Tenggerimyces mesophilus gen. nov., sp. nov., a member of the family Nocardioidaceae

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A novel aerobic actinomycete, designated strain I12A-02601T, was isolated from a desert soil crusts sample collected from the Shapotou region of Tengger Desert, north-west China. The substrate mycelia of this isolate were well-developed and branched, but not fragmented. The maturity aerial mycelia formed short chains of small, rod-shaped spores. The strain contained LL-diaminopimelic acid, d0-diaminopimelic acid, galactose, glucose, ribose and xylose in its whole-cell hydrolysates. The polar lipids consisted of diphosphatidylglycerol, N-acetylglucosamine-containing phospholipids, phosphatidylinositolmannoside and glycolipids. The predominant menaquinones were MK-10(H4) and MK-10(H6). The major fatty acids were iso-C15 : 0, anteiso-C15 : 0, C16 : 0, anteiso-C17 : 0 and iso-C16 : 0. The G+C content of the genomic DNA was 72.2 mol%. The 16S rRNA gene sequences comparison showed that strain I12A-02601T was most closely related to members of the family Nocardioidaceae, such as Actinopolymorpha alba YIM 48868T (93.3 % sequence similarity), Actinopolymorpha pittospori PIP 143T (93.2 %), and Flindersiella endophytica EUM 378T (93.2 %). In the phylogenetic tree based on 16S rRNA gene sequences, strain I12A-02601T formed a clade with the members of the genera Flindersiella, Thermasporomyces, and Actinopolymorpha in the family Nocardioidaceae.

Combined data from this taxonomic study using a polyphasic approach, led to the conclusion that strain I12A-02601T represents a novel species of a new genus in the family Nocardioidaceae, for which the name Tenggerimyces mesophilus gen. nov., sp. nov. is proposed. The type strain of the type species is I12A-02601T (=CPCC 203544T=DSM 45829T=NBRC 109454T).

The family Nocardioidaceae was described by Nesterenko et al. (1985), to accommodate two genera, Nocardioides (Prauser, 1976) and Pimelobacter (Suzuki & Komagata, 1983). Later, the species of the genus Pimelobacter were transferred into the genera Terrabacter and Nocardioides (Collins et al., 1989). At the time of writing, the family Nocardioidaceae comprises eight genera: Nocardioides (Prauser 1976), Aeromicrobium (Miller et al., 1991), Kribbella (Park et al., 1999), Marmoricola (Urzi et al., 2000), Actinopolymorpha (Wang et al., 2001), Thermasporomyces (Yabe et al., 2011), Flindersiella (Kaeckla & Franco, 2011) and Mumia (Lee et al., 2014). Members of the family Nocardioidaceae are found ubiquitously from various environments, including sub-zero-temperature terrestrial soil, mature compost, deep surface ecosystems, oligotrophic habitats, even tissues of plants or lichen. The family members share a common taxon-specific 16S rRNA signature nucleotides pattern (Zhi et al. 2009). Here, we report another novel member of the family Nocardioidaceae.

During our research on the diversity of culturable actinomycetes in the desert soil crusts of north-western China, a novel actinomycete, strain I12A-02601T was isolated from a sample of moss-dominated soil crusts in the Cuilu ditch (37° 25′ 37.85″ N 104° 35′ 08.26″ E, altitude 1701 m), Shapotou region, on the south-east edge of Tengger Desert and near to the Yellow River. Strain I12A-02601T was recovered on a 1/5-strength R2A agar plate (Difco) by using the dilution plating method. After incubation at 28 °C for approximately 4 weeks, the single colony of strain I12A-02601T was picked and purified, then maintained on PYG agar (peptone 0.3 %, Yeast extract 0.5 %, glycerol 1.0 %, glycine betaine 0.125 %, glutamate 10.0 % and agar 2.0 %) at 8 °C. Strain I12A-02601T was grown on PYG agar and then maintained on Trypticase soy agar slants. (Difco) at 28 °C. Strain I12A-02601T was grown on PYG agar and then maintained on Trypticase soy agar slants. (Difco) at 28 °C. Strain I12A-02601T was grown on PYG agar and then maintained on Trypticase soy agar slants. (Difco) at 28 °C.

