhizobium* (<80 %) were lower than the threshold value of 95 % ANI relatedness for species demarcation. Therefore, strain B7^T is concluded to represent a novel species of the genus *Mesorhizobium*, for which the name *Mesorhizobium oceanicum*sp. nov. is proposed. The type strain is B7^T (=KCTC 42783^T=MCCC 1K02305^T).

The genus *Mesorhizobium*, belonging to the family *Phyllobacteriaceae* was first proposed by Jarvis *et al.* [1] with type species *Mesorhizobium loti* which was isolated from a root nodule on *Lotus corniculatus* [2]. The genus was established to describe strains whose growth rate was slower than the fast-growing members of the genus *Rhizobium* [1]. At the time of writing, the genus *Mesorhizobium* consists of 41 species with validly published names [3] (http://www.bacterio.net/mesorhizobium.html). Members of the genus share the characteristics of being Gram-staining-negative, aerobic, rod-shaped and having a DNA G+C content around 57.8–65.0 mol%. At the time of writing, members of the genus *Mesorhizobium* have been isolated from root nodules of different leguminous plants such as *Sophora longicarinata* [4], *Anagyris latifolia* [5] and *Astragalus membranaceus* [6], and deep-sea sediment [7]. In this study, strain B7^T was isolated from deep seawater in the South China Sea. The aim of the present investigation was to determine the taxonomic position of strain B7^T based on analysis of phenotypic, phylogenetic, genomic and chemotaxonomic characteristics.

A bottle of seawater was collected in August 2012 from the South China Sea (15° 14′ N 117° 17′ E) at a depth of 1.0 km. The water sample was stored at 4 °C in the laboratory before use. Strain B7^T was isolated by the method described previously [8] and preserved by freeze-drying. For routine cultivation of strain B7^T, marine agar 2216 (MA; BD Difco) or marine broth 2216 (MB; BD Difco) were used. Four reference strains used in this study (*Mesorhizobium huakuii* LMG 14107^T, *M. loti* LMG 6125^T, *Mesorhizobium thiogangeticum* LMG 22697^T and *Mesorhizobium soli* JCM 19897^T) were purchased from the Belgian Co-ordinated Collections of Micro-organisms (BCCM/LMG) and the Japan Collection of Microorganisms (JCM), respectively.

Phenotypic tests were performed with cells grown on MB at 30 °C, unless otherwise indicated. The Gram reaction was tested by using the Gram staining method as described by Claus [9]. After incubation on MA at 30 °C for 3 days, cell morphology and the presence of flagella were observed using transmission electron microscopy (JEM-1230; JEOL) after uranyl

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Abbreviations: ANI, average nucleotide identity; DPG, diphosphatidylglycerol; PE, phosphatidylethanolamine; PC, phosphatidylcholine; PG, phosphatidylglycerol.
The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequence and complete genome sequence of strain B7^T are KT157593 and CP018171, respectively.
Three supplementary figures and two supplementary tables are available with the online Supplementary Material.

acetate staining. The motility test was performed by inoculation in semi-solid MB [MB medium with 0.5 % agar (w/v)]. The temperature range for growth was determined in MB at 4–55 °C (4, 10, 15, 20, 25, 28, 30, 35, 40, 45, 50 and 55 °C). The pH range for growth in MB was measured from pH 4.5 to pH 10.0, with an interval of 0.5 units, using appropriate biological buffers to maintain the stability of each pH system (40 mM MES for pH 4.5–6.0, PIPES for pH 6.5–7.5, Tricine for pH 8.0–8.5 and CAPSO for pH 9.0–10.0). Tolerance to NaCl was tested in medium containing (l^{-1}): 12.6 g $MgCl_2 . 6H_2O$, 6.64 g $MgSO_4 . 7H_2O$, 1.8 g $CaCl_2$, 0.55 g KCl, 0.08 g KBr, 0.025 g H_3BO_3, 0.04 g $SrCl_2 . 6H_2O$, 0.05 g NH_4Cl, 0.19 g K_2HPO_4, 5.0 g trypticase peptone (BD Difco) and 1.0 g yeast extract (BD Difco) (pH 7.0), with the concentrations of NaCl at 0, 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 % (w/v).

