***Devosia nitraria s*p. nov.，a novel species isolated from the roots of**

 ***Nitraria sibirica* in *China***

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**Abstract**

An aerobic, Gram-stain negative, short rod-shaped and motile strain, 36-5-1T, was isolated from the roots of *Nitraria sibirica* in Zhangye city, Gansu province, north-west of China. Phylogenetic analysis based on the 16S rRNA gene sequence and two housekeeping genes (*glnA* and *atpD*) indicated that the strain represents a novel species closely related to the *Devosia*, *Rhizobium* and *Devosia* genera with 98.3%, 96.2% and 91.1% similarities, respectively. The strain 36-5-1T contained Q-10 as the predominant ubiquinone and 16:0 (36.8%) as the major fatty acid; a large amount of unidentified glycolipid, diphosphatidylglycerol, phosphatidylglycerol and a small amount of unidentified polar lipids were present as polar lipids. In addition, the G*+*C content of the genomic DNA was 61.7 mol% and the DNA-DNA hybridization with type strains *D. geojensis* BD-c194T and *D. pacifica* NH131T 44.1±1.1 and 40.2±1.7, respectively. Based on chemotaxonomic data and molecular properties, strain 36-5-1T represents a novel species within the genus *Devosia*, for which the name *Devosia nitraria* sp. nov. is proposed. The type strain is 36-5-1T (= CGMCC1.15704T = NBRC112416T ).

**Keywords**: *Devosia*, novel species, *Nitraria sibirica*

**Introduction**

The genus *Devosia* was first described by Nakagawa et al. (1996) with the reclassification of ‘*Pseudomonas riboflavina*’ (Foster 1944) as *Devosia riboflavina*. Members of this genus are characterized as Gram stain negative, aerobic, oval or rod-shaped and non-spore forming bacteria. The genus is currently comprised of eighteen named species (<http://www.bacterio.net/devosia.html>).

*Nitraria* is a genus belonging to the family *Zygophyllaceae*. The genus consists of 15 species of deciduous shrubs. These shrubs are widely distributed in the Middle East, central Asia and in north-west region of China and they have special physiological properties in terms of drought and salt resistance. These properties have been shown to prevent soil desertification, by alleviating both the soil salinity and alkalinity ([Zhao et al. 2002](http://www.sciencedirect.com/science/article/pii/S037887411100585X#bib0170)), and consequently, the plants have significant ecological value. Five of the species are indigenous to northwestern China. Of the five *Nitraria sibirica* Pall. is the most common. The fruits of *Nitraria sibirica* Pall. are used in some traditional medicines and are particularly recommended for the treatment of hypertension ([Liu 1999](http://www.sciencedirect.com/science/article/pii/S037887411100585X#bib0095)). Several studies on *Nitraria* metabolites have shown obvious antihypertensive properties due to the regulation of digestion and spleen functioning ([Tulyaganov and Allaberdiev 2001](http://www.sciencedirect.com/science/article/pii/S037887411100585X#bib0145); [Tulyaganov and Allaberdiev 2003](http://www.sciencedirect.com/science/article/pii/S037887411100585X#bib0150); [Hadj Salem et al. 2011](http://www.sciencedirect.com/science/article/pii/S037887411100585X#bib0050); [Suo and Wang 2010](http://www.sciencedirect.com/science/article/pii/S037887411100585X#bib0130)). As yet, relatively little is known about the bacterial endophytes of *Nitraria*. Here we describe a novel bacterial endophyte of *Nitraria*, designated as 36-5-1T. A polyphasic approach was carried out to classify the bacterium, and according to our current analysis, the strain 36-5-1T likely belongs to the genus *Devosia*.

**Materials and methods**

**Strains and culture conditions**

A root sample was collected from *Devosia nitraria* grown in the sand soil of Hexi Corridor to isolate bacterial strains (GPS location: 39°16´N 100°18´E, 1398m). Endophytes were routinely cultured on either YMA (Vincent 1970) or LB (Bertani 1951) medium at 28°C and deposited as described by Xu (Xu et al. 2016). After primary analysis and sequencing of 16S rRNA, R2A agar (Reasoner & Geldreich, 1985) was used to culture the strain.

