**Devosia litorisediminis** sp. nov., isolated from a sand dune

Sooyeon Park¹ · Min Ju Seo¹ · Wonyong Kim² · Jung-Hoon Yoon¹

Received: 8 November 2021 / Revised: 3 January 2022 / Accepted: 19 January 2022 / Published online: 15 September 2022

© The Author(s), under exclusive licence to Springer-Verlag GmbH Germany, part of Springer Nature 2022

**Abstract**

A Gram-negative, aerobic, non-motile, and rod-shaped bacterial strain, designated BSSL-BM10\textsuperscript{T}, was isolated from sand of a dune that was collected from the Yellow Sea, Republic of Korea. It was subjected to a polyphasic taxonomic study. 16S rRNA gene sequence analysis showed that strain BSSL-BM10\textsuperscript{T} fell phylogenetically within the radiation comprising type strains of *Devosia* species. The 16S rRNA gene sequence of strain BSSL-BM10\textsuperscript{T} shared sequence similarities of 98.2% with the type strain of *D. naphthalenivorans* and 93.5–97.7% with type strains of other *Devosia* species. ANI and dDDH values between strain BSSL-BM10\textsuperscript{T} and type strains of 18 *Devosia* species were 71.0–78.4% and 18.8–21.5%, respectively. The DNA G + C content of strain BSSL-BM10\textsuperscript{T} was 60.9% based on its genomic sequence data. Strain BSSL-BM10\textsuperscript{T} contained Q-10 as the predominant ubiquinone and 11-methyl C\textsubscript{18:1} \(\omega7\text{c}\), C\textsubscript{18:1} \(\omega7\text{c}\), summed feature 3 (C\textsubscript{16:1} \(\omega7\text{c}\) and/or C\textsubscript{16:1} \(\omega6\text{c}\)), and C\textsubscript{16:0} as its major fatty acids. Major polar lipids of strain BSSL-BM10\textsuperscript{T} were phosphatidylglycerol and two unidentified glycolipids. Strain BSSL-BM10\textsuperscript{T} showed distinguishable phenotypic properties with its phylogenetic and genetic distinctiveness separated from recognized *Devosia* species. Based on data presented in this study, strain BSSL-BM10\textsuperscript{T} should be placed in the genus *Devosia*. The name *Devosia litorisediminis* sp. nov. is proposed for strain BSSL-BM10\textsuperscript{T} (= KACC 21633\textsuperscript{T} = NBRC 115152\textsuperscript{T}).

**Keywords** Sand dune · Polyphasic taxonomy · Genome · Novel species · *Devosia litorisediminis* sp. nov.

**Abbreviations**

ANI Average nucleotide identity
dDDH Digital DNA–DNA hybridization
GC Gas chromatograph

**Introduction**

The genus *Devosia*, a member of the family *Devi*osiaceae (Hördt et al. 2020) of the class *Alphaproteobacteria*, was proposed by Nakagawa et al. (1996) with the transfer of “*Pseudomonas riboflavina*” to *Devosia riboflavina* (type species). The genus *Devosia* currently comprises 28 species with validly published names (https://lpsn.dsmz.de/genus/devosia; Parte 2018). Members of the genus *Devosia* have been isolated from various habitats (Bautista et al. 2010; Galatis et al. 2013; Jia et al. 2014; Kumar et al. 2008; Lin et al. 2020; Park et al. 2016; Quan et al. 2020; Romanenko et al. 2013; Yoon et al. 2007; Zhang et al. 2012). Recently, in the course of screening novel bacteria from a sand dune close to the Yellow Sea of Republic of Korea, many bacterial isolates have been obtained followed by taxonomic characterization. One of these bacterial isolates, designated as BSSL-BM10\textsuperscript{T}, showed the closest affiliation to members of the genus *Devosia* from the result of 16S rRNA gene sequence comparison. In this study, strain BSSL-BM10\textsuperscript{T} is characterized further using a polyphasic approach.