For normal cultivation, all reference strains used in this study were cultured in Yeast Mannitol Broth (YMB) or on Yeast Mannitol Agar (YMA) [10] at 30 °C. To make the comparison more reliable, strain B7^T was cultured on modified YMA/YMB [YMA/YMB medium with 2 % NaCl (w/v)]. Catalase and oxidase activity were tested by the method as described by Sun *et al.* [11]. Degradation of L-tyrosine and hydrolysis of starch, Tweens 20, 40, 60 and 80 were examined as described by Sun *et al.* [12]. Hydrolysis of casein and gelatin were assayed according to Zhang *et al.* [13]. Nitrate reduction was tested according to Dong *et al.* [14]. H_2S and indole production were assayed as described by Zhu *et al.* [15]. The methyl red (MR) and Voges–Proskauer (VP) tests were examined as described by Lányí [16]. Other biochemical properties and enzyme activities were tested using API ZYM and API 20NE strips (bioMérieux) following the manufacturer's instructions. GN2 MicroPlates (Biolog) were used to detect the utilization of organic substrates according to the manufacturer's instructions. Utilization of complex compounds was determined using each optimal saline solution (YMB/modified YMB without mannitol, sodium glutamate and yeast) with 0.2 % (w/v) trypticase, peptone, polypeptone, soytone, casamino acids, yeast extracts and tryptone (BD Difco) after incubating at 30 °C for 3 days.

For chemotaxonomic studies, cell mass of strain B7^T was grown on modified YMA and four reference strains were grown on YMA at 30 °C. Cells used for the analysis of fatty acids and polar lipids were harvested from the third quadrants of agar plates [17]. Fatty acid methyl esters were extracted as described by Kuykendall *et al.* [18] and analysed by the Sherlock Microbial Identification System (MIDI; Microbial ID); results are shown in Table S1 (available in the online Supplementary Material). Polar lipids were extracted and separated by two-dimensional TLC on silica gel 60 F254 plates (Merck 5554) and then further analysed as described [19, 20]. After incubation in modified YMB at 30 °C for 3 days, cells of strain B7^T were collected for extraction of isoprenoid quinones which were subsequently purified by TLC and identified using the HPLC-MS system (Agilent) [21, 22].

Genomic DNA of strain B7^T was extracted using Quick Bacteria Genomic DNA Extraction kit (DongSheng Biotech). The 16S rRNA gene was amplified by PCR using universal primer pair 27F (5′-GAGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-GTCGTAACAAGGTAGCCGTA-3′). The purified PCR products were cloned into the vector pMD19-T (TaKaRa) and then sequenced. The almost-complete 16S rRNA gene sequence of strain B7^T (1449 bp) was identified by the EzTaxon-e tool [23]. Multiple sequences alignment was performed with the CLUSTAL X program of the MEGA 5 software package [24]. Phylogenetic trees were reconstructed using the neighbour-joining [25] and maximum-likelihood [26] methods with the MEGA5 program package. Bootstrap analysis was based on 1000 replications. To testify the phylogenetic tree reconstructed by MEGA5, All-Species Living Tree LTPs123 and database arb-6.0.6 were used as the reference, SINA webserver [27] and ARB software [28] were used for alignment of 16S rRNA gene sequences into LTPs123 and generation of a new maximum-likelihood phylogenetic tree, respectively. Genomic DNA of strain B7^T was extracted using CTAB methods [29]. The complete genome was sequenced by BGI Genome Institute (Beijing) with Illumina HiSeq 4000 and PacBio platform and assembled by SOAPdenovo 2.04 [30]. Average nucleotide identity (ANI) was calculated with OrthoANI [31]. DNA G+C content (mol%) was obtained from the genomic sequences. The gene sequences *atpD* (CP018171.1 from 290135 to 291688), *glnII* (CP018171.1 from 4538412 to 4537042) and *recA* (CP018171.1 from 3543275 to 3542229) were identified by Rapid Annotation System Technology (RAST) of the genome sequence of strain B7^T [32]. Corresponding sequences of recognized species of the genus *Mesorhizobium* were obtained from the GenBank database. The gene sequences were aligned using MAFFT [33] and poorly aligned positions were removed by Gblocks [34]. Then MEGA 5 was employed to reconstruct phylogenetic trees, using the maximum-likelihood method, with the Tamura-3-parameter model and G substitutions.

