Microvirga Jeongseonensis Sp. Nov., New Species Isolated From Soil in South Korea

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Abstract

A novel Gram-stain-negative, aerobic, rod-shaped, convex, and light pink-colored strain BT688^T was isolated from a soil sample collected in Jeongseon city, South Korea. Phylogenetic analysis based on 16S rRNA gene sequence revealed that strain BT688^T belongs to a distinct lineage within the genus *Microvirga* (family *Methylobacteriaceae*, order *Rhizobiales*, class *Alpha Proteobacteria*, phylum *Proteobacteria*). The 16S rRNA gene sequence similarity between strain BT688^T and *Microvirga aerilata* 5420S-16^T was 98.5%. Strain BT688^T had Q-10 as a major respiratory quinone and the major polar lipids of strain BT688^T was diphosphatidilglycerol (DPG), phosphatidylglycerol (PG), phosphatidylethanolamine (PE), and phosphatidylcholine (PC). The major cellular fatty acids of strain BT688^T were $C_{18:1}$ *ω7c* (76.0%) and summed feature 3 (9.6%).

Based on the polyphasic characteristics, strain BT688^T can be suggested as a novel bacterial species within the genus *Microvirga* and the proposed name is *Microvirga jeongseonensis*. The type strain of *Microvirga jeongseonensis* is BT688^T (= KCTC XXXX^T=NBRC 114857 ^{T}).

Introduction

The genus *Microvirga* was first described by Kanso and Patel (2003) belonged to the family *Methylobacteriaceae* order *Rhizobiales*. At the time of writing (April 2021), the genus comprises 18 validly published species (http://www.bacterio.net/Microvirga.html). *Microvirga* species have been retrieved from various polar environments including regoliths, 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. 2020), respectively. In genus *Microvirga*are, cells are Gram strain-negative, the average genome size is 3.53–9.63 Mb, and DNA G + C contents were 61.1–65.1% (Zhang et al. 2019). They contain $C_{18:1}$ *ω7c* and cyclo-$C_{19:0}$ *ω8c* as major fatty acids.

In this study, strain BT688^T was newly isolated from a soil sample collected in Jeongseon city, South Korea, and characterized. Phylogenetic analysis, phenotypic, genotypic, and chemotaxonomic characterization were performed to determine the taxonomic position of strain BT688^T. The results suggest that strain BT688^T represents a novel species of the genus *Microvirga*, for which the name *Microvirga jeongseonensis* sp. nov. is proposed.

Materials And Methods

Bacteria isolation and culture conditions

Strain BT688^T was isolated from Jeongseon city (37° 22' 38.1"N, 128° 47' 17.0" E) located in South Korea. A single colony was isolated using Reasoner's 2A (R2A) agar medium (Difco) after incubation at

25°C for 10 days. Then the strain was routinely cultured on R2A agar at 25°C, maintained at 4°C, and stored in 20% (w/v) glycerol suspension at − 80°C, respectively.

Morphology, physiology, and biochemical analysis

The cell morphology of strain BT688^T was examined using transmission electron microscopy (JEOL, JEM1010, Japan) by negative staining method. The Gram staining was performed using a kit follow the manufacturer's instruction (bioMérieux, France). Catalase activity was examined with 3% (w/v) H_2O_2 solution and oxidase activity was examined by adding 1% (w/v) tetramethyl-p-phenylenediamine (Cappuccino and Sherman 2002). The growth of strain BT688^T was examined on R2A agar, Luria-Bertani (LB) agar, Tryptic Soy Agar (TSA), Nutrient Agar (NA), and on MacConkey agar, respectively. In addition, growth was determined at different temperatures (4, 10, 15, 25 and 30°C), under various pH conditions (5 to 9, 1 pH intervals), and with different NaCl concentrations (1–5% [w/v %], 1% intervals), respectively. API 20NE and API ZYM tests were performed according to the manufacturer's instruction (bioMérieux, France).

Phylogenetic analysis

The 16S rRNA gene of strain BT688^T was amplified using two universal bacterial primers 27F and 1492R (Weisburg et al. 1991). The 16S rRNA gene sequence was identified using the EzBioCloud server (https://www.ezbiocloud.net/). The sequence was analyzed using the 337F, 518R, 785F and 926R universal primers (Macrogen, Korea). To determine the taxonomic position of strain BT688^T, 16S rRNA sequences of closely related species were obtained from EzBioCloud (Yoon et al. 2017) and compared with that of strain BT688^T using EzEditor2 program. 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 the 1,000 resampling method (Felsenstein 1985). Evolutionary distances were calculated according to the Kimura two-parameter model (Kimura 1983).