**Materials and methods**

**Bacterial strains and culture conditions**

Sand of a marine dune was collected from Boryeong (36°20′95″N, 126°53′38″E) close to the Yellow Sea of Republic of Korea. The sample (about 1–2 g) was serially
diluted with 0.85% (w/v) saline solution and spread onto marine agar 2216 (MA; BD Difco). After incubation at 25 °C for 7 days, strain BSSL-BM10T was isolated from the MA plate and spread onto trypticase soy agar (TSA; BD Bacto) at 30 °C. Cells of strain BSSL-BM10T were suspended in a sterile solution containing 20% (w/v) glycerol and stored at –80 °C for long-term preservation. *Devosia naphthalenivorans* JCM 32509T and *Devosia riboflava* DSM 7230T, the type strain of the type species, were obtained from the Japan Collection for Microorganisms (JCM; Japan) and Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ; Germany), respectively. Cells of strain BSSL-BM10T and *D. riboflava* DSM 7230T obtained from culture grown for 3 days in trypticase soy broth (BD Bacto) at 30 °C were used to extract DNA and to analyze isoprenoid quinones and polar lipids. Cell masses for cellular fatty acid analysis were obtained under the following conditions: strain BSSL-BM10T were harvested from TSA plates after cultivation for 3, 5, and 7 days at 30 °C, and *D. naphthalenivorans* JCM 32509T and *D. riboflava* DSM 7230T were harvested from MA and TSA plates, respectively, after cultivation for 5 days at 30 °C.

**Sequencing and phylogenetic analysis of 16S rRNA gene**

Chromosomal DNA extraction was performed using a Wizard Genomic DNA isolation kit (Promega) according to the manufacturer’s instruction. The 16S rRNA gene amplification was performed as described previously (Yoon et al. 1997) using PCR in which 9F (5′-GAGTTTGTACT GGTCAG-3′) and 1512R (5′-ACGGTACCTTGTTACGAGTT-3′) were used. Sequencing of the 16S rRNA gene followed by phylogenetic analysis were carried out as described by Yoon et al. (2003). Similarity between 16S rRNA gene sequences was calculated from alignment obtained using Clustal W program.

**Genomic analysis**

A TruSeq DNA LT Sample Prep kit (Illumina) was used to prepare a library for genomic sequencing. The library was sequenced using Illumina MiSeq platform. Sequencing data were assembled with SPAdes (Bankevich et al. 2012). Contamination of genome sequence was assessed using ContEst16S (Lee et al. 2017). Library construction and sequencing were performed by Chunlab Inc. (Republic of Korea). The ANI value based on BLAST + was calculated using JSpecies WS (http://jspecies.ribohost.com/jspeciesws/; Richter et al. 2015) or OrthoANI (Yoon et al. 2017) in EZBioCloud. The dDDH value was estimated using TYGS (https://tygs.dsmz.de/user_requests/new) with BLAST + in which the recommended formula 2 (Meier-Kolthoff et al. 2013) was used. Phylogenetic tree was constructed based on genomic sequences using previous methods (Lefort et al. 2015; Meier-Kolthoff et al. 2013) described in the TYGS. Intergenomic distances inferred under the algorithm 'trimming' and distance formula d5 (Meier-Kolthoff et al. 2013) and 100 distance replicates were calculated each. The resulting distances were used to infer a balanced minimum evolution tree with branch support via FASTME 2.1.6.1 including SPR post-processing (Lefort et al. 2015).

**Chemotaxonomic characterization**

Extraction and analysis of isoprenoid quinones were performed as described by Komagata and Suzuki (1987) and Park et al. (2014), respectively. Fatty acid analysis was performed as described by Park et al. (2014) using the standard MIDI protocol (Sherlock Microbial Identification System, version 6.2B), GC (Hewlett Packard 6890), and TSBA6 database of the Microbial Identification System (Sasser 1990). Extraction of polar lipids were carried out according to procedures described by Minnikin et al. (1984). They were separated by two-dimensional TLC using the solvent systems as described by Embley and Wait (1994). The TLC plates were sprayed with various reagents as described by Park et al. (2014) and individual polar lipids were visualized followed by identified with heating at 150 °C for 3 min.

**Morphological, cultural, physiological and biochemical characterization**

Cell morphology, Gram reaction, anaerobic growth, pH range for growth, growth at various concentrations of NaCl, hydrolysis of gelatin and urea and susceptibility to antibiotics were investigated as described by Park et al. (2014). For transmission electron microscopy (JEM1010; JEOL), cells were negatively stained with 1% (w/v) phosphotungstic acid and air-dried. Grids were then examined. Growths at 4, 10, 20, 25, 28, 30, 35, 37, and 40 °C on MA were measured to estimate the optimal temperature and temperature range for its growth. Nitrate reduction and hydrolysis of aesculin or Tween 80 were investigated as described previously (Lányi 1987) using artificial seawater (Bruns et al. 2001) for the preparation of the media. Hydrolysis of other substrates was tested as described by Barrow and Feltham (1993) with the modification that MA was used. Activities of catalase and oxidase were determined as described by Lányi (1987). Utilization of various substrates (each 0.2%) for growth was investigated as described by Kämpfer et al. (1991). Other biochemical and physiological properties were determined using API ZYM and API 20NE systems (bioMérieux;
France). Enzyme activities by the API ZYM system were determined after incubation at 30 °C for 8 h. Other physiological and biochemical properties by the API 20NE system were determined after incubation at 30 °C for 2 days.

