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ORIGINAL PAPER

Microvirga pudoricolor sp. nov., and *Microvirga alba* sp. nov., isolated from soil in South Korea

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Abstract

Two novel Gram-stain-negative, aerobic, rod-shaped, circular, convex, light-pink and white-colored bacterial strains BT291^T and BT350^T were isolated from soil collected in Uijeongbu city (37° 44′ 55″ N, 127° 2′ 20″ E) and Jeju island (33° 22′ 48″ N, 126° 31′ 48″ E), respectively, South Korea. Phylogenetic analysis based on 16S rRNA gene sequences revealed that each of the strains BT291^T and BT350^T belong to a distinct lineages within the genus *Microvirga* (family *Methylobacteriaceae*, order *Rhizobiales*, class *Alpha Proteobacteria*, phylum *Proteobacteria*, kingdom *Bacteria*). The 16S rRNA gene sequence similarity between the two strains BT291^T and BT350^T was 97.4%. The two strains were found to have the same quinone system, with Q-10 as the major respiratory quinone. The major polar lipids of strains BT291^T and BT350^T were phosphatidylethanolamine (PE), diphosphatydilglycerol (DPG), phosphatidylcholine (PC) and phosphatidylglycerol (PG). The major cellular fatty acids of strain BT291^T were $C_{18:1}$ $\omega 7c$ (58.2%) and cyclo-$C_{19:0}$ $\omega 8c$ (25.7%). The major cellular fatty acids of strain BT350^T were $C_{18:1}$ $\omega 7c$ (38.5%) and cyclo-$C_{19:0}$ $\omega 8c$ (27.7%). Based on the polyphasic analysis (phylogenetic, chemotaxonomic and biochemical), strains BT291^T and BT350^T can be suggested as two novel bacterial species within the genus *Microvirga* and the proposed names are *Microvirga pudoricolor* and *Microvirga alba*, respectively. The type strain of *Microvirga pudoricolor* is BT291^T (= KCTC 72368^T = NBRC 114845^T) and the type strain of *Microvirga alba* is BT350^T (= KCTC 72385^T = NBRC 114848^T).

Keywords *Methylobacteriaceae* · *Microvirga* · Taxonomy

Introduction

The genus *Microvirga* was first described by Kanso and Patel (2003) allocated to the family *Methylobacteriaceae*, order *Rhizobiales*. At the time of writing (August 2021), the genus comprises 18 published species as well as some non-validly published ones (https://lpsn.dsmz.de/genus/microvirga). *Microvirga* species have been retrieved from various polar environments in the last years, e.g., from regoliths from Tibet hot spring sediments (Liu et al. 2020), roots of rapeseed plants (Jimenez-Gomez et al. 2019), root nodule (Wang et al. 2019), forest soil (Zhang et al. 2019), rhizospheric soil (Li et al. 2020) and root nodule (Msaddak et al. 2019).

In this study, both strains BT291^T and BT350^T were isolated from a soil sample Uijeongbu city and Jeju island, respectively, South Korea. In the present study, we conducted a phylogenetic analysis based on the 16S rRNA gene sequences and phenotypic, genotypic and chemotaxonomic characteristics to determine the taxonomic position of strains BT291^T and BT350^T. The results suggested that strains BT291^T and BT350^T represent two novel species of the genus *Microvirga*, for which the name *Microvirga pudoricolor alba* sp. nov. and *Microvirga alba* sp. nov. are proposed.

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All authors contributed equally to this manuscript.

The 16S rRNA gene sequences of the strains BT291^T and BT350^T were deposited in GenBank/EMBL/DDBJ under the accession numbers are MT795755 and MT795757, respectively. The draft genome sequences of the strains BT291^T and BT350^T are available at the following accessions JAFEMB000000000 and JADQDO010000000, respectively.

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Materials and methods

Isolation and cultural conditions

Strain BT291^T was isolated from Uijeongbu city (37° 44′ 55″ N, 127° 2′ 20″ E) and strain BT350^T was isolated from Jeju island (33° 22′ 48″ N, 126° 31′ 48″ E) located in South Korea. Colonies were isolated using Reasoner's 2A (R2A) agar medium (Difco) after incubation at 25 °C for 9 days. The strains were routinely cultured on R2A agar at 25 °C, maintained at 4 °C and stored in 10% (w/v) glycerol suspension at − 80 °C.