Whole-genome sequence analysis

The genomic DNA of strain BT688^T was extracted using a genomic DNA extraction kit according to the manufacturer's instruction (Solgent, Korea). The sequencing libraries were prepared using the Nextera DNA Flex Library Prep Kit (Illumina) and whole-genome sequencing was performed using iSeq 100. The partial genome sequences were assembled by using the SPAdes algorism (ver. 3.10.1, Algorithmic Biology Lab, St. Petersburg Academic University of the Russian Academy of Sciences). The whole-genome sequence of strain BT688^T was deposited in GenBank (www.ncbi.nlm.nih.gov/) database. The genome sequence of strain BT688^T was 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 EzBioCloud (https://www.ezbiocloud.net) and the digital DNA-DNA

hybridization (dDDH) value was calculated using the Genome-to Genome Distance Calculator (GGDC) with the recommended formula 2 (Meier-Kolthoff et al. 2013).

Chemotaxonomic characteristics

To analyze the composition of cellular fatty acid, polar lipid, and quinone strain BT688^T was grown on R2A agar at 25°C for 3 days and collected cells were freeze-dried. Polar lipids of strain BT688^T were extracted as described by Minnikin et al. (1984). The total lipids, glycolipids, phosphatidylcholine, and amino lipid groups were separated using two-dimensional thin-layer chromatography (TLC) and detected by using proper detection reagents (Komagata and Suzuki 1987). Fatty acids were purified by saponification, methylation, and extraction procedures (Sasser 1990). Lipoquinones were extracted using the Sep-Pak Vac cartridges (Waters) and analyzed by high-performance lipid chromatography (HPLC) based on 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

Strain BT688^T was Gram-negative and had rod-shaped morphology (Fig. S1). Colonies of strain BT688^T were circular, convex, smooth, and light pink color after incubation for three days at 25°C. Cells of strain BT688^T could grow at 10 to 30°C (optimum 25°C) and pH 6.0–9.0 (optimum 7.0) on R2A agar. Different features between the newly isolated strain and reference strains were provided in Table 1. The negative reactions of strain BT688^T on API kits were shown in the supplementary table (Table S1).

Phylogenetic and genome sequence analysis

The length of the 16S rRNA gene of strain BT688^T was 1,428 bp. Based on the 16S rRNA gene sequence similarities, strain BT688^T was affiliated with the family *Methylobacteriaceae* and showed high sequence similarities with the genus *Microvirga*. The strain BT688^T was similar to *Microvirga aerilata* 5420S-16^T (98.5% of 16S rRNA gene similarity) and *Microvirga makkahensis* SV1470^T (98.2% of 16S rRNA gene similarity). These values were around or below the 98.7 % 16S rRNA gene sequence similarity recently used as the threshold for bacterial species classification (Chun et al. 2018). Analysis of neighbor-joining (Fig. 1), maximum-likelihood (Fig. S2), and maximum-parsimony (Fig. S3) trees clearly showed that strain BT688^T belongs to the genus *Microvirga*.

The draft genome length of strain BT688^T was 6.62 Mb (29.6×) and consisted of 6,356 protein-coding genes and 55 RNA genes including 2 rRNA genes and 53 tRNA genes. The genome sequence of strain BT688^T was deposited in GenBank under the accession number NZ_JACXAB000000000. The DNA G + C contents of strain BT688^T was 62.2 mol%. which value was within the range of the G + C contents of the genus *Microvirga* (63.5–64.3 mol%). The average nucleotide identity (ANI) value between strain BT688^T

and the most closely related type strain 5420S-16^T (genus *Microvirga*, *Microvirga aerilata*) was 83.3%. This value is below the ANI species threshold (95–96 % ANI value) as described by Ritcher and Rossello-Mora (2009).

The digital DNA-DNA hybridization value between strain BT688^T and *Microvirga aerilata* 5420S-16^T was 16%, which is below the cutoff (70 %) point (Meier- Kolthoff et al. 2013).

Chemotaxonomic characterization

The fatty acid profiles of strain BT688^T and two reference strains of genus *Microvirga* were presented in Table 2. The major fatty acid of strain BT688^T was $C_{18:1}\omega7c$ (76.0 %). Strain BT688^T had small amounts of $C_{19:0}$ cyclo $\omega8c$ (ND) and $C_{16:0}$ (5.8 %), whereas other closely related *Microvirga* species (*M. aerilata* 5420S-16^T and *M. makkahensis* SV1470^T) contained larger amounts of corresponding fatty acids ($C_{19:0}$ cyclo $\omega8c$: 1.6% and 18.8%, respectively; $C_{16:0}$: 6.3% and 9.0%, respectively). Strain BT688^T contained $C_{14:0}$ 3OH/$C_{16:1}$ iso I (2.8 %) and $C_{16:1}\omega7c$/$C_{16:1}\omega6c$ (9.6 %), while other closely related *Microvirga* species (*M. aerilata* 5420S-16^T and *M. makkahensis* SV1470^T) did not contain those type of fatty acids.

