Nocardioides perillae sp. nov., isolated from surface-sterilized roots of Perilla frutescens

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A Gram-stain-positive, rod-shaped actinobacterium, designated strain I10A-01402T, was isolated from surface-sterilized roots of a medicinal plant, Perilla frutescens, collected in a suburb of Beijing, China. Chemotaxonomically, the strain contained L-3-diaminopimelic acid as the diagnostic diamino acid and MK-8(H4) as the predominant menaquinone. The phospholipids were diphosphatidylglycerol, phosphatidylglycerol and phosphatidylinositol. The major fatty acids were C17 : 1ω9c, C18 : 1ω9c, C17 : 0, C16 : 0 and iso-C16 : 0. The genomic DNA G+C content was 70.4 mol%. 16S rRNA gene sequence analysis indicated that strain I10A-01402T belonged to the genus Nocardioides. Phylogenetic analyses based on 16S rRNA gene sequences showed that the isolate formed a robust cluster with Nocardioides ginsengisegetis Gsoil 48ST, N. koreensis MSL-09T and N. alcalitolerans KSL-1T. On the basis of the evidence from our polyphasic taxonomic study, a novel species, Nocardioides perillae sp. nov., is proposed. The type strain is I10A-01402T (=CPCC 203382T =DSM 24552T =KCTC 29022T).

The genus Nocardioides, first described by Prauser (1976), was assigned phylogenetically to the family Nocardioidaceae (Nesterenko et al., 1985) as the type genus. At the time of writing, including the recently described species Nocardioides ginsengisegetis (Im et al., 2010), N. alpinus (Zhang et al., 2012), N. ginsengagri (Lee et al., 2012) and N. daejeonensis (Woo et al., 2012), 57 species with validly published names were encompassed in the genus Nocardioides. One of these species, Nocardioides fastidiosus, has been transferred to the genus Aeromicrobium as Aeromicrobium fastidiosum (Tamura & Yokota, 1994).

The members of Nocardioides are characterized by irregular rod- or coccoid-shaped cells, a type I/C cell wall (L-3-diaminopimelic acid and the absence of diagnostic carbohydrates) and major menaquinone MK-8(H4). Isolates have been obtained from various environments such as ginseng fields (Cui et al., 2009; Yi & Chun, 2004a), tidal flat sediments (Yi & Chun, 2004b), sand (Lee et al., 2007; Kim et al., 2009), soil samples (Dastager et al., 2008) and seawater (Choi et al., 2007). In this study, a novel strain, I10A-01402T, isolated from surface-sterilized roots of a medicinal plant, Perilla frutescens (L.) Britt. var. frutescens, collected in a suburb of Beijing, China, was characterized by using a polyphasic approach.

The temperature range for growth was tested at 0, 4, 10, 28–37 (at intervals of 1°C), 40, 45 and 55°C on DSMZ 92 medium with swarming agar (0.3%, w/v). Cell morphology was examined using a JEOL JEM-1010 electron microscope (transmission electron microscopy mode) after incubation on DSMZ 92 medium at 32°C for 3 days. Before mounting on Formvar-coated copper grids 76 (Electron Microscopy Science), cells were negatively stained with 2% (w/v) uranyl acetate for 20 s.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain I10A-01402T is JN869461.

Two supplementary figures and a supplementary table are available with the online version of this paper.

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units) at 32 °C. Tolerance of NaCl was examined on DSMZ 92 at 32 °C with 0–20 % (w/v) NaCl (at intervals of 0.5 %). The above tests were observed from day 4 to day 10 of incubation. Metabolic characters were determined by using Biolog GEN III (MicroPlate) and API ZYM (bioMérieux) test kits according to the manufacturers’ instructions. The abilities of the strain to hydrolyse gelatin, Tween 80, L-tyrosine and starch and to produce H₂S and indole and its methyl red and Voges–Proskauer reactions were tested as described by Smibert & Krieg (1994). Catalase and oxidase activities were determined following a procedure described previously (Zhang et al., 2008). Nocardioides koreensis KCTC 19272ᵀ, N. ginsengisgetis KCTC 19469ᵀ and N. alkalitolerans KCTC 19037ᵀ were included in parallel physiological and biochemical tests.

