Devosia Sediminis sp. nov., Isolated from Fubterranean Sediment.

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Abstract

A novel Gram- negative, cream-colored, rod-shaped, aerobic, non-motile bacterium, designated MSA67^T, was isolated from a subterranean sediment sample of the Mohe Basin in Northeast China. Strain MSA67^T was detected to grow at 4–40°C (optimum, 28–30°C), pH 5.0–10.0 (optimum, pH 7.0) and in 0.0–8.0 % (w/v) NaCl (optimum, 2.0–3.0 %). Phylogenetic analysis based on 16S rRNA gene sequence revealed that strain MSA67^T was a member of the genus *Devosia*, with the highest similarity with *D. riboflavina* IFO13584^T (98.0 %) and *D. chinhatensis* IPL18^T (97.0 %). The major cellular fatty acids are $C_{16:0}$, $C_{18:1}\omega7c$ 11-methyl and $C_{18:1}\omega6c$ and/or $C_{18:1}\omega7c$. The major polar lipids are diphosphatidylglycerol, phosphatidylglycerol, glycolipids and three unidentified phospholipids. The major respiratory quinone is ubiquinone 10 (Q-10). The genomic size of strain MSA67^T is 4.1 MB and DNA G + C content is 63.6 %. Based on genotypic, phenotypic and phylogenetic results, strain MSA67^T is concluded to represent a novel species of the genus *Devosia*, for which the name *Devosia sediminis* sp. nov. is proposed. The type strain is MSA67^T (= CGMCC 1.18467^T = KCTC 82192^T).

Introduction

The genus *Devosia*, established by Nakagawa et al. (1996), belongs to the family *Devosiaceae*. Strains of the genus *Devosia* are rod-shaped, aerobic and oxidase-positive. They contain ubiquinone-10 (Q-10) as the predominant respiratory quinone and are Gram-negative. The G + C content of DNA of members of the genus *Devosia* ranges from 59.5 to 66.2 mol % (Yoon et al. 2007). The family comprises two genera with valid published names: genus *Devosia* and genus *Paradevosia* (Geng et al. 2015), and the genus *Devosia* contains 27 species with valid published names, which are listed in the LPSN (Parte et al. 2014). The genus *Devosia* are ubiquitous in diverse ecological environments, such as soil (Mohd et al. 2017), root nodules (Bautista et al. 2010), alpine glacier cryoconite (Zhang et al. 2012), deep-seawater (Liu et al. 2020), and surface seawater (Lin et al. 2020), which clearly indicates their excellent environmental adaptation.

Materials And Methods

Isolation and culturing conditions

Strain MSA67^T was isolated from a subterrestrial sediment sample collected from a borehole (53º 28' 37.80" N, 122º 19' 23.40" E; depth 358.5 m), which was sampled in Mohe Basin, Northeast China (Tian et al. 2015). The sediment was ground to powder under sterile conditions, and microbials were isolated and cultured according to a previously published method (Han et al. 2014). Strain MSA67^T was isolated by using the dilution plate technique on marine agar 2216 (MA; DB) at 28 ℃ for 7 days. The isolated strain was cultured at 28 ℃ on MA and maintained at -80 °C supplemented with a 15 % (v/v) glycerol suspension for extended preservation. The isolate was preserved at the Korean Collection for Type Cultures (KCTC) and the China General Microbiological Culture Collection Center (CGMCC). A deposit

number from each place has been allocated (KCTC 82192^T and CGMCC 1.18467^T, respectively). Reference strains *Devosia riboflavina* CGMCC 1.10398^T and *Devosia chinhatensis* CGMCC 1.10663^T were obtained from the CGMCC.

Phylogenetic analysis

Genomic DNA extraction, PCR amplification and sequencing of the 16S rRNA gene were done according to Zhou et al (2019). The 16S rRNA gene sequences of related taxa were extracted from the recently updated EzBioCloud (https://www.ezbiocloud.net/) (Kim et al. 2011). CLUSTAL W program was used to perform multiple sequence alignments under default parameters (Thompson et al. 1994), Neighbor-joining (Saitou et al. 1987), maximum-likelihood (Kishino et al. 1989) and maximum-parsimony (Walter et al. 1971) methods in MEGA 7 (Kumar et al. 2016) were used to reconstruct phylogenetic trees. Bootstrap analysis was performed based on 1,000 replicates (Felsenstein et al. 1985) with the distance option selection using Kimura's two-parameter model (Kimura et al. 1980). *Escherichia coli* ATCC 11775^T (X80725) was used in the phylogenetic analysis as an outgroup.