**Determination of 16S rRNA, recA and atpD gene sequences**

The extraction of genomic DNA and PCR amplification of the 16S rRNA gene were conducted as described by Terefework et al. (2001). Amplification of the 16S rRNA gene was performed with two universal primers, F’-27(5’-AGAGTTTGATCCTGGCTC

AGAACGAACGCT-3’) and R’-1492 (5’-TACGGCTACCTTGTTACGACTTCACCCC

-3’). Amplification of *glnA* and *atpD* genes were performed as described by Gaunt et al. (2001) and Turner & Young (2000), respectively. The gene sequences were compared with a BLAST search of the nucleotide database of the National Center for Biotechnology Information (Altschul et al. 1990) and EzTaxon server 2.1 (<http://www.ezbiocloud.net/>; Yoon et al., 2017). Sequences were aligned using the CLUSTAL W software (Thompson et al*.* 1994). Phylogenetic trees were then performed using neighbour-joining methods, maximum likelihood or maximum-parsimony with Kimura’s two-parameter substitution model (Saitou and Nei 1987). The robustness of the topology of the phylogenetic trees was evaluated by bootstrap analyses based on 1000 resamplings (Galtier et al. 1996; Felsenstein 1985).

**DNA base composition and DNA–DNA hybridization**

The G+C content of DNA was measured using thethermal denaturation method described by Marmur & Doty (1962) by using*E. coli* K-12 as the standard. DNA-DNA relatedness (hybridization) was determined by usingthe spectrophotometric method reported by De Ley *et al*. (1970)

**Amplification of symbiotic genes and *in vivo* symbiosis measured in cross-**

**nodulation tests**

Symbiotic genes, *nodA* and *nifH*, were amplified by using the primers *nifH*F (5’-TACGGNAARGGSGGNATCGGCAA-3’) /*nifH*I (5’-AGCATGTCYTCSAGY

TCNTCCA-3’) and *nodA*F(5’-TGCRGTGGARDCTRYGCTGGGAAA-3’) /*nodA*R(5’-GGNCCGTCRTCRAASGTCARGTA-3’) with PCR conditions previously described by Elliott et al. (2007), Laguerre et al. (2001) and Xu et al. (2013). Formation of symbiosis was also measured *in vivo* in greenhouse pot experiments by using eight different plant species including the original host plant (Graham et al. 1991): *Sophora alopecuroides*, *Medicago sativa*, *Phaseolus vulgaris*, *Pisum sativum*, *Vigna unguiculata*, *Trifolium repens*, *Glycine max*, *Galega oficinalis* and *Nitraria sibirica*. Briefly, plant seedlings were grown in pots filled with vermiculite moistened with N-free plant nutrient solution (Vincent 1970), inoculated with strain 36-5-1T and formation of nodules determined visually.

**Morphological tests and physiological characterization**

The bacterial strain was characterized on the basis of cell morphology including colony color, shape, size, and growth on certain media. To study the cell motility and shape, single colonies isolated from agar plates were prepared by using a JEM-1400, JEDL scanning electron microscope (SEM) and SU8010, Hitachi transmission electron microscope (TEM).

The ability to use common nitrogen and carbon sources, and resistance to common antibiotics, were tested as described by Gao et al. (1994). Additional enzyme activities and biochemical features were determined by using API ZYM, API 20NE kits and API 50 CH at 25°C as recommended by the manufacturer (bioMérieux). The temperature range and salinity tolerance was investigated by incubating the bacterium on R2A agar at different temperatures (4, 10, 20, 25, 28, 30, 37 and 40 °C) and in medium containing 1 %, 2 %, 3 %, 4 %, 5 %, 6 %, 7 % , 8 % (w/v) of NaCl. The pH range for strain 36-5-1T was measured in R2A broth adjusted with HCl or NaOH to different pH values across the range from 4.0 to 13.0 at intervals of 1.0 pH unit. Dye and chemical resistance were investigated by using methyl orange, methyl red, methylene blue, neutral red, congo red, malachite green, bromothymol blue and sodium deoxycholate at concentrations of 1% and 2% (w/v) in R2A medium.

