Nocardioides litorisoli sp. nov., isolated from lakeside soil

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Abstract

A Gram-stain-positive, rod-shaped, non-spore-forming bacterium, designated as x-2T, was isolated from lakeside soil of Sayram in the Xinjiang Uygur Autonomous Region, PR China. On the basis of 16S rRNA gene sequences, strain x-2T belongs to the genus Nocardioides in the family Nocardioidaceae, being most closely related to Nocardioides panacisoli Gsoil 346T (97.36 % 16S rRNA gene sequence similarity). Strain x-2T was characterized chemotaxonomically and found to have L,L-diaminopimelic acid in the cell-wall peptidoglycan, phosphatidylglycerol, diphosphatidylglycerol, glycolipids and another three unknown phospholipids as the major polar lipids, MK-8(H4) as the predominant menaquinone and C18:ω9c, iso-C15:0, C17:1ω8c and C16:0 as the major fatty acids. The genomic DNA G+C content of the novel strain was 71.1 mol%. The level of DNA–DNA relatedness between strain x-2T and N. panacisoli KCTC 19470T (=Gsoil 346T) was 29.8%. These chemotaxonomic characters support the position of strain x-2T within the genus Nocardioides. The results of physiological and biochemical tests, as well as phylogenetic analysis, suggest that strain x-2T represents a novel species of the genus Nocardioides, for which the name Nocardioides litorisoli sp. nov. is proposed. The type strain is x-2T (=KCTC 39865T=CCTCCAB 2016255T).

The genus Nocardioides was established by Prauser in 1976 [1]. This genus belongs to the phylum Actinobacteria, class Actinobacteria, order Actinomycetales and family Nocardiaceae. At the time of writing, there are 89 species with validly published names within this genus. Four novel species were reported in 2015 and seven in 2016 (http://www.microbiologyresearch.org/). The strains of species of this genus have been isolated from a wide range of habitats. For example, Nocardioides rotundus [2] and Nocardioides antarcticus [3] were isolated from the deep sea, Nocardioides baekrokdamensis [4] and Nocardioides ungokensis [5] were isolated from lakes. Other sources, such as tree roots, marine organisms, and crops have also been reported [6–8]. All strains of species of the genus Nocardioides are Gram-stain positive, strictly aerobic, rods or globular, and many strains have high DNA G+C contents. The major polar lipids are phosphatidylglycerol (PG), phosphatidylglycerol (PG) and/or phosphatidylglycerol (PI). The predominant menaquinone is MK-8 (H4). The cell-wall peptidoglycan contains L,L-diaminopimelic acid (LL-DAP) [4, 5, 9]. Strain x-2T was isolated from lakeside soil of Sayram (80°59′35″ E, 44°29′37.4″ N), which is located in the north Tian Shan Mountains of Bole city, Xinjiang Uygur Autonomous Region, PR China. The soil was suspended in normal saline and serial dilutions were spread on R2A agar (pH 7.0). The plates were incubated under aerobic conditions for 5–7 days at 28°C. A pure culture was isolated after several subcultivations on R2A agar. Further growth of strain x-2T was tested in LB (Luria-Bertani; Difco), TSB (tryptic soy broth; Difco), 1/10 TSB, NA (nutrient agar; Difco) and R2A (Difco). It grew well in R2A, but did not grow in other media. Polyphasic characterization was used to describe its typical characteristics. The strain was then cultured on R2A agar at 28°C and preserved in glycerol (20 %, v/v) at −70°C.

Cellular morphology was observed by transmission electron microscopy (Hitachi H-7650, Japan) and optical microscopy (Olympus BX51, Japan). Gram-staining was carried out using a Gram-staining kit (Jiangcheng Biotech) according to the method of Dussault [10]. The non-staining KOH method [11] was also used to check the result. Anaerobic growth of strain x-2T was determined in an anaerobic chamber (Mitsubishi Gas Chemical; O2<0.1 %, CO2>21 % and N2) for 7 days on R2A agar. Motility was tested by puncture inoculation in R2A with 0.3 % (w/v) agar. Colonies of strain x-2T on R2A agar were smooth, circular, white and convex after 5 days. The cells were Gram-stain-positive, aerobic, akinetic, rod-shaped, 0.4–0.5 μm in diameter and 1.2–2.2 μm in length (Fig. S1, available in the online Supplementary Material).

Keywords: Nocardioides; soil; Actinobacteria; polyphasic identification.

Abbreviations: DAP, Diaminopimelic acid; DPG, Diphosphatidylglycerol; GL, Glycolipid; ML, Maximum-likelihood; MP, Maximum-parsimony; NJ, Neighbour-joining; PS, Phosphatidylglycerol; PL, Phosphatidylglycerol. The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain x-2T is KY287237.