Genomic analysis

The genome of strain MSA67^T was sequenced using the Illumina Novaseq pe150 platform (Shanghai Majorbio Bio-Pharm Tech Ltd., Shanghai China). The sequenced reads were obtained and re-assembled using the SPAdes software (version 3.14.1) with default parameters based on a previously published report (Bankevich et al. 2012). The sequencing depth of coverage was 200X. The 16S rRNA fragment from the genome was detected on EZBioCloud (https://www.ezbiocloud.net/tools/contest16s). Results were deposited at NCBI (https://www.ncbi.nlm.nih.gov). The coding genes' functions were detected online (http://weizhong-lab.ucsd.edu/webMGA/server/cog/) by using a server database named COG as described previously (Galperin et al. 2015). The genomes of members of genus *Devosia* were download from NCBI Genbank, and were annotated by the NCBI Prokaryotic Genome Annotation Pipeline v5.0. OrthoFinder v2.2.6 was used to cluster the annotated genomes (Nguyen et al. 2015). Orthologs selected were aligned by Mafft software (Katoh et al. 2008), then all the alignments were trimmed by TrimAl, and LG+F+R5 was selected as the best model automatically. And iQ-tree v1.6.12 was used to reconstruct maximum-likelihood tree (Castresana et al. 2000) with bootstrap values set at 1,000 replications. EzGenome web service (www.ezbiocloud.net/tools/ani) and the Genome-to-Genome Distance Calcu-lator version 2.1 (http://ggdc.dsmz.de/distcalc2.php) were used to calculate the average nucleotide identity (ANI) (Konstantinidis et al. 2005) and digital DNA–DNA hybridization (dDDH) (Auch et al. 2010) values between strain MSA67^T and members of genus *Devosia*, respectively.

Physiological properties analysis

MSA67^T shared the highest similarity with *Devosia riboflavina* CGMCC 1.10398^T (Nakagawa et al. 1996) and *Devosia chinhatensis* CGMCC 1.10663^T (Kumar et al. 2008) based on 16S rRNA gene sequence. Therefore, *D. riboflavina* and *D. chinhatensis* were used as the reference strains. After growing cells for 3

days at 28 ℃ on MA, colonial and cell morphology were examined by eye and scanning electron microscopy (SU1510; Hitachi, Tokyo, Japan). Growth was tested on R2A agar (830 medium, DSMZ), tryptone soya agar (TSA, 535 medium, DSMZ), nutrient agar (NA, 101 medium, DSMZ) and Luria–Bertani agar (LB, 381 medium, DSMZ). Gram reactivity was determined according to Murray et al (1994). Anaerobic growth was determined using an anaerobic pouch (MGC; Mitsubishi, Tokyo, Japan) in MA medium for 7 days at 28 °C (Smibert et al. 1994). MSA67^T motility was tested in marine broth 2216 (MB; DB Bioscience, Franklin Lakes, NJ, USA) supplemented with 0.5% agar according to a previously published report (Bernardet et al. 2002). Oxidase test strips (BioMérieux SA, Marcy-l'Étoile, France) using 1 % (w/v) tetramethyl-*p*-phenylenediamine and bubble production using 3 % (v/v) H_2O_2 were used to measure oxidase and catalase activities. The suitable temperature range for growth were determined in MB when cells were incubated for 7 days at different temperatures, including 4, 10, 15, 22, 28, 30, 37, 40 ℃. The range of pH 4.0–11.0 (at intervals of 1 pH unit) was used to test the optimal growth in MB. A buffer system was used to set pH values: pH 3.0–5.0, 0.1 M citric acid/0.1 M sodium citrate; pH 6.0–8.0, 0.1 M KH_2PO^4/0.1 M K_2HPO_4; pH 9.0–10.0, 0.1 M $NaHCO_3$/0.1 M Na_2CO_3; pH 11.0, 0.05 M Na_2HPO_4/0.1 M NaOH (Zhang et al. 2016). NaCl tolerance was studied in modified MB with NaCl concentration range of 0–8.0 % (w/v, at intervals of 1.0 %). Published methods (Smibert et al. 1994) were used to investigate the hydrolysis of starch (0.2 %, w/v), casein (5 % skimmed milk, w/v), CM-cellulose (0.5 %, w/v), Tween 80 (1 %, w/v), Tween 20 (1 %, w/v) and Tween 40 (1 %, w/v) in MA as the basal medium . API 20NE, API ZYM and API 50CH kits (BioMérieux SA, Marcy-l'Étoile, France) were used to detect enzyme activities and assimilation of acid production according to the manufacturer's instructions.