Morphology, physiology and biochemical analysis

The cell morphology was examined using transmission electron microscopy (JEOL, JEM1010) with negative staining. The Gram-staining reaction was performed using a kit, following the manufacturer's instruction (bioMérieux). Catalase activity was examined with 3% (w/v) H_2O_2 solution and oxidase activity was examined by the addition of 1% (w/v) tetramethyl-p-phenylenediamine (Cappuccino and Sherman 2002). The growth of both of strains were tested on Reasoner's 2A (R2A) agar, Luria–Bertani (LB) agar, Tryptic Soy Agar (TSA), Nutrient Agar (NA) and on MacConkey (MAC) agar. Growth was tested at different temperatures (10, 15, 25 and 30 °C) under various pH conditions (5–9, 1 pH intervals) and different NaCl concentrations (1–5% [w/v %], 1% intervals). API 20NE and API ZYM tests were performed according to the manufacturer's instruction (bioMérieux).

Phylogenetic analysis

The 16S rRNA genes of strains BT291^T (1,428 bp) and BT350^T (1,435 bp) were amplified and sequenced using two universal bacterial primers 27F and 1492R (Weisburg et al. 1991) using the genomic DNA as a template. The sequencing was then done using the 337F, 518R, 785F, and 926R universal primers (Macrogen). To determine the taxonomic positions of both strains, 16S rRNA sequences of similarity searches were obtained from EzBioCloud (Yoon et al. 2017) and compared with those of both new strains using EzEditor2 server. Phylogenetic trees were reconstructed using the MEGAX program (Kumar et al. 2018) with the neighbor-joining (Saitou and Nei 1987), maximum-likelihood (Felsenstein 1981) and maximum-parsimony algorithms (Fitch 1971). The stability of the tree topologies was evaluated by bootstrap analysis based on 1000 resampling method (Felsenstein 1985). Evolutionary distances were calculated according to the Kimura two-parameter model (Kimura 1983).

Genome sequencing

The genomic DNA was extracted using a genomic DNA extraction kit according to the manufacturer's instruction (Solgent). The sequencing libraries were prepared using the Nextera DNA Flex Library Prep Kit (Illumina), and whole-genome sequencing was performed by iSeq 100. The genome sequences were assembled using SPAdes 3.10.1 (Algorithmic Biology Lab, St. Petersburg Academic University of the Russian Academy of Sciences). The whole-genome sequences of strains BT291^T and BT350^T were deposited in GenBank (www.ncbi.nlm.nih.gov/) database. The genome sequences of strains BT291^T and BT350^T were annotated by the National Center for Biotechnology Information Prokaryotic Genome Annotation Pipeline (PGAP) (Tatusova et al. 2016). The average nucleotide identity (ANI) was calculated using the EzBioCloud (https://www.ezbiocloud.net) and the digital DNA–DNA hybridization (dDDH) was calculated using the Genome-to-Genome Distance Calculator (GGDC) with the recommended formula 2 (Table S1) (Meier-Kolthoff et al. 2013).

Chemotaxonomic characteristics

For analysis of cellular fatty acid, polar lipid and quinone strains BT291^T and BT350^T were grown on R2A agar at 25 °C for 3 days and cells were freeze-dried. Polar lipids of strains BT291^T and BT350^T were extracted as described previously. The total lipids, glycolipids, phosphatidylcholine and amino groups were separated using two-dimensional thin-layer chromatography (TLC). The polar lipid spots were detected by spraying the proper detection reagents (Komagata and Suzuki 1987; Minnikin et al. 1984). The fatty acids were purified by saponification, methylation and extraction procedures (Sasser 1990). The quinones were extracted using the Sep-Pak Vac cartridges (Waters) and analyzed by high-performance lipid chromatography (HPLC) based on the previous methods (Hiraishi et al. 1996). The fatty acid methyl esters (FAME) were identified using the Sherlock Microbial Identification System V6.01 (MIS, database TSBA6, MIDI Inc).