**Predominant** **ubiquinone, fatty acid profile and polar lipids**

Ubiquinone is an essential component of electron transfer system in the plasma membrane of prokaryotes, while fatty acid profiling is widely used in the description of rhizobial species (Tighe et al. 2000). To measure these properties, strain 36-5-1T was grown on YMA medium with shaking at 170 rpm for 2 days at 25 °C. Cellular fatty acids were extracted and methylated according to the standard protocol described by Sasser (1990), analyzed by Gas Chromatography (GC) (model 6890; Agilent) and identified by using the TSBA6 database of the Microbial Identification System. Ubiquinone was analyzed by using reversed-phase High Performance Liquid Chromatography (HPLC) and a Diamonsil C18 chromatographic column (200 mm × 4.6 mm, i. d. 5 μm) with 300 ml methanol and 700 ml anhydrous ethanol as the mobile phase. Cellular polar lipids were extracted by using a chloroform-methanol system and separated by two-dimensional TLC using silica gel 60 F 254 aluminium-backed thin-layer plates (Merck) (Kates, 1986). Following ratios were used in the solvent system 65 : 24 : 4 by volume of chloroform/methanol/water and 80 : 12 : 15 : 4 by volume of chloroform/glacial acetic acid/methanol/water in the first and second dimensions, respectively. Separated components were visualized by treating the plates with a 50% (w/v) sulfuric acid ethanol solution followed by heating at 120 °C for 10 min. Zinzadze reagent was used to detect phospholipids.

**Sequence deposition**

The GenBank accession numbers for the 16S rRNA, *glnA* and *atpD* gene sequences of strains 36-5-1T are KU358684, KY523102 and KX095238, respectively.

**Results and discussion**

**Phylogenetic analysis based on 16S rRNA, *recA* and *atpD* sequence comparisons**

Based on the 16S rRNA gene sequence analysis, strain 36-5-1T was phylogenetically highly related to members of the genus *Devosia* and showed closest sequence similarity to *Devosia pacifica*NH131T (98.3%) and *D. geojensis* BD-c194T (98.6%) (Fig.1 and Fig S1)*.* The phylogenetic trees based on 16S RNA genes indicated that the strain clustered with species of the genus *Devosia*. In addition, the phylogenetic tree based on *glnA* and *atpD* gene sequences indicated that strain 36-5-1T fell into a coherent lineage with species of the genus *Rhizobium* and *Devosia*. The partial *glnA* and *atpD* gene sequences were 96.2% and 91.1% similar to type strains of *Rhizobium vitis*and *Devosia soli*, respectively (Fig. 2). Considering the relatively low sequence similarities of these two genes as a cut-off for genera delineation, this data suggests that strain 36-5-1T belongs to a novel species of the genus *Devosia*.

**DNA-DNA relatedness (hybridization)**

The G+C contentfor the representative strain 36-5-1T was 61.7mol % (Table 3). Two type strains of closely related species, *D. pacifica*NH131T and *D. geojensis* BD-c194T, were used as the reference strains for phenotypic and the hybridization studies. The DNA-DNA relatedness of the strain 36-5-1T with these two closely related species was 44.1% and 40.2%, respectively (Table 3).

**Amplification of symbiotic gene sequences and *in vivo* symbiosis measured in cross-nodulation tests**

Some species of thegenus *Devosia* have the ability to form a symbiosis with plants in order to fix nitrogen (Bautista et al. 2010, Rivas et al. 2003). The symbiosis encoding genes are adaptive, and in many cases, have an evolutionary history independent of the rest of the genome. We were not able to obtain PCR products for either *nifH* or *nodA* genes by using the corresponding primers and PCR conditions described previously. Similarly, strain 36-5-1T could not form nodules on the root of any of the tested plants including its original host plant. This suggests that 36-5-1T is not able to nodulate or fix nitrogen for the tested plants. However, considering there are more than 19000 legume species, strain 36-5-1T may not necessarily have the same symbiotic test results with other legumes.