16S rRNA gene sequence analysis using the EzTaxon-e tool indicated the 36 most related species of strain B7^T all belonged to genus *Mesorhizobium* (similarities ranged from 96.2 to 97.5 %), and based on the recommended threshold value of 98.65 % 16S rRNA gene sequence similarity [35], strain B7^T should be considered as the type strain of a supposed novel species of genus *Mesorhizobium*. The phylogenetic trees based on 16s rRNA gene sequences using both MEGA and ARB programs also indicated that strain B7^T fell in the clade which comprises species of the genus *Mesorhizobium*, supporting that strain B7^T is a novel species of the genus *Mesorhizobium* (Figs 1 and S1). The phylogenetic tree based on the concatenated *recA* [~205 bp], *atpD* [~306 bp] and *glnII* [~507 bp] sequences of strain B7^T and other strains revealed that strain B7^T was most closely related to *Mesorhizobium huakuii* USDA 4779^T (Fig. 2). Meanwhile, the OrthoANI values between strain B7^T and related type strains (Table S2) were lower than the threshold value of 95 % ANI relatedness for species demarcation [36, 37], which also confirmed that strain B7^T represents a novel species of the genus *Mesorhizobium*.

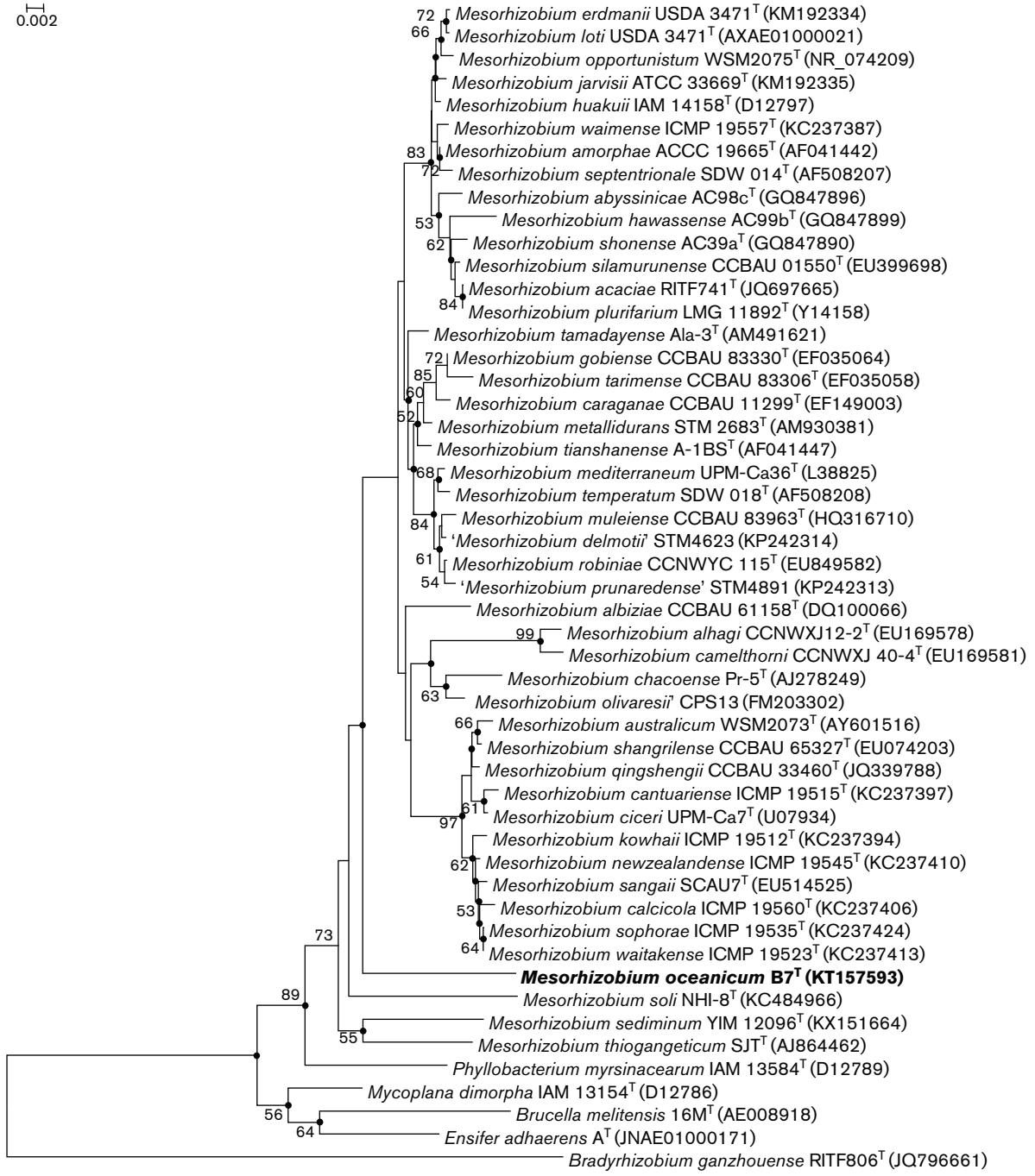

Fig. 1. Neighbour-joining tree using Kimura's two-parameter model based on 16S rRNA gene sequences, showing the phylogenetic relationships of the novel isolate and related members of the genus *Mesorhizobium* and other relative genera. Bootstrap values are based on 1000 replicates; only values >50 % are shown. Filled circles indicate nodes also obtained in the maximum-likelihood tree. Bar, 0.002 substitutions per nucleotide position.

Cells of strain B7^T were Gram-stain-negative, oval (0.4–0.6×0.8–1.0 µm), and non-motile without flagella (Fig. S2). Colonies were 1–2 mm in diameter, circular, light yellow, convex, opaque and smooth after growing on MA at 30 °C for 3 days. Strain B7^T grew at 25–40 °C (optimum 35 °C), at pH 5.5–9.0 (optimum pH 7.0) and with 0–8 % (w/v) NaCl (optimum 3 %). Detailed physiological and biochemical characteristics are displayed in Table 1 and the species description. Several phenotypic differences could be found between strain B7^T and the reference strains (Table 1). For