Four supplementary figures are available with the online Supplementary Material.

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002283 © 2017 IJMS

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DOI 10.1099/ijsem.0.002283
The nearly full-length 16S rRNA gene sequence of strain x-2T was amplified by PCR-mediated amplification using the universal primer pair, 27F and 1492R [12, 13]. After using T-A cloning with a pGEM-T Easy vector (Promega), the 1440 bp accurate sequence of the PCR product was acquired. The 16S rRNA gene sequence of strain x-2T was compared with all the related sequences on Eztaxon server (http://www.ezbiocloud.net/identify/) [14]. Sequences were aligned by using CLUSTAL X software [15]. Phylogenetic analysis was performed by using MEGA software version 6.0 [16]. Three methods, maximum-likelihood (ML) [17], neighbour-joining (NJ) [18] and maximum-parsimony (MP) [19] were used to describe phylogenetic trees. The ML tree was mainly based on the two-parameter calculation model [20] and the nearest-neighbour-interchange (NNI) heuristic search method. The NJ tree was built with Kimura’s two-parameter model [20] and the gamma distributed (G). Calculations for the MP tree were performed by using the Tree-Bisection-Reconnection heuristic search method with the number of initial trees (random addition) as 10 and partial deletion options. The tree topology was evaluated based on bootstrap analysis of 1000 replications [21]. The 16S rRNA gene sequence of Terrabacter tumescens DSM 20308T was used as an outgroup in the three different treeing methods.

The 16S rRNA gene sequence of strain x-2T was most closely related to Nocardioides panacisoli Gsoil 346T (97.36 %), followed by Nocardioides daecheonensis KIS2-16T (95.83 %), Nocardioides simplex KCTC 9106T (95.55 %), Nocardioides maradonensis RP-B30T (95.49 %) and Nocardioides aromaticivorans H-1T (95.49 %). Phylogenetic trees based on the NJ (Fig. 1), MP and ML methods (Figs S2 and S3) show that strain x-2T is grouped with members of the genus Nocardioides, and is most closely related to N. panacisoli Gsoil 346T. Accordingly, N. panacisoli KCTC 19470T (=Gsoil 346T) and the type species of the genus Nocardioides albus KCTC 9186T were selected as the reference strains.

To analyze the physiological and biochemical characteristics of strain x-2T, growth was tested at pH 3.0–11.0 (with a pH interval of 1.0) at 28°C in R2A with the appropriate buffer solutions. Salt tolerance was firstly assessed with 0–10 % (w/v) NaCl (at 1.0 % intervals) in R2A. Then it was assessed with 0–5 % (w/v) NaCl (at 0.5 % intervals) in R2A. The temperature range for growth was determined at 4, 10, 16, 28, 37 and 42°C on R2A agar. All these experiments were incubated for 7 days. Catalase activity was determined from the production of bubbles in 3 % (v/v) H₂O₂ by colonies. Oxidase activity was determined using tetramethyl-β-phenyle- nediamine. Hydrolysis of gelatin, starch, cellulose, casein and Tweens 20, 40, 60, 80 was determined as described by Cowan and Steel [22]. Degradation of DNA, xanthine, hypoxanthine and tyrosine was also investigated. Assimilation and acid production from carbohydrates were determined by means of API 50 CH kits (bioMérieux). Other enzyme activities and biochemical properties were determined with the API 20 NE and API ZYM systems (bioMérieux). All API tests were carried out in duplicate. Utilization of sole carbon sources was determined with basal culture medium (1 T: 1.8 g K₂HPO₄, 1.08 g KH₂PO₄, 0.5 g NH₄Cl, 0.5 g NaNO₃, 0.1 g KCl, 0.1 g MgSO₄ and 0.05 g CaCl₂, pH 7.0) at 28°C for 7 days. Strain x-2T had no nutritional requirements. The results of API ZYM tests were observed after incubation at 28°C for 6 h, API 50 CH and API 20 NE test systems were examined after incubation at 28°C for 24 and 48 h, respectively.

Growth of strain x-2T was observed at a temperature range from 16 to 37°C (optimum at 28°C), and a pH range from 6.0 to 9.0 (optimum at 8.0). It could only tolerate up to 1.0 % NaCl (optimum 0 %, w/v) in R2A medium. The strain could not grow in an anaerobic chamber or move when growing in R2A with 0.3 % agar (w/v). Strain x-2T showed a positive reaction for catalase and degradation of DNA, a weakly positive reaction for degradation of xanthine and tyrosine, but was negative for oxidase, gelatinase, amylase and cellulase. It could not hydrolyze the Tween series, casein or hypoxanthine. Differences between strain x-2T and the reference strains of the genus Nocardioides are shown in Table 1, while other characteristics are given in the species description.