Results and discussion

Morphology, physiology and biochemical analysis

Strains BT291^T and BT350^T were Gram-staining-negative bacteria and they showed rod-shaped morphology (Fig. S1). Differential features between the new strains and reference

Table 1 Different characteristics of *Microvirga pudoricolor* and *Microvirga alba* and closely related species of genus *Microvirga*

Characteristic	1	2	3	4	5
Size (µm long)	1.6–2.7	0.5–1.2	1.0–3.3	4.0	1.2–1.5
Size (µm wide)	0.6–1.3	0.4–0.9	1.2–1.5	1.0–1.5	0.7–0.9
Colony color	Light pink	White	Light pink	Light pink	white
Nitrate reduction to NO_2	−	−	−	+	+
Nitrate reduction to N_2	+	−	−	+	+
Production of acid from glucose	−	−	−	w	+
Enzyme activity					
Acid phosphatase	+	−	−	+	+
Alkaline phosphatase	−	+	−	w	+
Arginine dihydrolase	+	−	−	−	−
Esterase (C4)	+	+	−	+	+
Esterase (C8)	−	−	−	+	+
α-galactosidase	−	−	+	−	−
β-galactosidase (ONPG)	−	−	+	−	−
β-glucosidase (Esculin hydrolysis)	−	−	−	+	+
Leucine arylamidase	−	−	−	+	+
Lipase (C14)	−	−	−	+	−
Naphtol-AS-BI-phosphohydrolase	−	−	+	+	+
Protease (gelatin hydrolysis)	−	−	−	+	−
Urease	+	−	−	−	−
Assimilation					
Citrate	−	w	+	+	+
L-arabinose	−	−	+	−	−
D-glucose	−	−	−	+	−
D-maltose	−	−	−	+	+
D-mannose	−	−	+	+	−
D-mannitol	−	−	+	−	+
N-acetyl-D-glucosamine	−	−	−	−	+
G + C content	64.7%	61.9%	62.2%	63.5%	62.3%

Taxa: 1, strain BT291^T (data was obtained in this study); 2, strain BT350^T (data was obtained in this study); 3, *M. aerophila* 5420S-12^T (data was taken Tapase et al. 2017); 4, *M. subterranea* FaiI4^T (data was taken Tapase et al. 2017); 4, *M. brassicacearum* CDVBN77^T (data was taken Jimenez-Gomez et al. 2019). All strains were negative for production of indole, *N*-acetyl-*β*-glucosaminidase, *α*-fucosidase, *β*-galactosidase (PNPG), *α*-glucosidase (starch hydrolysis), *β*-glucosidase, *β*-glucuronidase, *α*-mannosidase and valine arylamidase

+ positive, − negative; *w* weak positive

strains were provided in Table 1. In addition, the negative reaction of strains BT291^T and BT350^T on API kits were given as supplementary tables (Table S3).

Phylogenetic and genome sequence analysis and analysis

Based on the 16S rRNA gene sequence similarities, strains BT291^T and BT350^T were affiliated with the family *Methylobacteriaceae* and showed high sequence similarities with the genus *Microvirga*. Strain BT291^T was closely related to *Microvirga aerophila* 5420S-12^T (97.5% 16S rRNA gene similarity) and *Microvirga subterranean* DSM 14364^T (97.2%). Strain BT350^T was closely related to *Microvirga aerophila* 5420S-12^T (97.6%) and *Microvirga brassicacearum* CDVBN77^T (96.8%). These values were around or below the 98.7% 16S rRNA gene sequence similarity recently used as the threshold for differentiating among bacterial species (Chun et al. 2018). The remaining *Microvirga* species exhibited sequence similarities lower than 97.0%. The phylogenetic analysis results clearly showed that strains BT291^T and BT350^T are two new species within the genus *Microvirga* (Figs. 1, S2, and S3).