Cells of strain I10A-01402ᵀ were Gram-stain-positive, rod-shaped and motile with a single polar flagellum (Fig. S1, available in IJSEM Online). Moist-surfaced, pale-yellow colonies with a maximum diameter of 1.5 mm were formed on DSMZ 92 medium after incubation for 96 h at 32 °C. Colonies on DSMZ 92 and ISP 2 were opaque and convex. Growth was observed at 20–37 °C, with optimum growth at 32 °C. The initial pH range for growth was 6.0–11.0, with optimum growth at pH 8.0. The detailed physiological and biochemical characteristics of the strain are given in Table 1 and in the species description.

Biomass for chemical (except for fatty acid analysis) and molecular studies was obtained by cultivation in DSMZ 92 broth at 32 °C for 5 days on a rotary shaker (about 150 r.p.m.). The isomer of diaminopimelic acid in whole-cell hydrolysates was determined by TLC as described by Lechevalier & Lechevalier (1980). Polar lipids were extracted, examined by two-dimensional TLC and identified according to previously described procedures (Minnikin et al., 1984). Menaquinones were extracted according to Collins et al. (1977) and analysed by HPLC (Groth et al. 1997). Cellular fatty acids were extracted from cells cultivated on trypticase soy agar (Difco) at 32 °C for 5 days (at the late-exponential phase of growth), methylated and analysed using the Sherlock Microbial Identification System (MIDI) according to the manufacturer’s instructions (Kroppenstedt, 1985; Meier et al., 1993). The ACTIN1 database in MIDI Sherlock version 6.0 was employed for this study.

LL-Diaminopimelic acid was detected as the diagnostic diamino acid in whole-cell hydrolysates of strain I10A-01402ᵀ. The polar lipids included diphosphatidylglycerol, phosphatidylglycerol, phosphatidylglycol and two unidentified aminolipids (Fig. S2). The major menaquinone was Q₁₀ (41.9 %); a small amount of Q₉ (32.4 %) was also detected. The saturated fatty acid profile was characterized by the presence of unsaturated, branched and saturated fatty acids; the major fatty acids were C₁₇:₁₀9C (26.6 %), C₁₄:₀ (15.6 %), C₁₇:₀ (15.0 %), C₁₆:₀ (10.3 %), iso-C₁₅:₀ (10.1 %), C₁₅:₀ (5.4 %), C₁₈:₀ (4.5 %), summed feature 8 (C₁₈:₁₀7C and/or C₁₈:₁₀6C, 3.6 %), C₁₆:₁₀9C (2.1 %), C₁₄:₀ (1.7 %), anteiso-C₁₇:₀ (1.0 %), C₁₉:₀ (0.8 %), iso-C₁₇:₀ (0.8 %), iso-C₁₄:₀ (0.7 %), iso-C₁₅:₀ (0.6 %), C₁₇:₀ 3-OH (0.5 %), iso-C₁₈:₀ (0.5 %) and C₁₃:₀ (0.4 %) (Table S1).

Extraction of genomic DNA and PCR amplification of the 16S rRNA gene were performed as described by Li et al. (2007). Purified PCR products were sequenced with an ABI PRISM automatic sequencer. The sequence obtained was compared with available 16S rRNA gene sequences from GenBank using the BLAST program and the EzTaxon-e server (http://www.eztaxon-e.ezbiocloud.net/; Kim et al., 2012) to determine an approximate phylogenetic affiliation. Multiple alignments with sequences of the most closely related taxa and calculations of levels of sequence similarity were carried out using CLUSTAL X (Thompson et al., 1997). A phylogenetic tree was constructed using the neighbour-joining method of Saitou & Nei (1987) from Kᵥₑ₅ values (Kimura, 1980, 1983) and MEGA version 4.0 (Tamura et al., 2007). The topology of the phylogenetic tree was evaluated by the bootstrap resampling method of Felsenstein (1985) with 1000 replicates. The G+C content of the genomic DNA was determined using the thermal denaturation method (Marmur & Doty, 1962) with DNA from Streptomyces griseus ATCC 23345ᵀ as a control. The genomic DNA G+C content was determined as 70.4 mol%.

A nearly complete 16S rRNA gene sequence was obtained for strain I10A-01402ᵀ (1454 nt). BLAST search results using the 16S rRNA gene sequence of strain I10A-01402ᵀ indicated that the new isolate showed similarities of 93.0–95.9 % with members of the genus Nocardioides, among which the highest similarities were 95.9, 95.6, 95.5 and 95.4 % with N. koreensis MSL-09ᵀ, N. basaltis J112ᵀ, N. salarius CL-Z59ᵀ and N. marinisabuli SBS-12ᵀ, respectively. In the phylogenetic tree reconstructed using the neighbour-joining method based on 16S rRNA gene sequences, strain I10A-01402ᵀ formed a robust cluster with N. ginsengisgetis Gsoil 485ᵀ, N. koreensis MSL-09ᵀ and N. alkalitolerans KSL-1ᵀ that was separate from the cluster consisting of N. basaltis J112ᵀ, N. salarius CL-Z59ᵀ and N. marinisabuli SBS-12ᵀ (Fig. 1). Meanwhile, strain I10A-01402ᵀ could be easily differentiated from its phylogenetic neighbours in chemotaxonomic characteristics, such as the cellular fatty acid composition (Table S1), as well as other phenotypic properties listed in Table 1.