The polar lipids of strain BT688^T consisted of one diphosphatidilglycerol (DPG), one phosphatidylglycerol (PG), one phosphatidylethanolamine (PE), one phosphatidylcholine (PC), one amino lipid (AL), one aminophospholipid (APL), one phospholipid (PL) and two unknown lipids (L) (Fig. S4). The major respiratory quinone of strain BT688^T was Q-10. The results of the chemotaxonomic analysis indicated that strain BT688^T is similar to those of the other species in the genus *Microvirga*. Based on phenotypic, phylogenetic, and biochemical characteristics, it can be concluded that strain BT688^T represents a novel species of the genus *Microvirga*, for which the name *Microvirga jeongseonensis* is proposed.

Description ofMicrovirga jeongseonensissp. nov.

Microvirga jeongseonensis (jeong.seon.en'sis. N.L. adj. *jeongseonensis* from Jeongseon in Korea, where the type strain was isolated).

Cells are Gram-negative, aerobic, rod-shaped, 0.5–1.2 µm in diameter and 1.2–2.5 µm in length. Colonies are circular, convex, and light pink colored on Reasoner's 2A (R2A) agar plates after growth for three 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 7.0) on R2A agar. 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 $C_{18:1}\omega7c$ (76.0 %). The major polar lipids are diphosphatidylglycerol (DPG), phosphatidylglycerol (PG), phosphatidylethanolamine (PE), and phosphatidylcholine (PC). Positive for nitrate reduction to N_2 (API 20NE). Positive for esterase (C4) and acid phosphatase (API ZYM). The whole-genome sequence of strain BT688^T has been deposited in GenBank under the accession number NZ_JACXAB000000000 (6.62 Mb). The genome-based G + C content is 62.2 mol%. The GenBank

accession number for the 16S rRNA gene sequence of strain BT688^T is MT795750 (1,419 bp). The type strain BT688^T (= KCTC XXXX^T=NBRC 114857^T) was isolated from a soil sample collected in Jeongseon city (37° 22' 38.1"N 128 °47' 17.0 "E), South Korea.

Declarations

The 16S rRNA gene sequence of strain BT688^T was deposited in GenBank/EMBL/DDBJ under the accession number MT795750. The draft genome sequence of BT688^T is available with the following accession number NZ_JACXAB000000000.

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Conflicts 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.

Author Contributions:

All authors contributed equally to this manuscript.

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Tables

Table 1. Different characteristics of *Microvirga jeongseonensis* and closely related species of genus *Microvirga*.

Taxa: 1, strain BT688^T (data was obtained in this study); 2, *M. aerilata* 5420S-16^T (data was taken Weon et al. 2010); 3, *M. makkahensis* SV1470^T (data was taken Veyisoglu et al. 2015

+, positive; -, negative; w, weak positive

	1	2	3
Urease	-	-	+
D-Glucose	-	-	+
L-Arabinose	-	-	+
D-Ribose	w	w	-
Acid phosphatase	+	+	-
Alkaline phosphatase	-	+	+
Lactic acid	+	-	-
3-Hydroxybenzoic acid	-	+	-
D-glucose	-	-	+
D-sorbitol	-	-	+
4-hydroxybenzoic acid	-	+	-
Esterase (C4)	-	+	-
Lipase (C14)	-	-	+
Trypsin	-	+	-
Naphtol-AS-BI-phosphohydrolase	-	+	+

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

Taxa: 1, strain BT688^T (this study); 2, *M. aerilata* 5420S-16^T (Weon et al. 2010); 3, *M. makkahensis* SV1470^T (Veyisoglu et al. 2015). All strains were grown on R2A agar at 25-28°C. ND, not detected; TR, trace amount (< 1%).

	1	2	3
Saturated			
$C_{14:0}$	TR	TR	1.9
$C_{16:0}$	5.8	6.3	9.0
$C_{18:0}$	2.2	8.6	4.5
Unsaturated			
$C_{16:1}$ sic 9	ND	2.5	4.8
$C_{18:1}$ *ω7c*	76.0	74.1	52.0
Branched-chain fatty acid			
$C_{18:1}$ *ω7c* 11-methyl	1.1	ND	ND
Hydroxy fatty acids			
$C_{18:0}$ 3OH	TR	1.2	2.7
Cyclo			
$C_{17:0}$ cyclo	ND	ND	2.8
$C_{19:0}$ cyclo *ω8c*	ND	1.6	18.8
Summed feature			
2; $C_{14:0}$ 3OH/$C_{16:1}$ iso I	2.8	ND	ND
3; $C_{16:1}$ *ω7c*/$C_{16:1}$ *ω6c*	9.6	ND	ND
4; $C_{17:1}$ iso I/$C_{17:1}$ anteiso B	ND	ND	1.8