The draft genome of strain BT291^T was 4.77 Mb (51.2×) long and consisted of 4,473 protein-coding genes, 57 RNA genes (6 rRNA genes, 50 tRNA genes) and 8 pseudogenes. The draft genome of strain BT350^T was 4.42 Mb (29.9×) long and consisted of 4,014 protein-coding genes, 51 RNA

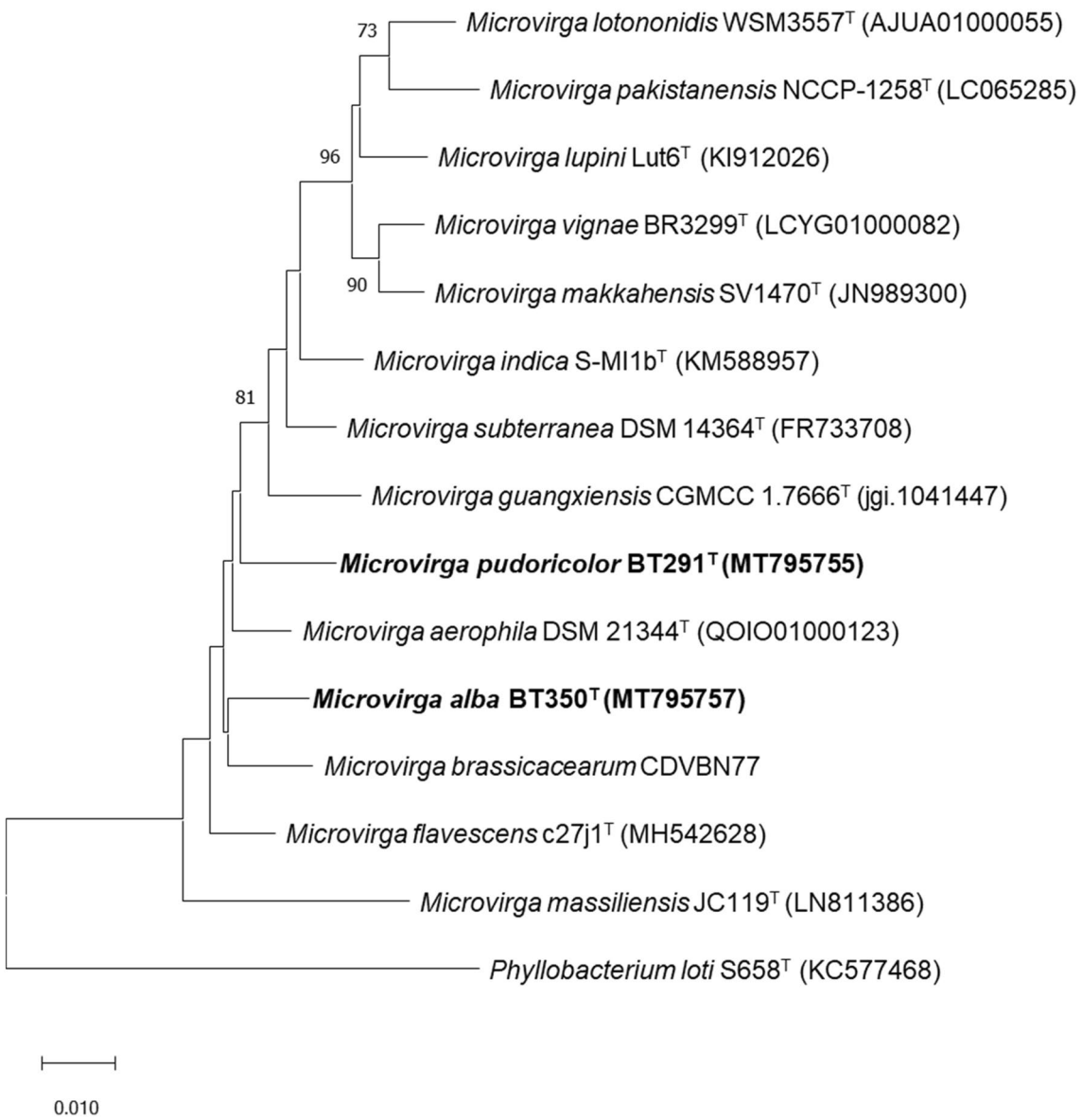

Fig. 1 Neighbor-joining phylogenetic tree reconstructed from a comparative analysis of 16S rRNA gene sequences showing the relationships of strains BT291^T and BT350^T with closely related validly published species. Bootstrap values (based on 1000 replications) greater than 70% based on neighbor-joining method is shown at the branch nodes. *Phyllobacterium loti* S658^T was used as an outgroup. Bar, 0.01 substitutions per nucleotide position

