Microvirga Puerhi Sp. Nov., Isolated from Puerh Tea Garden Soil

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Abstract

Strain WGZ8^T was isolated from a soil sample of Puerh tea garden in Puer city, Southwest China. The isolate was rod-shaped, Gram-stain negative, aerobic, non-motile. Growth occurred within 0-3.0% (w/v) NaCl (optimal concentration, 0-1.0%), pH 5.0-11.0 (optimal pH, 7.0) and 10-40°C (optimal temperature, 28°C). 16S rRNA gene sequences based phylogenetic and phylogenomic analysis revealed that WGZ8^T belonged to the genus *Microvirga*. Its major cellular fatty acids were C_{19:0} cyclo ω 8c, C_{16:0}, C_{18:1} ω 7c and/or C_{18:1} ω 6c. The profile of polar lipids included phosphatidylmethylethanolamine, phosphatidylmonomethylethanolamine, phosphatidylethanolamine, phosphatidylcholine, diphosphatidylglycerol and phosphatidyl-glycerol. The only respiratory quinone was detected as ubiquinone 10 (Q-10). The genome size of strain WGZ8^T was 5.17MB, and the content of DNA G+C was 61%. Based on the results of digital DNA-DNA hybridization and phenotypic results, strain WGZ8^T could be concluded as a novel species of the genus *Microvirga*, for which the name *Microvirga puerhi* sp. nov. is proposed. The type strain is WGZ8^T (=CGMCC 1.19171^T=JCM XXXX^T).

Introduction

The genus *Microvirga*, which was established by Kanso (Kanso et al. 2003), belongs to the family *Methylobacteriaceae*. According the LPSN database (<https://lpsn.dsmz.de/genus/microvirga>, Parte et al. 2020), 18 species with valid names were included in the genus *Microvirga*. Members of the genus *Microvirga* are aerobic, rod-shaped, oxidase negative and gram-negative. The predominant respiratory quinone is ubiquinone-10 (Q-10) and the G+C content of DNA is approximately 61.1 to 65.1 mol % (Table S1). Members of genus *Microvirga* can adapt to a variety of ecological environments: air (Weon et al. 2010), geothermal waters (Kanso et al. 2003), hot spring sediment (Liu et al. 2021), forest soil (Zhang et al. 2019), and metal industry waste (Tapase et al. 2017). In this study, we propose a novel species of genus *Microvirga*, designated as WGZ8^T which was isolated from Puerh tea garden soil, using polyphasic taxonomy and genomic approaches.

Materials And Methods

Isolation and culturing conditions

Strain WGZ8^T was isolated from a soil sample from a Puerh tea garden, located in Pu'er City, Yunnan Province, China (22° 42' 35" N, 100° 56' 10" E; altitude 1130 m). Isolation and cultivation of microbials based on previously reported methods (Han et al., 2014). Strain WGZ8^T was isolated on the oligotrophic Reasoner's 2A agar (R2A) at 30 °C for 3 days and then stored in 15 % (v/v) glycerol suspension at -80 °C for extended preservation.

Reference strains *Microvirga subterranea* DSM 14364^T and '*Microvirga rosea*' MAH-2 were obtained from the Guangdong Microbiological Culture Collection Center (GDMCC).

Phylogenetic analysis

16S rRNA gene of isolate WGZ8^T was amplified by PCR and sequenced as our previous report (Ma et al. 2021). EzBioCloud (Kim et al. 2011) (<https://www.ezbiocloud.net/>) was used to analyze the 16S rRNA gene sequence similarities with related strains. Maximum-parsimony (Walter et al. 1971), Neighbor-joining (Saitou et al. 1987) and maximum-likelihood (Kishino et al. 1989) methods in MEGA 7 (Kumar et al. 2016) were used to construct phylogenetic trees. *Enterovirga rhinocerotis* YIM 100770^T (KC992737) was used as an outgroup in the phylogenetic analysis. Other analysis methods were detailed in the footnotes of Fig. 1, Fig. S1 and S2.