Results and discussion

Phylogenetic analysis based on 16S rRNA gene sequence

The almost complete 16S rRNA gene sequence of strain BSSL-BM10T had a continuous stretch of 1421 nucleotides, corresponding to positions 28–1491 (95%) of the Escherichia coli 16S rRNA sequence. Phylogenetic trees using three different algorithms (neighbor joining, maximum-likelihood and maximum-parsimony) showed that strain BSSL-BM10T formed an independent lineage within the clade comprising type strains of Devosia species (Fig. 1; Figs. S1 and S2). Strain BSSL-BM10T shared the highest 16S rRNA gene sequence similarities (98.2%) with D. naphthalenivorans CM5-1T. It shared 93.5–97.7% 16S rRNA gene sequence similarities with type strains of other Devosia species. These sequence similarities indicated that strain BSSL-BM10T might be a species different from recognized Devosia species according to the threshold value (98.7%) recommended for delineation of a bacterial species by Kim et al. (2014).

Genomic features

The genome size of strain BSSL-BM10T obtained from the assembly of sequencing reads was 3,743,297 bp with a sequencing depth of coverage of 414.33X. The genomic sequence of strain BSSL-BM10T contained five contigs with N50 length of 2,671,820 bp. The complete 16S rRNA gene sequence from the genomic data of strain BSSL-BM10T was extracted using ContEst16S (Lee et al. 2017). It was found to be identical to respective 16S rRNA gene information previously obtained by Sanger sequencing. This indicated that strain BSSL-BM10T and its genomic data were not mislabeled. They did not originate from any source of contamination (Chun et al. 2018). Based on its genomic sequence data, the DNA G+C content of strain BSSL-BM10T was 60.9%, a value in the range reported for Devosia species (Jia et al. 2014; Quan et al. 2020). The phylogenetic tree based on genomic sequences showed that strain BSSL-BM10T formed a lineage within the clade comprising type strains of Devosia species (Fig. S3). The genomic sequence of strain BSSL-BM10T had an ANI value of 77.2% with that of D. naphthalenivorans CM5-1 and 71.0–78.4% with those of type strains of the other Devosia species as indicated in Table S1. Strain BSSL-BM10T had dDDH values of 21.0% with D. naphthalenivorans CM5-1T and 18.8–21.5% with type strains of the other 17 Devosia species (Table S1). These ANI values (71.0–78.4%) and dDDH values (18.8–21.5%) of genomic sequences between strain BSSL-BM10T and type strains of Devosia species were lower than ANI and dDDH values (95–96% and 70%, respectively) recommended for delineation of a bacterial species (Goris et al. 2007; Konstantinidis and Tiedje 2005; Richter and Rosselló-Móra 2009).

Chemotaxonomic characteristics

The predominant isoprenoid quinone detected in strain BSSL-BM10T was ubiquinone-10 (Q-10), consistent with results for members of Devosia species (Nakagawa et al. 1996; Quan et al. 2020). The major fatty acids (>10% of total fatty acids in all growth phases) found in strain BSSL-BM10T were 11-methyl C18:1ω7c, C18:1ω7c, C18:1ω6c, summed feature 3 (C16:1ω7c and/or C16:1ω6c), and C16:0 (Table S2). Fatty acid profiles of strain BSSL-BM10T were similar to those of type strains of D. naphthalenivorans and D. riboflava, with 11-methyl C18:1ω7c, C18:1ω7c, and C16:0 being the major fatty acids. Nevertheless, there were differences in proportions of some fatty acids, including summed feature 3 (C16:1ω7c and/or C16:1ω6c) and cyclo C19:0ω8c, between strain BSSL-BM10T and two reference strains (Table S2). Major polar lipids detected in strain BSSL-BM10T were phosphatidylglycerol and two unidentified glycolipids. Minor amounts of diphosphatidylglycerol, two unidentified...
lipids, another unidentified glycolipid, and one unidentified aminolipid were also present (Fig. S4). The polar lipid profile of strain BSSL-BM10T was similar to those of type strains of *D. naphthalenivorans* and *D. riboflava* in that phosphatidylglycerol and two unidentified glycolipids were major components (Fig. S4; Park et al. 2016).