Chemotaxonomic

MSA67^T cells and cells from the reference strains were cultured in MA for 7 days at 28 °C. Cells were collected at third quadrants. The harvested cells were saponified, methylated and extracted according to the procedure of the Sherlock Microbial Identification System (MIDI, version 6.1). Fatty acid methylesters extracted from the above steps were analyzed by GC (7890A GC system; Agilent, Santa Clara, CA, USA) and identities were confirmed using the Sherlock Aerobic Bacterial Database (TSBA 6). MSA67^T respiratory quinones were extracted and analyzed by using previously published HPLC methods (Da Costa et al. 2011a). The polar lipids on TLC plates were sprayed with molybdophosphoric acid, molybdenum blue, ninhydrin and anaphthol/sulfuric acid, and their identities identified (Minnikin et al. 1984; Da Costa et al. 2011b).

Results And Discussion

Molecular phylogenetic analysis

Almost full-length 16S rRNA gene sequence (1442 bp) of strain MSA67^T was identified and deposited into the GenBank database (accession number MN006580). MSA67^T 16S rRNA gene sequence shared the highest similarity with *D. riboflavina* IFO13584^T (98.0 %) and *D. chinhatensis* IPL18^T (97.0 %). These levels

of similarity were below the recently recommended cutoff of 98.65 % (Kim et al. 2014). Maximum-likelihood (Fig. 1), neighbor-joining algorithm (Fig. S1) and maximum-parsimony (Fig. S2) phylogenetic trees revealed that strain MSA67^T is a member of a putative novel species of the genus *Devosia*.

Genome analysis

The genome of strain MSA67^T has been deposited into the GenBank database (accession number JAEKMH000000000). One 16S rRNA fragment from the genome was detected, and it shared 100 % similarity with that from the PCR, and there are no contaminated sequences in the sample by analyzing the sequencing and GC depth (Fig. S3). The genomic size of strain MSA67^T was 4.1 MB. The DNA G+C content of strain MSA67^T was 63.6 % from the genomic sequence, which was in line with the genus *Devosia* range from 59.5 to 66.2 mol % (Table S1). The detailed analysis of the MSA67^T genome was presented in Table S2. Table S3 showed the distribution of genes into COG functional categories . Phylogenomic tree showed the relationship between related species (Fig. 2). The ANI and dDDH values between strain MSA67^T and members of the genus *Devosia* were presented in detail in Table S1. Both ANI and dDDH values were below the species delineation thresholds of 95–96 % ANI and 70 % dDDH, respectively. All above data supports that MSA67^T represents a novel genomospecies of the genus *Devosia*.

Physiological properties analysis

MSA67^T cells were Gram-negative. They are rod-shaped aerobic bacteria. After incubating for 3 days in MA, colonies were observed as smooth, opaque, convex and circular with cream-color and entire margins. Cells grew well in MA, R2A agar and LB agar media, however, they couldn't grow in TSA. Growth occurred at 4–40 °C (optimal temperature, 28-30 °C), at pH 5.0–10.0 (optimal pH, 7.0) and in 0–8 % (w/v) NaCl (optimal concentration, 2.0-3.0 %). More phenotypic differences are detailed in Table 1.

Chemotaxonomic characteristics

The major fatty acids, which represent more than 10 % of the total fatty acids, detected in strain MSA67^T were $C_{18:1}\omega6c$ and/or $C_{18:1}\omega7c$, 45.2 %, $C_{16:0}$ (22.1 %) and $C_{18:1}\omega7c$ 11-methyl (23.7 %). The MSA67^T fatty acid profile was similar to that of the two reference strains. Differences between them are detailed in Table 2. Respiratory quinone of strain MSA67^T was identified as Q-10 (100 %), similar to all members of the genus *Devosia*. The polar lipids of strain MSA67^T included diphosphatidylglycerol, phosphatidylglycerol, glycolipids and three unidentified phospholipids (Fig. S4).

According to the phylogenic, phenotypic and chemotaxonomic data, MSA67^T is considered to be a member of the genus *Devosia* within the family *Devosiaceae*, for which the name *Devosia sediminis* sp. nov. is proposed.

Description of *Devosia sediminis* sp. nov.

Devosia sediminis (se.di'mi.nis. L. gen. n. *sediminis* of sediment).

Cells are Gram-negative, rod-shaped, aerobic, devoid of gliding motility, approximately 0.5–0.7μm wide and 2.1–3.4 μm long. After growing in MA at 28 °C for 3 days, colonies are smooth, opaque, convex and circular with cream-color and entire margins, and approximately 1–2 mm in diameter . Growth occurs at 4–40 °C (optimal temperature, 28-30 °C), at pH 5.0–10.0 (optimal pH, 7.0) and in 0–8 % (w/v) NaCl (optimal concentration, 2.0-3.0 %). Cells grow well in MA, R2A agar and LB agar media, however, they cannot grow in TSA. They are positive for oxidase and catalase; negative for hydrolysis of Tweens 20, 40 and 80, starch, skimmed milk and CM- cellulose.