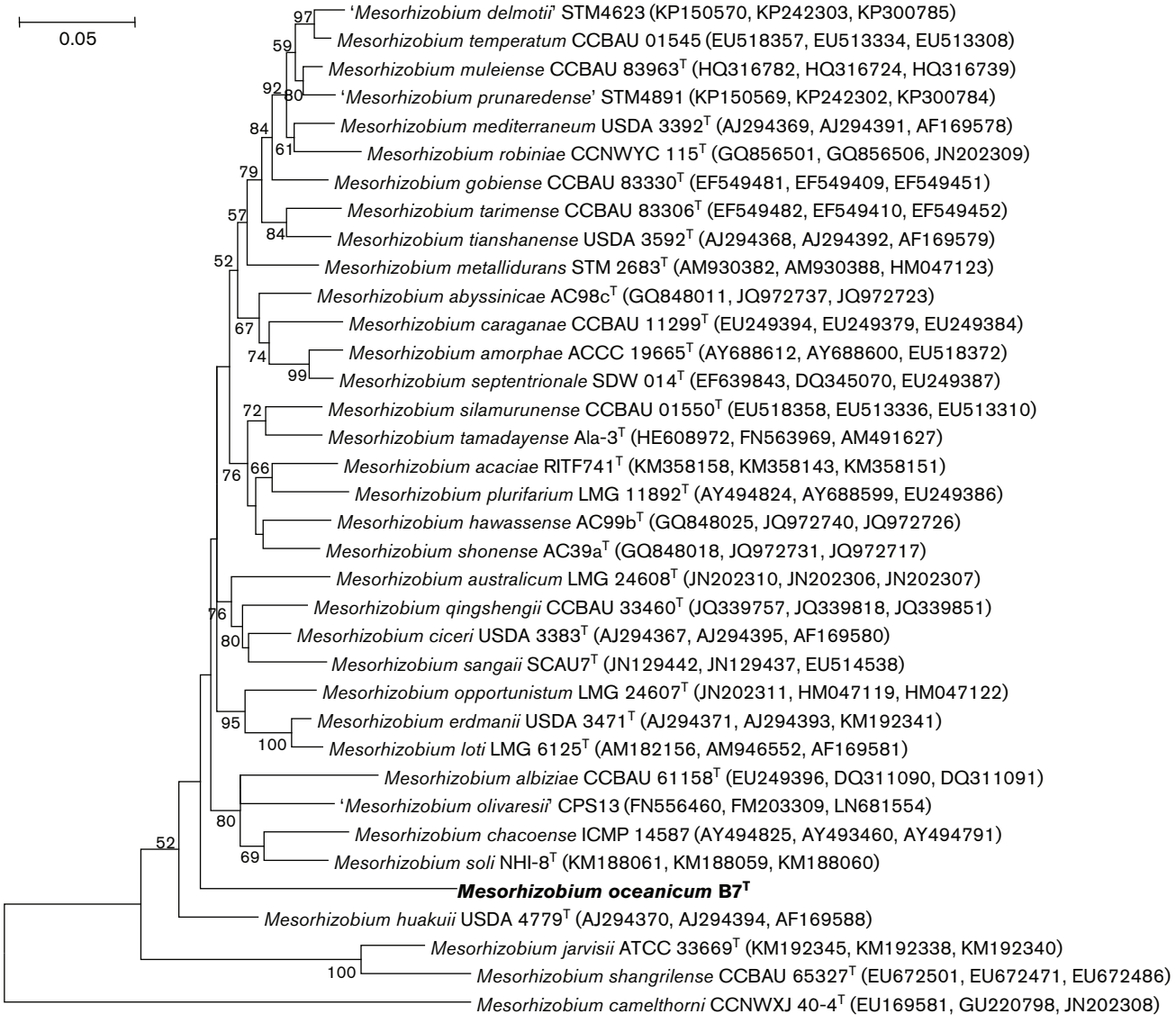

Fig. 2. Maximum-likelihood tree based on partial concatenated *recA*, *atpD* and *glnII* gene sequences of strain B7^T and the type strains of some closely related species within the genus *Mesorhizobium*. Bootstrap values calculated for 1000 replicates are indicated at nodes. Bar, 0.05 substitutions per nucleotide position.

example, strain B7^T has the ability to hydrolyse casein, gelatin and Tween 40 while the other four reference strains cannot; strain B7^T is unable to hydrolyse L-tyrosine nor produce H_2S, which are opposite to the four reference strains.

Respiratory quinone composition analysis showed that the only isoprenoid quinone in strain B7^T was Q-10, which was a common characteristic in the genus *Mesorhizobium*. Major fatty acids (>10 %) of strain B7^T were $C_{19:0}$ cyclo $\omega 8c$ and summed feature 8 ($C_{18:1}\omega 7c/C_{18:1}\omega 6c$), which were similar to most of the four reference strains though some proportional differences existed (Table S1). Meanwhile, $C_{17:0}$ which was detected in only in strain B7^T could differentiate the novel strain from the reference strains. The polar lipids of strain B7^T and the reference strains are shown in Fig. S3. The polar lipids of strain B7^T were diphosphatidylglycerol (DPG), phosphatidylglycerol (PG), phosphatidylethanolamine (PE), phosphatidylcholine (PC), one unknown aminophospholipid (APL1), one unknown glycolipid (GL2) and three unknown lipids (L1, L2 and L3). DPG, PG, PE and PC were also detected in the four reference strains and previously in the genus *Mesorhizobium* [38], which could be a significant feature to support that strain B7^T belonged to genus *Mesorhizobium*. The existence of GL2 in strain B7^T was consistent with that in reference strain *M. soli* JCM 19897^T. Meanwhile the