**Morphological, cultural, physiological and biochemical characteristics**

Strain BSSL-BM10T showed a Gram-negative, non-spore-forming, and non-flagellated properties and its cellular morphology was rod-shaped. Phenotypic characteristics of strain BSSL-BM10T are given in the species description, Table S3 and Fig. S5. Strain BSSL-BM10T grew well on TSA and MA. Although the type strain of *D. naphthalenivorans* also grew well on MA, it grew poorly on TSA. Strain BSSL-BM10T was resistant to gentamicin, whereas type strains of *D. naphthalenivorans* and *D. riboflava* were susceptible to gentamicin (Table 1). Strain BSSL-BM10T produced trypsin, α-glucosidase, and α-fucosidase, but the type strains of *D. naphthalenivorans* and *D. riboflava* did not produce these three enzymes (Table 1).

**Conclusion**

Combined results obtained from phylogenetic, genomic, and chemotaxonomic analyses made it reasonable to assign strain BSSL-BM10T as a member of the genus *Devosia* (Fig. 1; Figs. S1, S2 and S3; Table S2). Strain BSSL-BM10T was distinguished from type strains of *D. naphthalenivorans* and *D. riboflava* by differences in several phenotypic characteristics, including nitrate reduction, acid production from D-glucose, utilization of some substrates, susceptibility to some antibiotics, and activities of some enzymes (Table 1).

Based on polyphasic taxonomic data presented, strain BSSL-BM10T is considered to represent a novel *Devosia* species, for which we propose the name *Devosia litorisediminis* sp. nov.

**Description of *Devosia litorisediminis* sp. nov.**

*Devosia litorisediminis* (li.to.ri.se.di’mi.nis, L. neut. n. *litus*, the seashore, coast; L. neut. n. *sedimen*, sediment; N.L. gen. n. *litorisediminis*, of a coastal sediment, tidal flat sediment).

Cells are rod-shaped measuring approximately 0.3–0.8 μm in diameter and 0.8–4.0 μm in length. Gram-staining reaction is negative. Spores are not formed. No flagellum is found. Colonies on TSA are circular, convex, smooth, glistening, grayish yellow in color, and 0.5–1.0 mm in diameter after incubation at 30 °C for 5 days. Grows optimally at 30 °C and pH 7.0–8.0. Growth occurs at 4 °C to 37 °C, but not at 40 °C and occurs at pH 5.0, but not at pH 4.5. Growth occurs in the presence of 0.5–5.0% (w/v) NaCl with an optimum of approximately 1.0–2.0% (w/v) NaCl. Anaerobic growth does not occur on TSA or TSA supplemented with nitrate. Catalase- and oxidase-positive. Nitrate is not reduced to nitrite. Aesculin, hypoxanthine, urea, and xanthine are hydrolyzed, while casein, gelatin, starch, Tween 80, and L-tyrosine are not. L-Arabinose,
D-galactose, D-glucose, maltose, D-cellubiose, D-fructose, D-mannose, sucrose, D-trehalose, D-xylene, acetate, citrate, succinate, L-malate, pyruvate, and salicin are utilized as carbon and energy sources, but benzoate, formate, and L-glutamate are not. Susceptible to ampicillin (10 μg), carbenicillin (100 μg), chloramphenicol (100 μg), neomycin (30 μg), novobiocin (5 μg), oleandomycin (15 μg), penicillin G (20 IU), and tetracycline (30 μg), but resistant to cephalothin (30 μg), gentamicin (30 μg), kanamycin (30 μg), lincomycin (15 μg), polymyxin B (100 IU), and streptomycin (50 μg). In assays with API 20NE system, it is positive for activity of urease and hydrolysis of 4-nitrophenyl-β-D-galactopyranoside. In assays with the API ZYM system, activities of esterase (C4), esterase lipase (C8), leucine and valine arylamidases, trypsin, alkaline and acid phosphatases, naphthol-AS-BI-phosphohydrolase, β-galactosidase, α-glucosidase, β-glucosidase, N-acetyl-β-glucosaminidase, and α-fucosidase are present. The predominant ubiquinone is Q-10. The major fatty acids (> 10% of total fatty acids) are 11-methyl C₁₈:₁ ω₇c, C₁₈:₁ ω₇c, summed feature 3 (C₁₆:₁ ω₇c and/or C₁₆:₁ ω₆c), and C₁₆:₀. The major polar lipids are phosphatidylglycerol and two unidentified glycolipids. The DNA G+C content of the type strain is 60.9% (from genome sequence data).

The type strain, BSSL-BM10T (=KACC 21633T =NBRC 115152T), was isolated from a sand dune at Boryeong on the Yellow Sea, Republic of Korea.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence and GenBank accession number for the whole genome shotgun sequence of strain BSSL-BM10T are MN872411 and JAGXTP0000000000, respectively.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00203-022-03181-5.