In API 20NE tests, they are positive for urease activity, *β*-galactosidase, *β*-glucosidase (aesculin hydrolysis), L-arabinose, *N*-acetylglucosamine, D-glucose, D-mannose and maltose. They are negative for nitrate reduction to nitrite, D-glucose fermentation, indole production, arginine dihydrolase, D-mannitol, gelatin hydrolysis, trisodium citrate, potassium gluconate, adipic acid, capric acid, malic acid and phenylacetic acid. In API ZYM tests, they are positive for esterase lipase(C8), esterase (C4), leucine arylamidase, alkaline phosphatase, acid phosphatase, valine arylamidase, naphthol-AS-BI-phosphohydrolase, *α*-Glucosidase, *α*-Mannosidase, *β*-Galactosidase, *β*-Glucosidase, *N*-acetyl-*β*-glucosaminidase and trypsin. They are weakly positive for cystine arylamdase, *α*-chymotrypsin; and negative for lipase (C14), *α*-galactosidase, *β*-glucuronidase and *β*-fucosidase. In API 50CH tests, they are positive for D-fructose, D-ribose, D-glucose, *N*-acetylglucosamine, arbutin, aesculin ferric citrate, salicin, D-cellobiose, D-maltose, D-lactose (bovine origin), D-trehalose, starch, glycogen; weakly positive for potassium 5-ketogluconate and glycerol; and negative for erythritol, D-arabinose, D-adonitol, D-xylose, D-galactose, D-arabitol, D-melezitose, D-raffinose, D-melibiose, D-mannose, D-mannitol, D-turanose, D-lyxose, D-tagatose, D-fucose, D-sorbitol, L-arabinose, L-sorbose, L-rhamnose, L-fucose, L-arabitol, L-xylose, methyl-*β*-D-xylopyranoside, dulcito, inositol, methyl-*α*-D-mannopyranoside, methyl-*α*-D-glucopyranoside, amygdalin, inulin, xylitol, gentiobiose, potassium gluconate and potassium 2-ketogluconate. The major fatty acids are $C_{16:0}$, $C_{18:1}\omega7c$ 11-methyl and $C_{18:1}\omega6c$ and/or $C_{18:1}\omega7c$. The polar lipids are glycolipids, phosphatidylglycerol, diphosphatidylglycerol, and three unidentified phospholipids. Respiratory quinone is identified to be Q-10. The genomic size of the strain is 4.1 MB. The DNA G+C content of the strain is 63.6 %.

In summary, the type strain, MSA67^T (=CGMCC 1.18467^T=KCTC 82192^T) has been isolated from the sediment of the Mohe Basin in Northeast China. The GenBank/EMBL/DDBJ accession numbers for the genome and 16S rRNA gene sequence of the type strain are JAEKMH000000000 and MN006580, respectively.

Abbreviations

ANI, average nucleotide identity; dDDH, digital DNA-DNA hybridization; COG, cluster of orthologous groups of proteins; RNA, ribonucleic acid: tRNA, transfer ribonucleic acid; sRNA, small ribonucleic acid;

LINE, long-interspersed nuclear elements; SINE, short interspersed nuclear elements; RC, rolling circle; DPG, diphosphatidylglycerol; PG, phosphatidylglycerol; GL, glycolipids; L, unidentified lipids.

Declarations

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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References