Table 1. Differential phenotypic characteristics of strain B7^T and the type strains of related species

Strains: 1, B7^T; 2, *M. soli* JCM 19897^T; 3, *M. huakuii* LMG 14107^T; 4, *M. loti* LMG 6125^T; 5, *M. thiogangeticum* LMG 22697^T. All data are obtained from this study unless otherwise indicated. +, Positive; −, negative; W, weakly positive; ND, no data available.

Characteristic	1	2	3	4	5
Habitat	Seawater	Forest soil sample*[a]	Root nodule of *Astragalus sinicusb*	Root nodule on *Lotus corniculatus*[c]	Soil adjacent to root of *Clitoria ternatea*[d]
Cell morphology	Oval	Rod[a]	Rod[b]	Rod [c]	Irregularly elongated or rod-shaped[d]
Flagella	−	ND	One polar or subpolar [b]	one polar or subpolar[c]	ND
Motility	−	−	+[b]	+[c]	−[d]
Temperature for growth (°C)	25–40	14–40[a]	25–30[b]	<42[c]	30–37[d]
pH for growth	5.5–9.0	6.0–9.0[a]	5.0–9.5[b]	4.0–10.0[c]	5.5–8.5[d]
Salinity for growth	0–8	0–2[a]	<2[b]	<3[c]	<2[d]
Nitrate reduction to nitrite	W	−	W	W	+
H_2S production	−	+	+	+	+
Methyl red reaction	−	−	+	+	+
Enzyme activity					
Acid phosphatase	W	+	+	+	+
Caseinase	+	−	−	−	−
Esterase(C4)	+	W	W	W	+
Esterase lipase(C8)	+	+	W	+	+
Gelatinase	W	−	−	−	−
α-Glucosidase	−	+	+	+	W
Leucine arylamidase	−	+	−	−	−
Urease	+	+	+	+	−
Valine arylamidase	W	+	W	−	−
Hydrolysis of:					
Aesculin	−	−	+	−	+
L-Tyrosine	−	+	+	+	+
Tween 20	−	−	−	−	+
Tween 40	+	−	−	−	−
Tween 60	+	−	−	−	+
Utilization of:					
Potassium gluconate	−	+	+	+	+
Adipic acid	W	+	+	+	+
Citrate	+	+	+	−	+
Trypticase	W	+	+	+	+
Casamino acids	−	W	+	+	+
Peptone	−	+	+	+	+
Tryptone	−	+	+	+	+
DNA G+C content (mol%)	65.1	64.4[a]	59–64[b]	59–64[c]	59.6[d]