Genomic analysis

Illumina Novaseq pe150 platform (Beijing Novogene Bioinformatics Technology Co., Ltd. Beijing China) was used to sequence the genome of strain WGZ8^T. The sequences were obtained and re-assembled as reported previously (Ma et al., 2021). As previously described (Galperin et al. 2015), the coding genes' functions were searched online using the COG server database (<http://weizhong-lab.ucsd.edu/webMGA/server/cog/>). The NCBI Prokaryotic Genome Annotation Pipeline v5.0 was used to annotate, and OrthoFinder v2.2.6 was used to cluster the annotated genomes as described by Nguyen et al. (2015), and 1018 single copy orthologs were identified. Mafft software (Kato et al. 2008) was used to align orthologs selected, then TrimAl was used to trim all the alignments. The best model automatically is GTR+F+R5. Maximum-likelihood tree was reconstructed using iQ-tree v1.6.12 as described by Castresana et al. (2000). ANI (average nucleotide identity, Konstantinidis et al. 2005) and dDDH (DNA-DNA hybridization, Auch et al. 2010) values between the strain WGZ8^T and members of genus *Microvirga* was calculated using the web service www.ezbiocloud.net/tools/ani and <http://ggdc.dsmz.de/distcalc2.php>, respectively.

Physiological properties analysis

Colonies and cells of WGZ8^T were observed by eyes and the scanning electron microscopy (SU1510; Hitachi, Tokyo, Japan) after growing for 3 days at 28 °C on R2A agar. Nutrient agar (NA), Luria-Bertani agar (LB) and tryptone soya agar (TSA) were also used to test cell growth. Tests of anaerobic growth, Gram reaction, motility, oxidase, catalase, growth temperature, pH and NaCl tolerance were carried out using the methods reported previously (Ma et al., 2021). API ZYM, API 50CH and API 20NE kits were used to detect assimilation of acid production and enzyme activities, according to the manufacturers' instructions (BioMérieux SA, Marcy-l'Étoile, France). To investigate the hydrolysis of Tween 40 (w/v, 1 %), Tween 80 (1 %), Tween 20 (1 %), CM-cellulose (0.5 %), casein (5 % skimmed milk), and starch (0.2 %), R2A agar used as the basal medium, based on conventional methods (Smibert et al. 1994).

Chemotaxonomic analysis

Cells of reference strains and WGZ8^T were cultured and collected at 28 °C for 3 days on R2A. The collected cells were extracted, methylated and saponified as described before (Ma et al., 2021). 7890A GC

system (Agilent, Santa Clara, USA) was used to analyze the fatty acid methyl-esters extracted from the above steps and the Sherlock Aerobic Bacterial Database (TSBA 6) was used to confirm compounds' identities. Ninhydrin, α -naphthol-sulfuric acid, molybdophosphoric acid and molybdenum blue were sprayed on TLC plates to identify polar lipids (Costa et al. 2011b; Minnikin et al. 1984). HPLC was used to extract and analyzed respiratory quinones (Costa et al. 2011a).

Results And Discussion

Phylogenetic and genomic analysis

16S rRNA gene of strain WGZ8^T was sequenced (1451 bp). '*M. rosea*' MAH-2 shared the highest similarity (98.0 %) with WGZ8^T, followed by *M. subterranea* DSM 14364^T (97.6 %). The 16S rRNA gene sequence similarity values were all lower than 98.65% of the recommended species cutoff (Kim et al. 2011). Fig. 1 (maximum-likelihood), Fig. S1 (neighbor-joining) and Fig. S2 (maximum-parsimony) revealed that strain WGZ8^T should be a member of the genus *Microvirga*.

The genome size and DNA G+C content of strain WGZ8^T were 5.17 MB and 61.0 %, respectively (Table S1). One 16S rRNA with 100% similarity to PCR results was detected from the genome. According to the analysis sequencing and GC depth, there was no contaminated sequence in the sample (Fig. S3). Table S2 showed the detail statistics of the genome analysis. The COG functional categories were listed in Table S3. The relationship between WGZ8^T and related species was showed in phylogenomic tree (Fig. 2). Table S1 showed the ANI and dDDH values between strain WGZ8^T and members of the genus *Microvirga*, ANI and dDDH values were all lower than the species delineation thresholds of 95-96 % and 70 %, respectively. All these trees and analysis results support that WGZ8^T should be a new species of the genus *Microvirga*.