genes (4 rRNA genes, 47 tRNA genes) and 66 pseudogenes. Genome properties of the strains BT291^T and BT350^T based on RAST annotations are detailed in Table S2. The DNA G + C contents of strains BT291^T and BT350^T were 64.7 mol% and 61.9 mol%, respectively. These values were within the range of the G + C contents for the genus *Microvirga* as previously reported (63.5–64.3 mol%). The digital DNA–DNA hybridization values between strains BT291^T and BT350^T and other related type strains of genus *Microvirga* were less than 23.1%, respectively (Table S1), which are below the cutoff (70%) point (Meier-Kolthoff et al. 2013). Average nucleotide identity (ANI) values between strains BT291^T and BT350^T and other related type strains of genus *Microvirga* were less than 79.1%, respectively (Table S1). These values are below the ANI species threshold (95–96% ANI value) as described by Ritchter and Rossello-Mora (2009).

Chemotaxonomic characterization

The fatty acid profiles of strains BT291^T and BT350^T and three reference strains of genus *Microvirga* were presented

in Table 2. The major fatty acids of strain BT291^T $C_{18:1}$ $\omega 7c$ (58.2%) and cyclo-$C_{19:0}$ $\omega 8c$ (25.7%).

The major fatty acid profiles of strain BT350^T were $C_{18:1}$ $\omega 7c$ (38.5%) and cyclo-$C_{19:0}$ $\omega 8c$ (27.7%).

The polar lipids of strain BT291^T consisted of a phosphatidylethanolamine (PE), an unknown diphosphatydilglycerol (DPG), unknown phosphatidylcholine (PC), an unknown phosphatidylglycerol (PG), an unknown aminolipid (AL), an unknown aminophospholipid (APL), an unknown phospholipid (PL), an unknown glycolipid (GL) and two unknown lipids (L) (Fig. S4). Besides, strain BT350^T consisted of a phosphatidylethanolamine (PE), an unknown diphosphatydilglycerol (DPG), an unknown phosphatidylglycerol (PG), unknown phosphatidylcholine (PC), an unknown amino lipid (AL) and an unknown lipid (L) (Fig. S5). The dominant respiratory quinone of strains BT291^T and BT350^T was Q-10. These results supported that chemotaxonomic characteristic of strains BT291^T and BT350^T are similar to those of the other species in the genus *Microvirga*.

Table 2 Cellular fatty acid profiles of *Microvirga pudoricolor* sp. nov., and *Microvirga alba* sp. nov., and closely related species of genus *Microvirga*

Fatty acids	1	2	3	4	5
Saturated					
14:0	tr	tr	1.1	–	–
16:0	5.8	10.4	7.6	5.5	9.2
17:0	–	2.7	–	6.7	–
17:0 cyclo	1.4	6.4	1.1	–	3.8
18:0	1.5	2.2	1.0	3.8	6.1
19:0 10-methyl	1.1	–	–	–	–
19:0 cyclo $\omega 8c$	25.7	27.7	3.6	2.4	24.3
Unsaturated					
17:1 $\omega 8c$	–	–	–	2.0	–
17:1 $\omega 6c$	–	–	–	1.4	–
18:1 $\omega 7c$ 11-methyl	–	–	–	–	4.2
18:1 $\omega 7c$	58.2	38.5	69.6	73.4	39.3
18:0 3OH	1.2	1.8	tr	–	1.7
Summed feature 2 (16:1 iso I / 14:0 3OH)	2.2	3.5	4.3	3.3	4.5
Summed feature 3 (16:1 $\omega 6c$ / 16:1 $\omega 7c$)	2.1	6.1	11.0	1.5	4.5

Taxa: 1, strain BT291^T (data was obtained in this study); 2, strain BT350^T (data was obtained in this study); 3, *M. aerophila* 5420S-12^T (data was taken Weon et al. 2010); 4, *M. subterranean* DSM 14364^T (data was taken Weon et al. 2010); 4, *M. subterranea* FaiI4^T (data was taken Jimenez-Gomez et al. 2019). All strains were grown on R2A agar at 25–28 °C. For unsaturated fatty acids, the location of the double bond was presented by counting the number from the methyl (ω) end of the carbon chain

– not detected, *tr* trace amount (< 1%)

Based on phenotypic, phylogenetic, and biochemical features, it is concluded that strains BT291^T and BT350^T represent two novel species of the genus *Microvirga*, for which the name *Microvirga pudoricolor* and *Microvirga alba* are proposed.