*Data from: *a*, Nguyen *et al.* [39]; *b*, Chen *et al.* [40]; *c*, Jarvis *et al.* [2]; *d*, Ghosh *et al.* [41].

compositions of L1, L2 and L3 in strain B7^T could differentiate the isolate from reference strains.

Based on the phylogenetic, genomic, chemotaxonomic and phenotypic characteristics, it is concluded that strain B7^T represents a novel species of the genus *Mesorhizobium*, for which the name *Mesorhizobium oceanicum* sp. nov. is proposed.

DESCRIPTION OF *MESORHIZOBIUM OCEANICUM* SP. NOV.

Mesorhizobium oceanicum (o.ce.a′ni.cum. L. neut. adj. *oceanicum* belonging to the sea).

Cells are Gram-staining-negative, aerobic, non-motile and oval-shaped, measuring 0.4–0.6 µm wide by 0.8–1.0 µm long. Colonies on MA after 4 days of incubation at 30 °C are

circular, opaque, smooth, light yellow, convex and 1.0–2.0 mm in diameter. Growth occurs at 25–40 °C (optimum 35 °C), at pH 5.5–9.0 (optimum pH 7.0) and with 0–8 % NaCl (optimum 3 %). Oxidase- and catalase-positive. Nitrate is weakly reduced to nitrite. Nitrite is not reduced to N_2. Casein, Tween 40, Tween 60 and urea are hydrolysed, gelatin is weakly hydrolysed, but esculin, tyrosine, starch, Tween 20 and Tween 80 are not hydrolysed. Negative for H_2S production, indole production, Voges–Proskauer test and methyl red test. Utilization of L-arabinose, *N*-acetyl-glucosamine, D-glucose, maltose, D-mannitol, D-mannose, citrate, malate, yeast extracts, polypeptone, soytone are positive, utilization of peptone, casamino acids and tryptone are negative; utilization of adipic acid and trypticase is weak. Fermentation of glucose and assimilation of potassium gluconate, n-capiric acid and phenylacetic acid are negative. Production of alkaline phosphatase, esterase (C4), esterase lipase (C8) and trypsin are positive; production of acid phosphatase, naphthol AS-BI phosphohydrolase and valine arylamidase are weakly positive; and production of chymotrypsin, cystine arylamidase, β-fucosidase, α-galactosidase, β-galactosidase, α-glucosidase, β-glucosidase, β-glucuronidase, α-mannosidase, leucine arylamidase, lipase (C14) and *N*-acetyl-β-glucosaminidase are negative. The predominant isoprenoid quinone is Q-10. Major fatty acids (>10 %) are summed feature 8 ($C_{18:1}\omega7c/C_{18:1}\omega6c$) and $C_{19:0}$ cyclo $\omega8c$. The polar lipids consist of DPG, PG, PE, PC, one unknown aminophospholipid, one unknown glycolipid and three unknown lipids.

The type strain is B7^T (=KCTC 42783^T=MCCC 1K02305^T), isolated from deep seawater in the South China Sea. The DNA G+C content of the genomic DNA of the type strain is 65.1 mol%.