Abbreviations: LL-DAP, D0-diaminopimelic acid; LL-DAP, LL-diaminopimelic acid; DPG, diphosphatidylglycerol; GluNu, N-acetylgulcosamine-containing phospholipids; ISP, International Streptomycetes Project; PIM, phosphatidylinositolmannoside.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain I12A-02601T is KP284443.

Two supplementary tables and two supplementary figures are available with the online Supplementary Material.
sodium pyruvate 0.125 %, agar 1.5 %, pH 7.2) at 4 °C and as glycerol suspensions (20 %, v/v) at −80 °C.

Growth of the isolate was tested in a range of temperatures (4–45 °C) and pH (4.0–11.0). NaCl tolerance was examined on PYG agar medium supplemented with 0, 1, 2, 3, 4, 5, 7, 10, 12 and 15 % (w/v) NaCl. Morphological properties were examined on International Streptomyces Project (ISP) medium 4 agar (Shirling & Gottlieb, 1966) after 10, 14, 21 and 28 days at 28 °C, using light microscopy (Axio scope A1; Zeiss) and scanning electron microscopy (Quanta 200; FEI). Cultural characteristics were recorded using ISP2, ISP3, ISP4, ISP5, ISP6, ISP7 (Shirling & Gottlieb, 1966), R2A, PYG and Bennett’s agar (glucose 1 %, yeast extract 1 %, beef extract 1 %, casein-enzyme-hydrolysate 2 %, agar 2 %; pH 7.2) slants at 28 °C, for 7–28 days. Physiological and biochemical characteristics tests were performed at 28 °C. H2S production, gelatin liquefaction, nitrate reduction and starch hydrolysis were examined by methods described previously (MacFaddin, 1980). Catalase activity was determined by assessing bubble production in 3 % (v/v) H2O2 (Kovács, 1956). Carbon source utilization was tested using Biolog GEN III MicroPlates according to the manufacturer’s instructions. All the above physiological character tests were observed consistently for 30 days. Qualitative enzyme tests and acid production from carbohydrates were determined using the API ZYM and API 50CH systems (bio-Mérieux) according to the manufacturer’s instructions.

Optimum growth occurred at 28–30 °C and pH 6.0–8.0. Substrate mycelia developed well on ISP2, ISP4, ISP6, ISP7, R2A and PYG agar, while aerial mycelia were observed on ISP4, R2A and PYG agar (Table S1, available in the online Supplementary Material). Aerial mycelia of strain I12A-02601T fragmented into short chains with small, rod-shaped spores (Fig. S1). No diffusible pigment was produced on any of the tested media. Detailed physiological and biochemical characteristics of strain I12A-02601T are given in the species description.

The whole-cell sugar composition and diagnostic isomers of diaminopimelic acid were analysed by TLC as described by Lechevalier & Lechevalier (1965, 1980). Menaquinones were extracted by using the method of Collins et al. (1977) and analysed by HPLC (Groth et al., 1997). Polar lipids were extracted, examined by two-dimensional TLC and identified as described by Minnikin et al. (1984). Cellular fatty acids were extracted from cells collected in late-exponential phase after cultivation on tryptic soy agar (Difco) at 28 °C for 5 days, following the instructions of the standard Sherlock Microbial Identification System (Kroppenstedt, 1985; Meier et al., 1993). MIDI Sherlock Version 6.0 and ACTIN1 database were employed for analysis.

Strain I12A-02601T contained LL-diaminopimelic acid (LL-DAP) and DD-diaminopimelic acid (DD-DAP) as the diamino acid, and galactose, glucose, ribose and xylose as the whole-cell sugars. The menaquinone profile contained MK-10(H4), MK-10(H8) and MK-10(H12), at a ratio of 9 : 66 : 25. The phospholipids comprised diphosphatidylglycerol (DPG), N-acetylglucosamine-containing phospholipid (GluNu), and phosphatidylinositolmannoside (Fig. S2). The fatty acids profile is given in Table S2, with iso-C15 : 0 (13.0 %), anteiso-C15 : 0 (10.1 %), iso-C16 : 0 (16.3 %), C16 : 0 (27.6 %) and anteiso-C17 : 0 (13.1 %) as the major fatty acids. The chemotaxonomic characteristics of strain I12A-02601T distinguished this strain from members of the family Nocardioidaceae are shown in Table 1. According to the data stated above, strain I12A-02601T exhibited the typical characteristics of the family Nocardioidaceae, containing LL-DAP in the cell wall, DPG in the phospholipids profile, and major amounts of saturated fatty acids. However, strain I12A-02601T also contained DD-DAP in the cell wall, GluNu in the polar lipids profile, and MK-10(H8) in the main menaquinones composition that were not detected in any other genera in the family Nocardioidaceae, and which differentiated strain I12A-02601T from species of the genera Flindersiella, Thermasporomyces and Actinopolymorpha (Table 1).