Physiological properties analysis

WGZ8^T is Gram-negative and rod-shaped aerobic bacteria. It could grow well on R2A, TSA, NA and LB agar. Growth occurred at 0-3 % (w/v) NaCl (optimal concentration, 0-1 %), pH 5.0-11.0 (optimal pH, 7.0) and 10-40 °C (optimal temperature, 28 °C). After 3 days of incubation on R2A agar, colonies were smooth, translucent, dense and mass, wet, bright, convex, and circular with entire margins and milky white. Table 1 shows the phenotypic differences with reference strains. All the negative properties of strain WGZ8^T in commercial kits were listed in table S4.

Chemotaxonomic characteristics

The major fatty acids (> 10 % of total) of the strain WGZ8^T were detected as C_{19:0} cyclo ω 8c (43.3 %), C_{16:0} (18.8 %) and C_{18:1} ω 6c and/or C_{18:1} ω 7c (16.4 %). The two reference strains fatty acid profile was similar to that of the WGZ8^T (Table 2). The polar lipids of strain WGZ8^T included phosphatidyl dimethylethanolamine, phosphatidyl monomethylethanolamine, phosphatidylethanolamine,

phosphatidylcholine, diphosphatidylglycerol and phosphatidylglycerol (Fig. S4). The respiratory quinone of strain WGZ8^T was the same to all members of the genus *Microvirga*, and was identified as Q-10 (100 %).

According to the chemotaxonomic, phenotypic and phylogenetic data, WGZ8^T can be considered as a new member of the genus *Microvirga*, for which the name *Microvirga puerhi* sp. nov. is proposed.

Description of *Microvirga puerhi* sp. nov.

Microvirga puerhi (pu.erh'i. N.L. gen. n. puerhi of Pu-erh, referring to Pu-erh tea garden soil where the type strain was isolated).

Cells are rod-shaped, Gram-negative, aerobic, devoid of motility, approximately 0.4-0.8 µm wide and 1.5-2.5 µm long. After growing in R2A agar at 28 °C for 3 days, colonies are smooth, translucent, dense and mass, wet, bright, convex, and circular with entire margins, milky white., and 1-2 mm in diameter. Growth occurs within 0-3.0 % (w/v) NaCl (optimal concentration, 0-1.0 %), pH 5.0-11.0 (optimal pH, 7.0) and 10-40 °C (optimal temperature, 28 °C). It grows well on R2A, TSA, NA and LB agar. Positive for catalase and negative for oxidase; negative for hydrolysis of CM-cellulose, Tweens (20, 40 and 80) and skim milk. Positive for starch and gelatin hydrolysis.

In API 20NE tests, it is positive for urease activity, D-glucose fermentation, assimilation of D-mannose, potassium gluconate, D-glucose and L-arabinose. It is positive in API ZYM tests for naphthol-AS-BI-phosphohydrolase, trypsin, esterase (C4), acid phosphatase, alkaline phosphatase and leucine arylamidase; weakly positive for valine arylamidase, esterase lipase (C8), β-glucosidase and β-galactosidase. In API 50CH tests, it is positive for acid production from L-arabinose, D-ribose, D-xylose, D-adonitol, aesculin ferric citrate, D-lyxose, D-fucose, L-fucose and potassium 5-ketogluconate; weakly positive for D-glucose. Negative properties in commercial kits are listed in table S4. The major cellular fatty acids are C_{19:0} cyclo ω8c, C_{16:0}, C_{18:1} ω7c and/or C_{18:1} ω6c. The major polar lipids are phosphatidylethanolamine, phosphatidylcholine, diphosphatidylglycerol, phosphatidylglycerol, phosphatidylmethylethanolamine and phosphatidylmonomethylethanolamine. Ubiquinone 10 (Q-10) is the only respiratory quinone.

The type strain, WGZ8^T (=CGMCC1.16488^T =JCMXXXX^T) was isolated from a soil sample of Puerh tea garden in Puer city, Southwest of China. The genome size and DNA G+C content of the type strain is 5.17 MB and 61.0 % respectively.

Abbreviations

ANI, average nucleotide identity; dDDH, digital DNA-DNA hybridization; COG, cluster of orthologous groups of proteins; RNA, ribonucleic acid.

Declarations

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Conflict of interest

The authors declare that there is no conflict of interest.

Ethical statement

This study does not describe any experimental work related to human.