**Morphological tests and physiological characterization**

Distinctive phenotypic characteristics for the strains of the novel species and the type strains of the phylogenetically closest species are shown in Table 1. An electron micrograph is shown in Fig. 4.

**Ubiquinone, fatty acid analysis and polar lipids**

The predominant ubiquinone of strain 36-5-1T is Q-10. The major fatty acid is C16:0 (36.8%) with minor amounts of C16:0 N alcohol (14.6%), 10-methyl C17:0 (12.6%), C18:1*ω*7*c* (9.4%), C18:0 (7.3%), C19:0 cyclo ω8*c* (5.5%), 11-Methyl C18:1 ω7c (4.7%) and C18 : 0 3-OH (2.5%). The cellular fatty acid profile was similar to the reference strains *D. geojensis* BD-c194 T (Ryu et al., 2008) and *D. pacifica*NH131T (Jia et al., 2014) that have C16:0 and C18:1*ω*7*c* as their main lipids, respectively.A large amount of unidentified glycolipid, diphosphatidylglycerol, phosphatidylglycerol and a small amount of unidentified polar lipids were present as polar lipids for strain 36-5-1T.

**Description of *Devosia nitraria* sp. nov.**

*Devosia nitraria* (Ni.tra'ri. N.L. gen. n. *nitraria*, referring to the host plant *Devosia nitraria*) is a Gram-negative, aerobic, motile (flagella), non-spore forming rod, which is 0.3-0.5 μm wide and 1.0-1.5 μm long. Colonies on R2A medium are circular, convex, white and semitranslucent, with a typical diameter of 3-5 mm after 3 days of growth at 25 °C. Cells are positive for utilization of inositol, L-arabinose, D-mannitol, D-sorbitol, gentiobiose, glucose, sodium citrate, sodium malonate, pyruvic acid sodium, sucrose, D-xylose, D-ribose, D-fructose, D-mannose, D-maltose, sodium acetate, esculine, salicine, amygdaline, D-melibiose, D-saccharose, D-trehalose, D-raffinose, and D-tyranose as sole carbon sources but negative for D-galactose, L-rhamnose, erythritol, D-arabinose, D-adonitol, L-xylose, L-sorbose, inuline,  Dulcitol, xylitol, L-fucose, D-fucose, D-arabitol, D-lactose and mannopyranose as the sole carbon sources. Cells are positive for utilization of L-homocysteine, L-alanine, DL-histidine, L-tyrosine, L-leucine, L-threonine, methionine, L-phenylalanine, L-arginine, L-glutamic, aspartic acid and xanthine but negative for tryptophan as a sole nitrogen source. Optimum growth occurs at 30°C and the strain’s growth is inhibited at temperature extremes of 4°C and 40 °C in R2A medium, as well as at 37°C and 40°C when grown in LB and YMA media, respectively. Optimum pH for growth is at 7.0 while some growth was observed up to pH levels of 11.0. Cells grow on YMA in the presence of 7% (w/v) NaCl but do not grow in media supplemented with 0.1% malachite green, 0.1% methylene blue and 0.1% neutral red. Cells are sensitive to 10 μg ml-l tetracycline, gentamicin, chloramphenicol, ampicillin, streptomycin and kanamycin, but resistant to polymyxin and neomycin. Cells are negative for casein hydrolysis, litmus milk alkali production, nitrate reduction, Voges-Proskauer, D-melezitose, potassium gluconate, potassium 2-ketogluconate and potassium 5-ketogluconate, but positive for D-cellobiose, urease, cytochrome oxidase, catalase oxidase and hydrolysis of gelatin. Cells can assimilate N-acetyl glucosamine but not phenylacetic acid. The predominant ubiquinone is Q-10. The main cellular fatty acid is C16:0.

The type strain, 36-5-1T (= CGMCC1.15704T = NBRC112416T), was isolated from the nodules of *Devosia nitraria* grown in Hexi corridor in Zhangye city, Gansu province of China.