Extraction of genomic DNA from strain I12A-02601T, amplification and sequencing of the 16S rRNA gene were carried out as described previously by Xu et al. (2003). The nearly complete resultant 16S rRNA gene sequence (1526 bp) of strain I12A-02601T was analysed using BLAST (Altschul et al., 1997) and subsequently aligned with the 16S rRNA gene sequences of representatives of related genera available from the GenBank database by using the clustal x program (Thompson et al., 1997). Phylogenetic trees were reconstructed with the neighbour-joining (Saitou & Nei, 1987), maximum-parsimony (Kluge & Farris, 1969) and maximum-likelihood (Felsenstein, 1981) methods using the software package MEGA version 5 (Tamura et al., 2011). Distances were calculated using distance options according to Kimura’s two-parameter model (Kimura, 1980, 1983). Bootstrap resampling analysis, according to the method of Felsenstein (1985), with 1000 resamplings was used to evaluate the phylogenetic tree topology.

BLAST search results using the 16S rRNA gene sequence of strain I12A-02601T showed it was the most similar with members of the family Nocardioidaceae, i.e. Actinopolymorpha alba YIM 48868T (93.3 % sequence similarity), Actinopolymorpha pittospori PIP 143T (93.2 %), and Flindersiella endophytica EUM 378T (93.2 %). After alignment of the 16S rRNA gene sequence (1526 bp) of strain I12A-02601T with the collected sequences of the representative members of the family Nocardioidaceae, the 1390 bp-length common sequences were used in phylogenetic analyses. In the phylogenetic tree based on 16S rRNA gene sequences, strain I12A-02601T formed a robust clade with recognised species of the genera Flindersiella, Thermasporomyces and Actinopolymorpha within the family Nocardioidaceae (Fig. 1). Analysis of 16S rRNA signatures showed that strain I12A-02601T shared the signature nucleotides pattern defined for the family Nocardioidaceae (Zhi et al., 2009). However,
Table 1. Differential characteristics of *Tenggerimyces mesophilus* I12A-02601T and other genera of the family Nocardioidaceae

Genera: 1, *Tenggerimyces* gen. nov. (data from this study); 2, *Flindersiella* (Kaewkla & Franco, 2011); 3, *Thermasporomyces* (Yabe et al., 2011); 4, *Actinopolymorpha* (Cao et al., 2009; Wang et al., 2001, 2008; Yuan et al., 2010); 5, *Kribbella* (Park et al., 1999); 6, *Nocardioides* (Collins et al. 1989); 7, *Aeromicrobium* (Miller et al., 1991); 8, *Marmoricola* (Urzi et al., 2000); 9, *Mumia* (Lee et al., 2014). DPG, Diphosphatidylglycerol; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PG-OH, phosphatidylglycerol containing 2-hydroxy fatty acids; PI, phosphatidylinositol; GluNu, *N*-acetylglucosamine-containing phospholipid; PIM, phosphatidylinositolmannosides; PGL, phosphoglycolipid; GL, glycolipid; PL, unknown phospholipid(s); UL, unknown polar lipid; Rib, ribose; Xyl, xylose; Glc, glucose; Rha, rhamnose; Ara, arabinose; Man, mannose; Gal, galactose; DAP, diaminopimelic acid; ND, not determined.