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Tables

Table 1. Differential characteristics between strain WGZ8^T and reference strains of species of the genus *Microvirga*.

Characteristic	1	2	3
Colony colour	Cream-colored	light pink color	light pink color
pH range for growth	5-11	5-7*	6-9*
Assimilation of (API 20NE):			
Gelatin hydrolysis	-	-	+
D-glucose	+	-	+
L-arabinose	+	-	+
D-mannose	+	-	+
D-mannitol	-	+	-
N-acetylglucosamine	-	+	-
Maltose	-	+	-
Potassium gluconate	+	-	+
Enzyme activities (API ZYM):			
Esterase lipase (C8)	w	w	+
β -Galactosidase	w	-	-
β -Glucuronidase	-	-	w
β -Glucosidase	w	w	-
<i>N</i> -acetyl- β -glucosaminidase	-	w	w
Acid production from (API 50CH):			
Glycerol	-	w	-
D-arabinose	-	w	-
D-adonitol	+	-	+
D-glucose	w	+	+
D-fructose	-	-	w
D-trehalose	-	+	-
D-tagatose	-	w	+
DNA G+C content (%)	61.0	61.4 $\pm$	65.1 $\pm$

Strains: 1, WGZ8^T; 2, '*Microvirga rosea*' MAH-2; 3, *Microvirga subterranea* DSM 14364^T. Symbol: +, positive; -, negative; w, weakly positive. Data were taken from this study unless otherwise indicated. All strains were positive for the following characteristics: D-glucose fermentation, alkaline phosphatase, esterase (C4), leucine arylamidase, valine arylamidase, trypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, L-arabinose, D-ribose, D-xylose, aesculin ferric citrate, D-lyxose, D-fucose, L-fucose, potassium 5-ketogluconate.

*Results from the previously reports (Huq, MA 2018; Sungwan et al. 2003)

‡Data from the genome sequences.

Table 2. Cellular fatty acid profiles of strain WGZ8^T and reference strains Strains: 1, WGZ8^T; 2, '*Microvirga rosea*' MAH-2; 3, *Microvirga subterranea* DSM 14364^T. All data were from this study. Values are percentages of the total fatty acids; fatty acids amounting to <1 % of the total fatty acids are not listed; TR, trace (<1 %); ND, not detected.

Fatty acid	1	2	3
Straight-chain			
C _{16:0}	18.8	5.2	8.2
C _{17:0}	1.9	TR	6.4
C _{18:0}	4.4	TR	3.9
Cyclo			
15:1 ω 8c	ND	1.3	TR
C _{17:0} cyclo	4.2	TR	ND
C _{17:1} ω 6c	ND	ND	1.5
C _{18:1} ω 7c 11-methyl	3.8	TR	1.4
C _{19:0} cyclo ω 8c	43.3	17.8	29.4
C _{20:2} ω 6,9c	2.5	TR	TR
Summed features*			
2	3.3	3.2	3.1
3	1.3	2.8	1.2
8	16.4	66.6	41.7

*Fatty acids that could not be separated by GC using the MIDI system. summed feature 2, C_{12:0} aldehyde, C_{14:0} 3OH and/or C_{16:1} iso I; summed feature 3, C_{16:1} ω 7c and/or C_{16:1} ω 6c; summed feature 8, C_{18:1} ω 7c and/or C_{18:1} ω 6c.

Figures

Figure 1

Maximum-likelihood phylogenetic tree based on 16S rRNA gene sequences showing the taxonomic position of strain WGZ8^T in the genus *Microvirga*. Numbers at nodes indicate bootstrap percentages (based on 1,000 replications); values >50 % are shown. Bar, 0.01 substitutions per nucleotide position. *Enterovirga rhinocerotis* YIM 100770^T (KC992737) was used as an outgroup.

Figure 2

Phylogenetic relationship of strain WGZ8^T and closely related strains in the genus *Microvirga*. The phylogenetic tree of strain WGZ8^T and related type strains of the genus *Microvirga* based on genes with homology and 1018 single copies in 22 genomes sequences. Bar, 0.1 nucleotide substitution rate (Knuc) units. The relationships were inferred using the maximum-likelihood algorithm using iQ-tree v1.6.12. *Enterovirga rhinocerotis* YIM 100770^T was used as an outgroup.

Supplementary Files

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