Figures

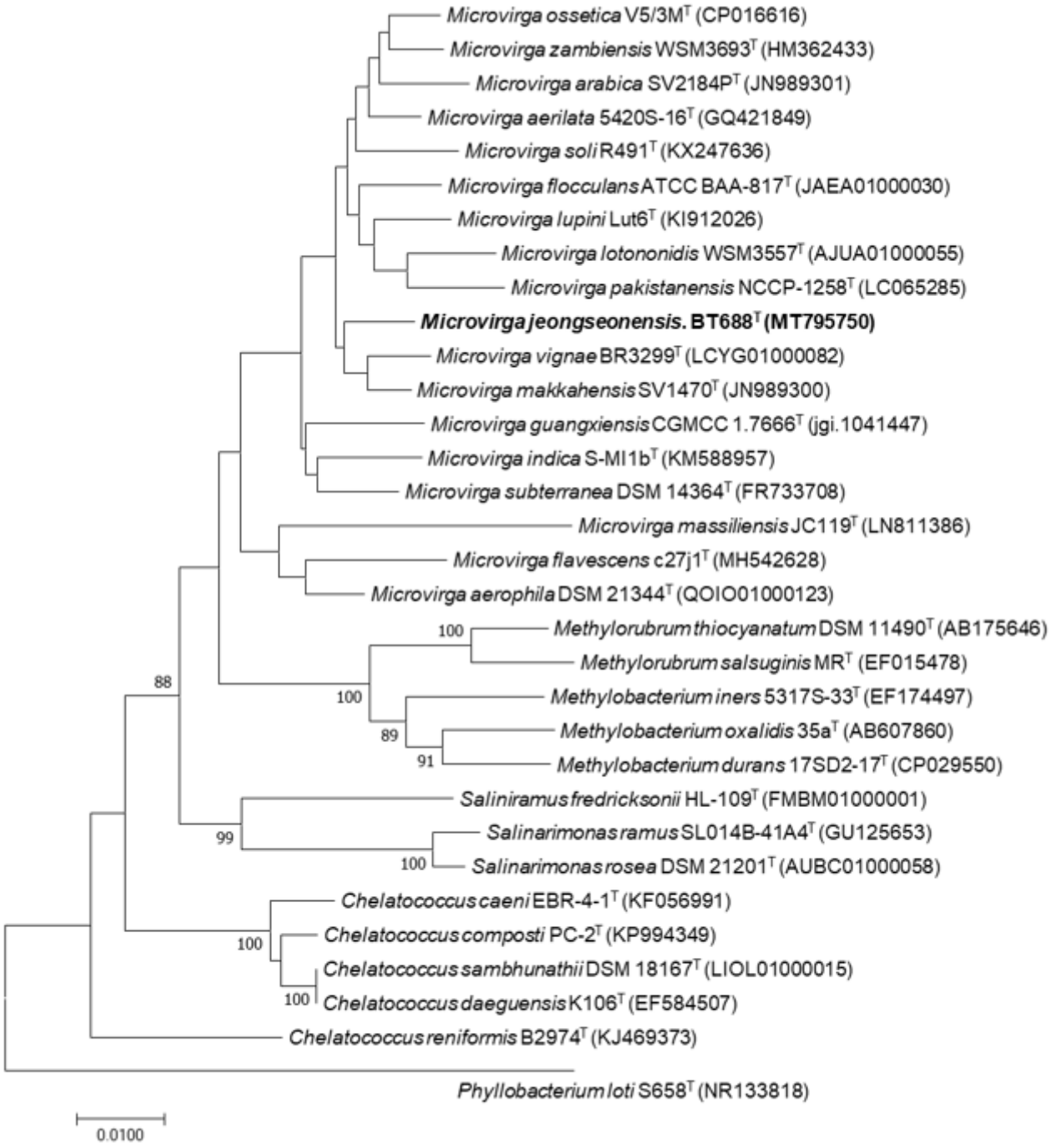

Figure 1

Neighbor-joining phylogenetic tree constructed from a comparative analysis of 16S rRNA gene sequences showing the relationships of strain BT688T with closely related validly published species. Bootstrap values (based on 1,000 replications) greater than 70% are indicated at the branch nodes. Phyllobacterium loti S658T was used as an outgroup. Bar, 0.01 substitutions per nucleotide position.

Supplementary Files

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