1. Nakagawa Y, Sakane T, Yokota A (1996) Transfer of "*Pseudomonas riboflavina*" (Foster 1944), a Gram-negative, motile rod with long-chain 3-hydroxy fatty acids, to *Devosia riboflavina* gen. nov. sp. nov. nom. rev. Int J Syst Bacteriol 46:16–22
2. Yoon J-H, Kang S-J, Park S, Oh T-K (2007) *Devosia insulae* sp. nov., isolated from soil, and emended description of the genus *Devosia*. Int J Syst Evol Microbiol 57:1310–1314
3. Geng S, Pan X, Mei R, Wang Y, Sun J, Liu XY, Tang YQ, Wu XL (2015) *Paradevosia shaoguanensis* gen. nov. sp. nov. Isolated from a Coking Wastewater. Curr Microbiol 70:110–118
4. Parte AC (2014) LPSN—list of prokaryotic names with standing in nomenclature. Nucleic Acids Res 42:613–616
5. Mohd Nor M, Sabaratnam V, Tan G (2017) *Devosia elaeis* sp. nov., isolated from oil palm rhizospheric soil. Int J Syst Evol Microbiol 67:851–855
6. Bautista V, Monsalud R, Yokota A (2010) *Devosia yakushimensis* sp. nov., isolated from root nodules of *Pueraria lobata* (Willd.) Ohwi. Int J Syst Evol Microbiol 60:627–632
7. Zhang D, Redzic M, Liu H, Zhou Y, Schinner F (2012) *Devosia psychrophila* sp. nov. and *Devosia glacialis* sp. nov., from alpine glacier cryoconite, and an emended description of the genus *Devosia*. Int J Syst Evol Microbiol 62:710–715
8. Liu Y, Du J, Zhang J, Lai Q-L, Shao Z-G, Zhu H-H (2020) *Devosia marina* sp. nov., isolated from deep seawater of theSouth China Sea, and reclassification of *Devosia subaequoris* as alater heterotypic synonym of *Devosia soli*. Int J Syst Evol Microbiol 70:3062–3068
9. Lin D, Huang Y, Chen Y, Zhu S, Yang J (2020) *Devosia indica* sp. nov., isolated from surface seawater in the Indian Ocean. Int J Syst Evol Microbiol 70:340–345

10. Tian M, Zhang R-G, Han L, Zhao X-M, Lv J (2015) *Dyadobacter sediminis* sp. nov. isolated from a subterranean sediment sample. Int J Syst Evol Microbiol 65:827–832
11. Han L, Wu S-J, Qin C-Y, Zhu Y-H, Lu Z-Q, Xie B, Lv J (2014) *Hymenobacter qilianensis* sp. nov., isolated from a subsurface sandstone sediment in the permafrost region of Qilian Mountains, China and emended description of the genus *Hymenobacter*. Antonie Van Leeuwenhoek 105:971–978
12. Zhou D, Tan X, Zhang W, Chen H-Y, Fan Q-M, Lv J (2019) *Rhodoferax bucti* sp. nov. isolated from fresh water. Int J Syst Evol Microbiol 69:3903–3909
13. Kim O-S, Cho Y-J, Lee K, Yoon S-H, Kim M, Na H, Park SC, Jeon YS, Lee JH, Yi H (2011) Introducing eztaxon-e: a prokaryotic 16s rrna gene sequence database with phylotypes that represent uncultured species. Int J Syst Evol Microbiol 62:716–721
14. Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res 22:4673–4680
15. Saitou N, Nei MC (1987) The neighbor-joining method-a new method for reconstructing phylogenetic trees. Mol Biol Evol 4:406–425
16. Kishino H, Hasegawa M (1989) Evaluation of the maximum likelihood estimate of the evolutionary tree topologies from dna sequence data, and the branching order in hominoidea. J Mol Evol 29:170–179
17. Walter M, Fitch (1971) Toward defining the course of evolution: minimum change for a specific tree topology. Syst Zool 20:406–416
18. Kumar S, Stecher G, Tamura K (2016) MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. Mol Biol Evol 33:1870–1874
19. Felsenstein J (1985) Confidence limits on phylogenies: an approach using the Bootstrap. Evolution 39:783–791
20. Kimura M (1980) A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J Mol Evol 16:111–120
21. Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M (2012) SPAdes: a new genome assembly algorithm and its applications to single- cell sequencing. J Comput Biol 19:455–477
22. Galperin MY, Makarova KS, Wolf YI, Koonin EV (2015) Expanded microbial genome coverage and improved protein family annotation in the COG database. Nucleic Acids Res 43:261–269
23. Nguyen LT, Schmidt HA, von Haeseler A, Minh BQ (2015) IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Mol Biol Evol 32:268–274
24. Katoh K, Toh H (2008) Recent developments in the MAFFT multiple sequence alignment program. Brief Bioinform 92:86–98
25. Castresana J (2000) Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. Mol Biol Evol 17:540–552