Description of *Microvirga pudoricolor* sp. nov.

Microvirga pudoricolor **(pu.do.ri'co.lor. N.L. fem. adj. *pudoricolor* light-pink colored).**

Cells are Gram-stain-negative, aerobic, rod-shaped, 0.6–1.3 µm in diameter and about 1.6–2.7 µm in length, non-spore forming and non-motile. Colonies are irregular, convex and light-pink-colored on Reasoner's 2A (R2A) agar plates after growth for 3 days at 25 °C. Growth is observed at temperatures ranging from 10 to 30 °C (optimum 25 °C). The pH range for growth is 6.0–9.0 (optimum pH 8.0) on R2A agar. Normal cell growth occurs at 10–30 °C (optimum 25 °C) and pH 6.0–9.0 (optimum 8.0). Cells grow on Reasoner's 2A agar (R2A), Luria–Bertani agar (LB), Tryptic Soy Agar (TSA), Nutrient Agar (NA) and Macconkey (MAC) agar (weakly). Cells are positive for oxidase and catalase activity. The major respiratory quinone is Q-10. The dominant cellular fatty acids are $C_{18:1}$ $\omega 7c$ (58.2%) and cyclo-$C_{19:0}$ $\omega 8c$ (25.7%). The major polar lipids are phosphatidylethanolamine (PE), diphosphatidylglycerol (DPG), phosphatidylcholine (PC), phosphatidylglycerol (PG). The genome-based G + C content is 64.7 mol%. Positive for nitrate reduction, arginine dihydrolase and urease (API 20NE). Positive for esterase (C4) and acid phosphatase (API ZYM).

The type strain BT291^T (= KCTC 72368^T = NBRC 114845^T) was isolated from a soil sample collected in Uijeongbu city (37° 44′ 55″ N, 127° 2′ 20″ E), South Korea.

The whole-genome sequence of strain BT291^T has been deposited in GenBank under the accession number NZ_JAFEMB000000000 (4.77 Mb). The GenBank accession number for the 16S rRNA gene sequence of strain BT291^T is MT795755 (1,422 bp).

Description of *Microvirga alba* sp. nov.

Microvirga alba **(al'ba. L. fem. adj. *alba* white).**

Cells are Gram-stain-negative, aerobic, rod-shaped, 0.4–0.9 µm in diameter and about 0.5–1.2 µm in length, non-spore forming and non-motile. Colonies are irregular, convex and white colored on Reasoner's 2A (R2A) agar plates after growth for 3 days at 25 °C. Growth is observed at temperatures ranging from 10 to 30 °C (optimum 25 °C). The pH range for growth is 5.0–9.0 (optimum pH 8.0) on R2A agar. Cells grow on Reasoner's 2A agar (R2A),

Tryptic Soy Agar (TSA) and Nutrient Agar (NA) but not on Luria–Bertani agar (LB) and Macconkey (MAC) agar. Cells are positive for oxidase and catalase activity. The major respiratory quinone is Q-10. The dominant cellular fatty acids are $C_{18:1}$ $\omega 7c$ (38.5%) and cyclo-$C_{19:0}$ $\omega 8c$ (27.7%). The major polar lipids are phosphatidylethanolamine (PE), diphosphatydilglycerol (DPG), phosphatidylcholine (PC) and Phosphatidylglycerol (PG). The genome-based G + C content is 61.9 mol%. Weakly positive for trisodium citrate (API 20NE). Positive for alkaline phosphatase and esterase (C4) (API ZYM).

The type strain BT350^T (= KCTC 72385^T = NBRC 114848^T) was isolated from a soil sample collected in Jeju island (33° 22′ 48″ N, 126° 31′ 48″ E), South Korea.

The whole-genome sequence of strain BT350^T has been deposited in GenBank under the accession number NZ_JADQDO010000000 (4.42 Mb). The GenBank accession number for the 16S rRNA gene sequence of strain BT350^T is MT795757 (1416 bp).

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00203-021-02569-z.

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Declarations

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

Ethical approval This article does not contain any studies with human participants or animals.

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