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<td>Hyphae, rods</td>
<td>Hyphae, rods</td>
<td>Coccoid or short rods</td>
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<td>Hyphae, rods, cocci</td>
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<td>MK-9(H4), MK-10(H4), MK-11(H3)</td>
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strain I12A-02601\textsuperscript{T} possessed its own signature nucleotides distinguished from the genera with validly published names in the family Nocardioidaceae at the sites 831 : 855 (U–U), 836 : 850 (G–C), 896 : 903 (U–A), 968 (G), 1001 : 1039 (U–A) and 1189 (C). The specific diagnostic nucleotides signature patterns of genera of the family Nocardioidaceae are listed in Table 2. The genomic DNA G\textsubscript{+}C content of strain I12A-02601\textsuperscript{T} was determined using the thermal denaturation (\(T_m\)) method (Marmur & Doty, 1962) with Actinopolymorpha pittospori DSM 45354\textsuperscript{T} as a reference. The DNA G\textsubscript{+}C content of strain I12A-02601\textsuperscript{T} was 72.2 mol\%.

Therefore, on the basis of phenotypic and phylogenetic characteristics, strain I12A-02601\textsuperscript{T} is truly different from any existing genera in the family Nocardioidaceae and represents a novel species of a new genus in this family, for which the name Tenggerimyces mesophilus gen. nov., sp. nov. is proposed.

**Description of Tenggerimyces gen. nov.**

Tenggerimyces [Teng.ge.ri.my\textsuperscript{es}. Chin. n. Tenger a desert located at west of China, N.L. n. -myces (from Gr. mykes fungus) a fungus-like organism; N.L. n. Tenggerimyces a fungus-like organism isolated from Tenger desert].

Gram-positive, aerobic actinomycetes. Catalase-positive. The whole-cell hydrolysates contain LL-DAP and DD-DAP as the diagnostic diamino acid, and galactose, glucose, ribose and xylose as the diagnostic sugars. Phospholipids consist mainly of DPG, GluNu and PIM. The unsaturated MK-10 is the predominant menaquinone. The major fatty acids are iso-C\textsubscript{15} : 0, anteiso-C\textsubscript{15} : 0, iso-C\textsubscript{16} : 0, C\textsubscript{16} : 0 and anteiso-C\textsubscript{17} : 0. Possesses signature nucleotides pattern at the following sites: 831 : 855 (U–U), 836 : 850 (G–C), 896 : 903 (U–A), 968 (G), 1001 : 1039 (U–A) and 1189 (C). Phylogenetically, the genus is placed in the family Nocardioidaceae.

The type species is Tenggerimyces mesophilus.
Table 2. Differentiation of signature nucleotides pattern between the genus Tenggerimyces gen. nov. and other genera of the family Nocardioidaceae

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Description of Tenggerimyces mesophilus sp. nov.

Tenggerimyces mesophilus [me.so.phi’lus. Gr. adj. mesos middle; Gr. adj. philos loving; N.L. masc. adj. mesophilus middle (temperature) -loving, mesophilic].

Displays the following characteristics in addition to those given in the genus description. Produces aerial mycelium on ISP4, R2A and PYG agar. Spores are tiny rods on short chains that develop from aerial mycelia. No diffusible pigment is detected on any of the tested media. Grows between 10 and 37 °C, pH 6.0–10.0 and in the presence of up to 4 % (w/v) NaCl. Optimum growth is achieved at 28–30 °C and pH 6.0–8.0. Positive for catalase, liquefaction of gelatin, starch hydrolysis and nitrate reduction. Negative for oxidase, urea hydrolysis and production of H2S. Arabinose, xylose, d-arabinose, l-arabinose, aesculin ferric citrate, d-fucose, l-fucose, d-glucose, melibiose, l-rhamnose, d-ribose, sucrose, trehalose and d-xylose. Positive for activities of acid phosphatase, alkaline phosphatase, chymotrypsin, cystine arylamidase, esterase (C4), esterase lipase (C8), a-galactosidase, b-galactosidase, a-glucosidase, b-glucosidase, lipase (C14), a-mannosidase and trypsin.

The type strain is I12A-02601T (=CPCC 203544T=DSM 45829T=NBRC 109454T), which was isolated from desert soil crusts from the Shapotou region of Tengger Desert, north-west China. The DNA G+C content of the type strain is 72.2 mol%.

Acknowledgements

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