26. Konstantinidis KT, Tiedje JM (2005) Genomic insights that advance the species definition for prokaryotes. Proc Natl Acad Sci U S A 102:2567–2572
27. Auch AF, von Jan M, Klenk H-P, Göker M (2010) Digital DNA- DNA hybridization for microbial species delineation by means of genome-to-genome sequence comparison. Stand Genomic Sci 2:117–134
28. Kumar M, Verma M, Lal R (2008) *Devosia chinhatensis* sp. nov. isolated from a hexachlorocyclohexane (HCH) dump site in India. Int J Syst Evol Microbiol 58:861–865
29. Murray R, Doetsch RN, Robinow CF (1994) Murray R, Doetsch RN, Robinow CF. Determinative and cytological light microscopy. Meth Gen Mol Bacterio pp:21–41
30. Smibert RM, Krieg NR (1994) Phenotypic characterization. In Methods for General and Molecular Bacteriology pp:607–654
31. Bernardet JF, Nakagawa Y, Holmes B (2002) Proposed minimal standards for describing new taxa of the family *Flavobacteriaceae* and emended description of the family. Int J Syst Evol Microbiol 52:1049–1070
32. Zhang W, Yuan X, Feng Q-Q, Zhang R-G, Zhao X-M, Lv J (2016) *Altererythrobacter buctense* sp. nov., isolated from mudstone core. Antonie Van Leeuwenhoek 109:793–799
33. Da Costa MS, Albuquerque L, Nobre MF, Wait R (2011a) The extraction and identification of respiratory lipoquinones of prokaryotes and their use in taxonomy. Methods Microbiol 38:197–206
34. Minnikin DE, O'Donnell AG, Goodfellow M, Alderson G, Athalye M, Schaal A, Parlett JH (1984) An integrated procedure for the extraction of bacterial isoprenoid quinones and polar lipids. J Microbiol Methods 2:233–241
35. Da Costa MS, Albuquerque L, Nobre MF, Wait R (2011b) The identification of polar lipids in prokaryotes. Method Microbiol 38:165–181
36. Kim M, Oh H-S, Park S-C, Chun J (2014) Towards a taxonomic coherence between average nucleotide identity and 16S rRNA gene sequence similarity for species demarcation of prokaryotes. Int J Syst Evol Microbiol 64:346–351

Tables

Table 1. Differential characteristics between strain MSA67^T and reference strains of species of the genus *Devosia*.

Strains:1, MSA67^T; 2, *Devosia riboflavina* CGMCC 1.10398^T; 3, *Devosia chinhatensis* CGMCC 1.10663^T. Symbol: +, positive; -, negative; w, weakly positive. Data were taken from this study unless otherwise indicated. All strains were positive for the following characteristics: urease activity, *β*-glucosidase (aesculin hydrolysis), *β*-galactosidase, D-glucose, L-arabinose, D-mannose, *N*-acetylglucosamine, maltose, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, trypsin, naphthol-AS-BI-phosphohydrolase, *β*-galactosidase, *β*-glucosidase, arbutin, aesculin ferric citrate, salicin. All strains were negative for the following characteristics: D-glucose fermentation, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, phenylacetic acid, lipase (C14), *α*-galactosidase, *β*-

glucuronidase, erythritol, D-adonitol, methyl-*β*-D-xylopyranoside, D-galactose, D-mannose, L-sorbose, L-rhamnose, dulcitol, inositol, D-mannitol, D-sorbitol, methyl-*α*-D-mannopyranoside, methyl-*α*-D-glucopyranoside, amygdalin, D-melibiose, inulin, D-melezitose, D-raffinose, xylitol, gentiobiose, D-turanose, D-tagatose, D-arabitol, L-arabitol, potassium gluconate, potassium 2-ketogluconate.

Characteristic	1	2	3
Colony colour	Cream-colored	Cream-colored*	Cream-colored
Growth at			
28°C	+	+*	+*
pH range for growth	5-10	7 *	5-9 *
Hydrolysis of:			
Casein	-	-*	-*
Assimilation of (API 20NE):			
Reduction of nitrate to nitrite	-	+	-
Indole production	-	-	+
Arginine dihydrolase	-	+	+
Gelatin hydrolysis	-	+	+
D-mannitol	-	+	+
Enzyme activities (API ZYM):			
Alkaline phosphatase	+	+	-
Cystine arylamdase	w	w	+
α-Chymotrypsin	w	w	-
Acid phosphatase	+	+	w
α-Glucosidase	+	-	+
N-acetyl-*β*-glucosaminidase	+	+	-
α-Mannosidase	+	+	-
β-Fucosidase	-	+	-
Acid production from (API 50CH):			
Glycerol	w	-	-
D-arabinose	-	+	-
L-arabinose	-	+	+
D-ribose	+	w	+

D-xylose	-	+	+
L-xylose	-	+	-
D-glucose	+	-	-
D-fructose	+	w	w
N-acetylglucosamine	+	-	-
D-Cellobiose	+	-	-
D-Maltose	+	-	-
D-lactose (bovine origin)	+	-	-
Sucrose	+	-	-
D-trehalose	+	-	-
Starch	+	-	-
Glycogen	+	-	-
D-lyxose	-	+	+
D-fucose	-	+	+
L-fucose	-	+	-
Potassium 5-ketogluconate	w	+	+
DNA G+C content (%)	63.6	61.8 ‡	62.4‡
*Different from the results previously reported (Nakagawa et al. 1996; Kumar et al. 2008). ‡Data from the genome sequences.			