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**The authors declare no conflict of interest.**

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**Table 1.** Distinctive features of *D. nitraria* 36-5-1T and the closest related species, *D. pacifica* and *D. geojensis*. Taxa: 1, *D. pacifica*NH131T; 2, *D. geojensis* BD-c194 T；3. *D. nitraria* 36-5-1T. +, positive; -, negative.

|  |  |  |  |
| --- | --- | --- | --- |
| **Characteristic** | **1** | **2** | **3** |
| **Nitrate reductase:** | **+** | **-** | **+** |
| **Hydrolysis of:** |
| Casein | **+** | **+** | **-** |
| Aesculin | **+** | **+** | **+** |
| Salicine | **+** | **+** | **+** |
| Urea | **+** | **+** | **+** |
| Casei | **-** | **-** | **-** |
| Tween 80 | **-** | **-** | **-** |
| Gelatin | **+** | **-** | **+** |
| Starch | **-** | **-** | **-** |
| L-Tyrosine | **-** | **+** | **+** |
| Inuline | **+** |  | **-** |
| N-acetyl glucosamine | **+** | **+** | **+** |
| Phenylacetic acid | **-** | **-** | **-** |
| **Assimilation of:** |
| Fructose | **+** | **+** | **+** |
| Gentiobiose | **+** | **+** | **+** |
| Sucrose | **-** | **+** | **+** |
| Asparagine  | **-** | **+** | **+** |
| Arginine  | **+** | **-** | **+** |
| Erythritol | **-** | **-** | **-** |
| Sodium glutamate | **-** | **+** | **+** |
| Sodium malate  | **-** | **+** | **+** |
| Sodium citrate | **-** | **-** | **+** |
| Pyruvic acid sodium | **+** | **+** | **+** |
| Rhamnose  | **-** | **+** | **-** |
| Ribose | **-** | **+** | **+** |
| D-Arabinose  | **+** | **-** | **-** |
| L-Arabinose | **+** | **+** | **+** |
| Salicin | **-** | **+** | **+** |
| Raffinose | **-** | **+** | **+** |
| D-trehalose | **+** | **+** | **+** |
| Succinic acid | **+** | **+** | **+** |
| D-galactose | **-** | **-** | **-** |
| D- adonitol | **+** | **-** | **-** |
| Xylitol | **-** | **-** | **-** |
| Mannopyranose | **-** | **-** | **-** |
| L-homocysteine | **+** | **+** | **+** |
| L-alanine | **+** | **+** | **+** |
| DL-histidine | **+** | **+** | **+** |
| L-glutamic | **+** | **+** | **+** |
| Methionine | **+** | **+** | **+** |
| L-phenylalanine | **+** | **+** | **+** |
| L-threonine | **+** | **+** | **+** |
| L-leucine | **-** | **+** | **+** |
| Xanthine | **+** | **+** | **+** |
| Tryptophan | **-** | **-** | **-** |
| D-melezitose | **+** | **-** | **-** |
| Potassium gluconate | **-** | **-** | **-** |
| Potassium 2-ketogluconate  | **-** | **-** | **-** |
| Potassium 5-ketogluconate | **-** | **-** | **-** |
| **Acid production from:** |
| D-Xylose | **+** | **+** | **+** |
| D-cellobiose | **+** | **+** | **+** |
| L-Xylose | **+** | **-** | **-** |
| D-Glucose  | **+** | **+** | **+** |
| L-fucose | **+** | **-** | **-** |
| D-fucose | **-** | **-** | **-** |
| D-sorbitol  | **+** | **+** | **+** |
| Sorbose  | **+** | **-** | **+** |
| Inositol  | **-** | **+** | **+** |
| Mannitol | **-** | **+** | **+** |
| Methyl a-D-mannose  | **+** | **+** | **+** |
| N-acetylglucosamine  | **+** | **+** | **+** |
| Amygdalin  | **+** | **-** | **-** |
| Maltose  | **+** | **+** | **+** |
| Lactose | **-** | **+** | **-** |
| D-Tagatose  | + | **-** | **+** |
| Sucrose | **+** | **+** | **+** |
| D-Melibiose, | **-** | **+** | **+** |
| **Resistance to antibiotics:** |
| Polymyxin  | **-** | **+** | **+** |
| Streptomycin  | **-** | **+** | **-** |
| Gentamicin  | **-** | **+** | **-** |
| Neomycin  | **+** | **+** | **+** |
| Tetracycline | **+** | **-** | **-** |
| Chloramphenicol | **-** | **+** | **-** |
| Ampicillin | **+** | **-** | **-** |
| Kanamycin | **-** | **+** | **-** |
| **Production of:** |  |  |  |
| Oxidase | **+** | **+** | **+** |
| Cytochrome oxidase | **+** | **+** | **+** |
| Catalase | **+** | **+** | **+** |