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

References

1. Jarvis BDW, van Berkum P, Chen WX, Nour SM, Fernandez MP *et al.* Transfer of *Rhizobium loti*, *Rhizobium huakuii*, *Rhizobium ciceri*, *Rhizobium mediterraneum*, and *Rhizobium tianshanense* to *Mesorhizobium* gen. nov. *Int J Syst Bacteriol* 1997;47:895–898.
2. Jarvis BDW, Pankhurst CE, Patel JJ. *Rhizobium loti*, a new species of legume root nodule bacteria. *Int J Syst Bacteriol* 1982;32:378–380.
3. Parte AC. LPSN—list of prokaryotic names with standing in nomenclature. *Nucleic Acids Res* 2014;42:D613–D616.
4. De Meyer SE, Tan HW, Heenan PB, Andrews M, Willems A. *Mesorhizobium waimense* sp. nov. isolated from *Sophora longicarinata* root nodules and *Mesorhizobium cantuariense* sp. nov. isolated from *Sophora microphylla* root nodules. *Int J Syst Evol Microbiol* 2015;65:3419–3426.
5. Ramírez-Bahena MH, Hernández M, Peix A, Velázquez E, León-Barrios M. Mesorhizobial strains nodulating *Anagyris latifolia* and *Lotus berthelotii* in Tamadaya ravine (Tenerife, Canary Islands) are two symbiovars of the same species, *Mesorhizobium tamadayense* sp. nov. *Syst Appl Microbiol* 2012;35:334–341.
6. Zhao CT, Wang ET, Zhang YM, Chen WF, Sui XH *et al.* *Mesorhizobium silamurunense* sp. nov., isolated from root nodules of *Astragalus* species. *Int J Syst Evol Microbiol* 2012;62:2180–2186.
7. Yuan CG, Jiang Z, Xiao M, Zhou EM, Kim CJ *et al.* *Mesorhizobium sediminum* sp. nov., isolated from deep-sea sediment. *Int J Syst Evol Microbiol* 2016;66:4797–4802.
8. Sun C, Wang RJ, Su Y, Fu GY, Zhao Z *et al.* *Hyphobacterium vulgare* gen. nov., sp. nov., a novel alphaproteobacterium isolated from seawater. *Int J Syst Evol Microbiol* 2017;67:1169–1176.
9. Claus D. A standardized Gram staining procedure. *World J Microbiol Biotechnol* 1992;8:451–452.
10. Vincent J. The cultivation, isolation and maintenance of rhizobia. *A Manual for the Practical Study of the Root-Nodule Bacteria*. Oxford: Blackwell Scientific; 1970. pp. 1–13.
11. Sun C, Pan J, Zhang XQ, Su Y, Wu M. *Pseudoroseovarius zhejiangensis* gen. nov., sp. nov., a novel alpha-proteobacterium isolated from the chemical wastewater, and reclassification of *Roseovarius crassostreae* as *Pseudoroseovarius crassostreae* comb. nov., *Roseovarius sediminilitoris* as *Pseudoroseovarius sediminilitoris* comb. nov. and *Roseovarius halocynthiae* as *Pseudoroseovarius halocynthiae* comb. nov. *Antonie van Leeuwenhoek* 2015;108:291–299.
12. Sun C, Huo YY, Liu JJ, Pan J, Qi YZ *et al.* *Thalassomonas eurytherma* sp. nov., a marine proteobacterium. *Int J Syst Evol Microbiol* 2014;64:2079–2083.
13. Zhang XQ, Sun C, Wang CS, Zhang X, Zhou X *et al.* *Sinimarinibacterium flocculans* gen. nov., sp. nov., a gammaproteobacterium from offshore surface seawater. *Int J Syst Evol Microbiol* 2015;65:3541–3546.
14. Dong X, Cai M. *Determinative Manual for Routine Bacteriology*. Beijing: Scientific Press (English translation); 2001.
15. Zhu X, Jia X, Zhang X, Wu Y, Chen Z *et al.* *Modern Experimental Technique of Microbiology*. Hangzhou: Zhejiang University Press (English translation); 2011.
16. Lányí B. Classical and rapid identification methods for medically important Bacteria. *Methods Microbiol* 1988;19:1–67.
17. Xu L, Wu YH, Jian SL, Wang CS, Wu M *et al.* *Pseudohongiella nitratireducens* sp. nov., isolated from seawater, and emended description of the genus *Pseudohongiella*. *Int J Syst Evol Microbiol* 2016;66.
18. Kuykendall LD, Roy MA, O'Neill JJ, Devine TE. Fatty acids, antibiotic resistance, and deoxyribonucleic acid homology groups of *Bradyrhizobium japonicum*. *Int J Syst Bacteriol* 1988;38:358–361.
19. Minnikin DE, O'Donnell AG, Goodfellow M, Alderson G, Athalye M *et al.* An integrated procedure for the extraction of bacterial isoprenoid quinones and polar lipids. *J Microbiol Methods* 1984;2:233–241.
20. Fang MX, Zhang WW, Zhang YZ, Tan HQ, Zhang XQ *et al.* *Brassicibacter mesophilus* gen. nov., sp. nov., a strictly anaerobic bacterium isolated from food industry wastewater. *Int J Syst Evol Microbiol* 2012;62:3018–3023.
21. Komagata K, Suzuki K. Lipid and cell-wall analysis in bacterial systematics. *Methods Microbiol* 1988;19:161–207.
22. Tindall BJ, Sikorski J, Smibert RA, Krieg NR. Phenotypic characterization and the principles of comparative systematics. In: Reddy CA, Beveridge TJ, Breznak JA, Marzluf GA, Schmidt TM *et al.* (editors). *Methods for General and Molecular Microbiology*, 3rd ed. Washington, DC: American Society of Microbiology; 2007. pp. 330–393.