Table 2. Cellular fatty acid profiles of strain GSA243-2^T and reference strains

Strains:1, MSA67^T; 2, *Devosia riboflavina* CGMCC 1.10398^T; 3, *Devosia chinhatensis* CGMCC 1.10663^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			
$C_{14:0}$	TR	TR	TR
$C_{16:0}$	22.1	26.1	13.1
$C_{17:0}$	1.2	TR	3.3
$C_{18:0}$	2.9	2.8	6.5
Hydroxy			
$C_{10:0}$ 3-OH	ND	TR	ND
$C_{17:0}$ 3-OH	TR	ND	TR
$C_{18:0}$ 3-OH	1.9	TR	1.1
Cyclo			
$C_{17:0}$ cyclo	ND	1.6	ND
Summed features*			
3	TR	1.3	1.4
7	TR	TR	1.4
8	45.2	18.5	65.7

*Fatty acids that could not be separated by GC using the MIDI system. summed feature 3, $C_{16:1}\omega 6c$ and/or $C_{16:1}\omega 7c$; summed feature 7 $C_{19:1}\omega 6c$ and/or $C_{19:0}$ cyclo; summed feature 8, $C_{18:1}\omega 7c$ and/or $C_{18:1}\omega 6c$.

Figures

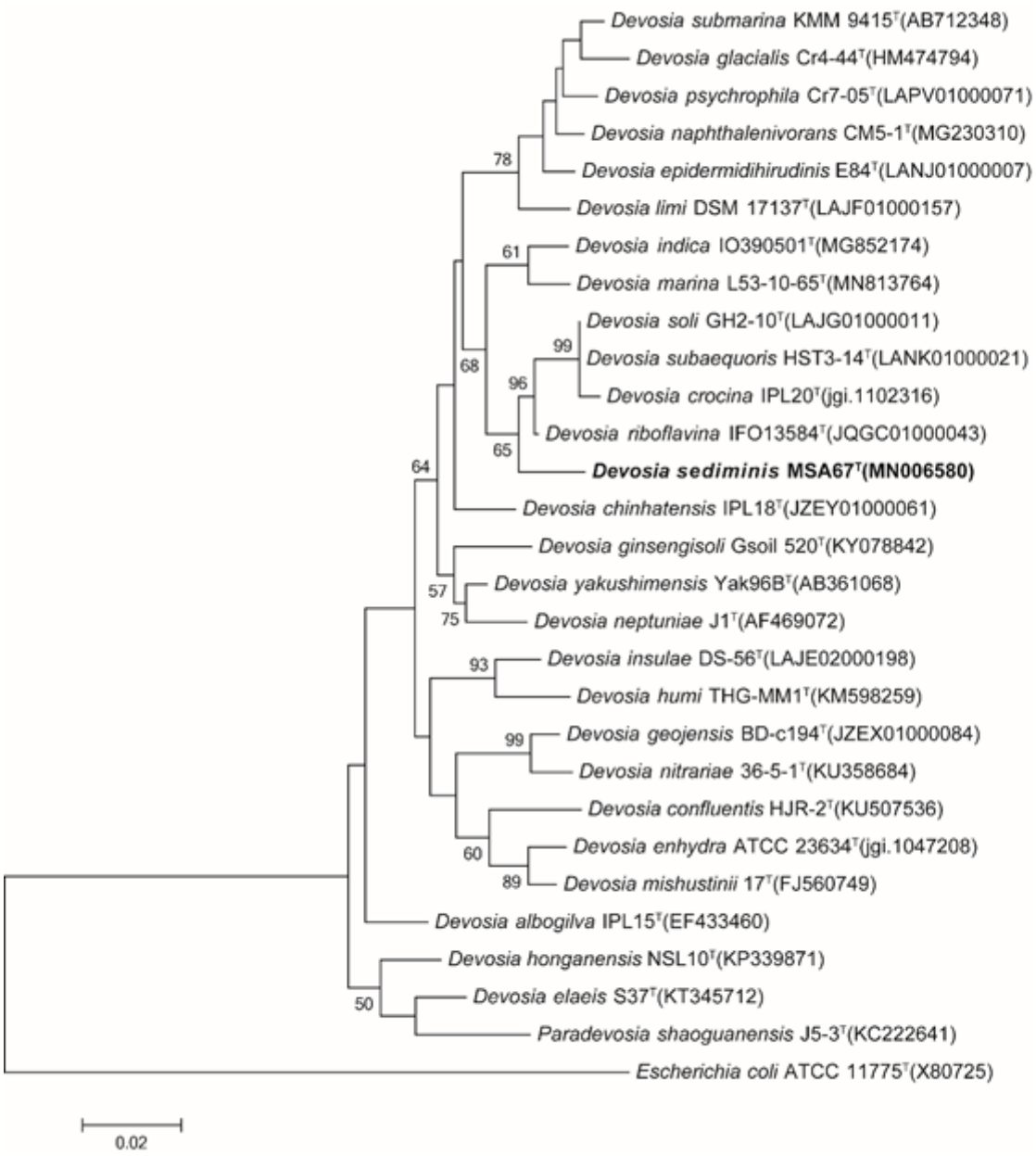

Figure 1