**Table 2.** Cellular fatty acids of 36-5-1T.

|  |  |
| --- | --- |
| **Fatty acid** | ***D. nitraria* 36-5-1T** |
| C14: 0 | 1.6 |
| C15: 0 | - |
| C16: 0 | 36.8 |
| C17:0 | 0.6 |
| C18 : 0 | 7.3 |
| C19 : 0 | - |
| C16:0 N alcohol | 14.6 |
| C17: 0 cyclo | 0.7 |
| C16:1*ω*7*c*/16:1*ω*6*c* | 1.9 |
| C17:1*ω*8*c* | - |
| C17:1*ω*6*c* | - |
| C18:1*ω*5*c* | - |
| C18:1*ω*7*c* | 9.4 |
| C18:1*ω*9*c* | - |
| C20:1*ω*7*c* | - |
| C18:3*ω*6c (6,9,12) | 1.3 |
| C8:0 3-OH | - |
| C18 : 0 3-OH | 2.5 |
| 10-methyl C17:0  | 12.6 |
| 11-Methyl C18:1 ω7c | 4.7 |
| C19:0 cyclo ω8*c* | 5.5 |
| ECL18.814 | - |

**Table 3.** Isolates and reference strains in the genus *Devosia* used in this study and DNA–DNA relatedness among them.

|  |  |  |
| --- | --- | --- |
| Strain | G+C mol% | DNA relatedness with 36-5-1T (%) |
| *D. nitraria* 36-5-1T | 61.7 | 100% |
| Reference strains*D. geojensis* BD-c194 T*D. pacifica*NH131T | 60.863.0 | 44.1±1.140.2±1.7 |

**Fig. 1** Comparison of 16S rRNA gene sequences. Phylogenetic tree constructed by the NJ method showing the relationships between the novel species and reference strains. Bootstrap percentages above 50% are indicated. Bar denotes for 0.1 substitutions per nucleotide position.

**Fig. 2** **(a)** Comparison of partial *gln*A sequences. Phylogenetic tree was constructed by the neighbor-joining method from Jukes-Cantor distance matrices of the sequences. Bootstrap percentages above 50% are indicated. Bar denotes for 0.1 substitutions per nucleotide position.

**(b)** Comparison of partial *atp*Dsequences. The phylogenetic tree was constructed by the neighbor-joining method from Jukes-Cantor distance matrices of the sequences. Bootstrap percentages above 50% are indicated. Bar denotes for 0.1 substitutions per nucleotide position.

**Fig. 3** Polar lipid profile of strain 36-5-1T after two-dimensional TLC and staining with molybdatophosphoric acid. DPG, diphosphatidylglycerol; PG, phosphatidylglycerol; GL, unidentified glycolipid L; 1-3, unidentified polar lipids.

**Fig. 4 (a)** Scanning electron micrograph of strain 36-5-1T grown on YMA media for 48h. Barindicates 1.0 μm.

**(b)** Transmission electron micrograph of strain 36-5-1T grown on YMA media for 48h.

Bar indicates 1.0 μm.

**Fig. S1** (a) Maximum Likelihood tree reconstructed from 16S rRNA gene sequences of 36-5-1T and related type strains. Bootstrap values (based on 1000 replicates) above 50 % are indicated at the nodes. Bar denotes for 0.1 substitutions per nucleotide position.

 **(b)** Maximum Parsimony tree reconstructed from 16S rRNA gene sequences of 36-5-1T and related type strains. Bootstrap values (based on 1000 replicates) above 50 % are indicated at the nodes.