Acknowledgements This work was supported by the project on survey of indigenous species of Korea of the National Institute of Biological Resources (NIBR) under the Ministry of Environment (MOE) and “Cooperative Research Program for Agriculture Science and Technology Development (Project No. PJ014442)” of Rural Development Administration, Republic of Korea.

Funding National Institute of Biological Resources, project on survey of indigenous species of Korea, Jung-Hoon Yoon, Rural Development Administration, PJ014442, Jung-Hoon Yoon.

Declarations

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 performed by any of the authors.

References

Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kuliko AS, Levin VM, Nikolenko SI, Phan S, Prjibelski AD, Pyshkin AV, Sirotkin AV, Vyahhi N, Tesler G, Alekseyev MA, Pevzner PA (2012) SPAdes: A new genome assembly algorithm and its applications to single-cell sequencing. J Comput Biol 19:455–477. https://doi.org/10.1089/cmb.2012.0021

Barrow GI, Feltham RKA (1993) Cowan and Steel’s Manual for the Identification of Medical Bacteria In: G. I. Barrow, R. K. A. Feltham Cambridge University Press, Cambridge. https://doi.org/10.1017/CBO9780511527104

Bautista VV, Monsalud RG, Yokota A (2010) Devosia yakushimensis sp. nov., isolated from root nodules of Pueraria lobata (Willd.) Ohwi. Int J Syst Evol Microbiol 60:627–632. https://doi.org/10.1099/ijs.0.011254-0

Bruns T, Rohde M, Berthe-Corti L (2001) Muricauda ruestrinensis gen. nov., sp. nov., a facultatively anaerobic, appendaged bacterium from German North Sea intertidal sediment. Int J Syst Evol Microbiol 51:1997–2006. https://doi.org/10.1099/00207135-51-6-1997

Chun J, Oren A, Ventosa A, Christensen H, Arahal DR, da Costa MS, Rooney AP, Yi H, Xu XW, De Meyer SD, Trujillo ME (2018) Proposed minimal standards for the use of genome data for the taxonomy of prokaryotes. Int J Syst Evol Microbiol 68:461–466. https://doi.org/10.1099/ijsem.0.02516

Embley TM, Wait R (1994) Structural lipids of eubacteria. In: Goodfellow M, O’Donnell AG (eds) Modern Microbial Methods. Chemical Methods in Prokaryotic Systematics. John Wiley & Sons, Chichester, pp 121–161

Galatis H, Martin K, Kämpfer P, Glaeser SP (2013) Devosia epidermidisirudinis sp. nov. isolated from the surface of a medical leech. Antonie Van Leeuwenhoek 103:1165–1171. https://doi.org/10.1007/s10482-013-9895-3

Goris J, Konstantinidis KT, Klappenbach JA, Coenye T, Vandamme P, Tiedje JM (2007) DNA–DNA hybridization values and their relationship to whole-genome sequence similarities. Int J Syst Evol Microbiol 57:81–91. https://doi.org/10.1099/ijs.0.002516

Hördt A, López MG, Meier-Kolthoff JP, Schleuning M, Weinhold LM, Tindall BJ, Gronow S, Kyrpides NC, Woyke T, Göker M (2020) Analysis of 1000+ type-strain genomes substantially improves taxonomic classification of Alphaproteobacteria. Front Microbiol 11:468. https://doi.org/10.3389/fmicb.2020.00468

Jia YY, Sun C, Pan J, Zhang WY, Zhang XQ, Huo YY, Zhu XF, Wu M (2014) Devosia pacifica sp. nov., isolated from deep-sea sediment. Int J Syst Evol Microbiol 64:2637–2641. https://doi.org/10.1099/ijs.0.059626-0

Kämpfer P, Steif M, Dott W (1991) Microbiological characterization of a fuel-oil contaminated site including numerical identification of heterotrophic water and soil bacteria. Microb Ecol 21:227–251. https://doi.org/10.1007/BF02539156

Kim M, Oh HS, Park SC, 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. https://doi.org/10.1099/ijs.0.059774-0

Komagata K, Suzuki K (1987) Lipid and cell-wall analysis in bacterial taxonomy of prokaryotes. Int J Syst Evol Microbiol 68:461–466. https://doi.org/10.1099/ijs.0.064483-0

Kumar M, Verma M, Lal R (2008) Devosia chinhatensis sp. nov., isolated from a hexachlorocyclohexane (HCH) dump site in India.

Springer