Maximum-likelihood phylogenetic tree based on 16S rRNA gene sequences showing the taxonomic position of strain MSA67T in the genus Devosia. Numbers at nodes indicate bootstrap percentages (based on 1000 replications); values >50% are shown. Bar, 0.02 substitutions per nucleotide position. Escherichia coli ATCC 11775T (X80725) was used as an outgroup.

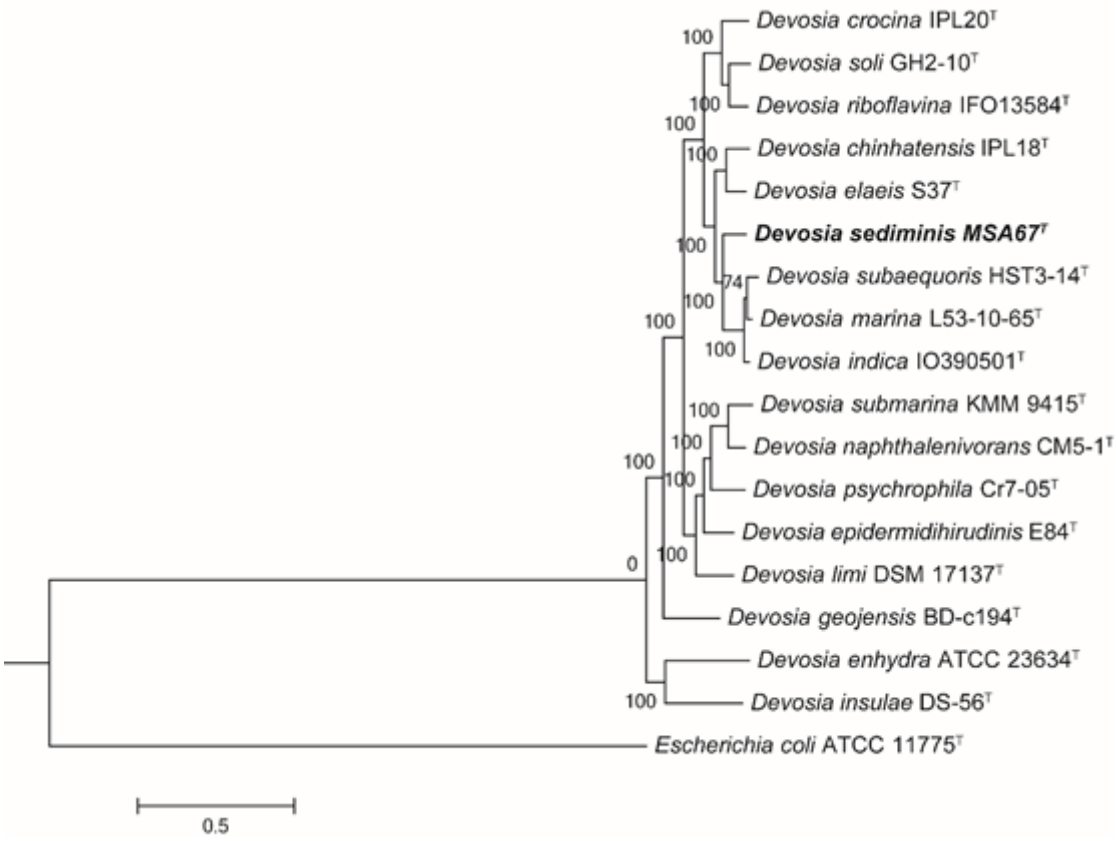

Figure 2

Phylogenetic relationship of strain MSA67T and closely related strains in the genus Devosia. The phylogenemic tree of strain MSA67T and related type strains of the genus Devosia based on genes with homology and 671 single copies in 18 genomes sequences. Bar, 0.5 nucleotide substitution rate (Knuc) units. The relationships were inferred using the maximum-likelihood algorithm using iQ-tree v1.6.12. Escherichia coli ATCC 11775T (X80725) was used as an outgroup.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- MSA67SuppplementarymaterialArch.docx