Whole-cell fatty acid compositions of the strains were analyzed by using the Sherlock Microbial Identification System (version 4.5; database TSBA40 4.10) [23, 24]. Polar lipids of strain x-2T were examined by two-dimensional TLC [25]. Respiratory quinones were analyzed by HPLC [23]. The cell-wall peptidoglycan was analyzed using the method described by Schumann [26]. DNA–DNA hybridization between strain x-2T and N. panacisoli KCTC 19470T was performed using the thermal denaturation and renaturation method [27]. The genomic DNA G+C content was determined according to the reversed-phase HPLC method [28]. All three strains were cultivated and then analyzed under the same conditions. Analyses were carried out in duplicate. Cell biomass of the three strains for chemotaxonomic analysis was collected after 5 days of incubation under aerobic conditions in R2A at 28°C with shaking at 120 r.p.m.

The major fatty acids (>5 %) of strain x-2T were C₁₈:₁ω₉c (31.6 %), iso-C₁₆:₀ (20.0 %), C₁₇:₁ω₈c (5.7 %) and C₁₆:₀ (5.6 %) (Table 2). Comparison of these three species of the genus Nocardioides showed that the predominant fatty acids were similar, but differed in the proportions present. The major polar lipids of strain x-2T were PG, DPG, glycolipids (GL) and three unknown phospholipids (PL1, PL2 and PL3) (Fig. S4). MK-8(H₄) (88.5 %) was the major menaquinone and the cell-wall peptidoglycan contained LL-diaminopimelic acid, similarly to other species of genus Nocardioides. For DNA–DNA hybridization tests, the level of DNA–DNA relatedness between strain x-2T and N. panacisoli KCTC 19470T was 29.8 %, which was less than the threshold value of 70 % recommended for species delineation [29]. The DNA G+C content of strain x-2T was 71.1 mol%, which was consistent with that of species of the genus Nocardioides with validly published names.
Fig. 1. A neighbour-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the relationship of strain x-2ᵀ with related species of the genus Nocardioides. The sequence of Terrabacter tumescens DSM 20308ᵀ was used as an outgroup. Bootstrap values > 50 %, expressed as percentages of 1000 replications, are shown at branching points. Filled circles indicate that the corresponding nodes were also recovered in maximum-parsimony and maximum-likelihood trees. Bar, 0.01 substitutions per nucleotide position.
Strain x-2T shared many features with its most closely related strain, *N. panacisoli* Gsoil 346T, and the type species of this genus, *N. albus* KCTC 9186T, including the major fatty acids, major polar lipids, major menaquinone, DNA G+C content, cell-wall peptidoglycan, and many physiological and biochemical characteristics. All these features support strain x-2T belonging to the genus *Nocardioïdes*. However, strain x-2T differs from the reference strains with respect to the 16S rRNA gene, DNA–DNA relatedness values, hydrolysis of casein and gelatin, utilization of galactose (Table 1), and a differential fatty acid content (Table 2). So, it is proposed here that strain x-2T represents a novel species of the genus *Nocardioïdes*.

**DESCRIPTION OF Nocardioïdes litorisoli sp. nov.**

*Nocardioïdes litorisoli* sp. nov. (li.to.ri.so’li. L. n. *litus*, -oris, shore; L. *n. solum*, soil; N.L. gen. *n. litorisoli* from soil of a shore, referring to the source of isolation).

Cells are Gram-stain-positive, oxidase-negative, catalase-positive, non-spore-forming, non-motile and short rods (0.4–0.5 µm in diameter and 1.2–2.2 µm in length after 5 days of growth at 28 °C on R2A agar). Colonies on R2A agar are smooth, circular, light white and convex after 5 days. Grows at 16–37 °C (optimum at 28 °C), but not at 4 or 42 °C. The pH range for growth is 6.0–9.0 (optimum at pH 8.0). Tolerates up to 1.0 % NaCl (optimum 0 %, w/v). Negative reactions for amylase, gelatinase or urease. Hydrolyses DNA, xanthine and tyrosine, but not casein, cellulose,
The type strain, x-2T (=KCTC 39845T=CCTCCAB 2016255T), was isolated from the lakeside soil of Sayram in Xinjiang Uygur Autonomous Region, PR China. The genomic DNA G+C content of the type strain is 71.1 mol%.

References