23. **Kim OS, Cho YJ, Lee K, Yoon SH, Kim M *et al.*** Introducing EzTaxon-e: a prokaryotic 16S rRNA gene sequence database with phylotypes that represent uncultured species. *Int J Syst Evol Microbiol* 2012;62:716–721.
24. **Tamura K, Peterson D, Peterson N, Stecher G, Nei M *et al.*** MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol Biol Evol* 2011;28:2731–2739.
25. **Saitou N, Nei M.** The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* 1987;4:406–425.
26. **Felsenstein J.** Evolutionary trees from DNA sequences: a maximum likelihood approach. *J Mol Evol* 1981;17:368–376.
27. **Pruesse E, Peplies J, Glöckner FO.** SINA: accurate high-throughput multiple sequence alignment of ribosomal RNA genes. *Bioinformatics* 2012;28:1823–1829.
28. **Ludwig W, Strunk O, Westram R, Richter L, Meier H *et al.*** ARB: a software environment for sequence data. *Nucleic Acids Res* 2004; 32:1363–1371.
29. **Clarke JD.** Cetyltrimethyl ammonium bromide (CTAB) DNA miniprep for plant DNA isolation. *Cold Spring Harb Protoc* 2009;2009: pdb.prot5177.
30. **Luo R, Liu B, Xie Y, Li Z, Huang W *et al.*** SOAPdenovo2: an empirically improved memory-efficient short-read de novo assembler. *Gigascience* 2012;1:18.
31. **Lee I, Kim YO, Park SC, Chun J.** OrthoANI: an improved algorithm and software for calculating average nucleotide identity. *Int J Syst Evol Microbiol* 2015;66:1100–1103.
32. **Aziz RK, Bartels D, Best AA, Dejongh M, Disz T *et al.*** The RAST server: rapid annotations using subsystems technology. *BMC Genomics* 2008;9:1.
33. **Katoh K, Standley DM.** MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Mol Biol Evol* 2013;30:772–780.
34. **Castresana J.** Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Mol Biol Evol* 2000; 17:540–552.
35. **Kim M, Oh HS, Park SC, Chun J.** Towards a taxonomic coherence between average nucleotide identity and 16S rRNA gene sequence similarity for species demarcation of prokaryotes. *Int J Syst Evol Microbiol* 2014;64:346–351.
36. **Goris J, Konstantinidis KT, Klappenbach JA, Coenye T, Vandamme P *et al.*** DNA-DNA hybridization values and their relationship to whole-genome sequence similarities. *Int J Syst Evol Microbiol* 2007;57:81–91.
37. **Richter M, Rosselló-Móra R.** Shifting the genomic gold standard for the prokaryotic species definition. *Proc Natl Acad Sci USA* 2009;106:19126–19131.
38. **Choma A, Komaniecka I.** Analysis of phospholipids and ornithine-containing lipids from *Mesorhizobium* spp. *Syst Appl Microbiol* 2002;25:326–331.
39. **Nguyen TM, Pham VH, Kim J.** *Mesorhizobium soli* sp. nov., a novel species isolated from the rhizosphere of *Robinia pseudoacacia* L. in South Korea by using a modified culture method. *Antonie van Leeuwenhoek* 2015;108:301–310.
40. **Chen WX, Li GS, Qi YL, Wang ET, Yuan HL *et al.*** *Rhizobium huakuii* sp. nov. isolated from the root nodules of *Astragalus sinicus*. *Int J Syst Bacteriol* 1991;41:275–280.
41. **Ghosh W, Roy P.** *Mesorhizobium thiogangeticum* sp. nov., a novel sulfur-oxidizing chemolithoautotroph from rhizosphere soil of an Indian tropical leguminous plant. *Int J Syst Evol Microbiol* 2006;56: 91–97.