Based on the morphological characteristics, chemotaxonomic analyses and genotypic data presented above, it is proposed that strain I10A-01402ᵀ represents a novel species of the genus Nocardioides, with the name Nocardioides perillae sp. nov.

### Description of Nocardioides perillae sp. nov.

Nocardioides perillae [pe.r’il.lae. L. fem. gen. n. perillae of Perilla, referring to the isolation of the type strain from a root of *Perilla frutescens* (Linn.) Britt. var. *frutescens*],

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**On:** Mon, 17 Dec 2018 23:20:20
Cells are aerobic, Gram-stain-positive, rod-shaped (0.6–0.9 × 1.5–1.8 μm) and motile with a single polar flagellum. Colonies are convex, pale yellow to deep yellow. Soluble pigments are not found on test media. Growth occurs at 20–37 °C (optimum 32 °C) and at pH 6–11 (optimum pH 8.0) with 0–3% (w/v) NaCl. Oxidase-positive, catalase-negative. Hydrolyses starch and Tweens 40 and 80, but not cellulose, milk, gelatin, urea, l-tyrosine, hypoxanthine or

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
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<td>Cell morphology</td>
<td>Rods</td>
<td>Short rods</td>
<td>Short rods</td>
<td>Rods, cocci</td>
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<tr>
<td>Cell width (μm)</td>
<td>0.6–0.9</td>
<td>0.2–0.7</td>
<td>0.3–0.4</td>
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<td>Cell length (μm)</td>
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<td>0.8–3.2</td>
<td>0.9–1.3</td>
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<td>Colony colour</td>
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<td>Cream–white</td>
<td>Milky white</td>
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<tr>
<td>Motility</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Catalase</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Oxidase</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Temperature for growth (°C)</td>
<td>Range</td>
<td>20–37</td>
<td>27–37</td>
<td>4–37</td>
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<td>pH range for growth</td>
<td>6.0–11.0</td>
<td>7.0–8.0</td>
<td>5.5–9.5</td>
<td>5.5–12.0</td>
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<tr>
<td>Maximum NaCl concentration for growth (%) (w/v)</td>
<td>3</td>
<td>5</td>
<td>3</td>
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<td>Nitrate reduction</td>
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<td>–</td>
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<td>+</td>
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<tr>
<td>l-Tyrosine</td>
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<td>Enzyme activity in API ZYM strip</td>
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<td>Cystine arylamidase</td>
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<td>–</td>
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<td>Esterase (C4)</td>
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<td>+</td>
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<td>β-Galactosidase</td>
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<td>Lipase (C14)</td>
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<td>Naphthol-AS-BI-phosphohydrolase</td>
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<td>Valine arylamidase</td>
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<td>–</td>
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<td>Utilization in Biolog GEN III MicroPlate</td>
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<tr>
<td>Celllobiose</td>
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<td>+</td>
<td>+</td>
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<tr>
<td>Citric acid</td>
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<td>–</td>
<td>–</td>
<td>+</td>
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<tr>
<td>D-Fructose</td>
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<td>–</td>
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<tr>
<td>D-Galactose</td>
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<td>–</td>
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<td>a-D-Glucose</td>
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<tr>
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<tr>
<td>l-Lactic acid</td>
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<td>Lactose</td>
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<td>+</td>
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<td>Maltose</td>
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<td>D-Mannitol</td>
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<tr>
<td>D-Mannose</td>
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<td>Melibiose</td>
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<td>myo-Inositol</td>
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<td>Raffinose</td>
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<td>–</td>
<td>+</td>
</tr>
<tr>
<td>l-Rhamnose</td>
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<td>–</td>
<td>–</td>
<td>+</td>
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<tr>
<td>Sucrose</td>
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<td>+</td>
<td>–</td>
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<tr>
<td>Trehalose</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
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<tr>
<td>Tween 40</td>
<td>w</td>
<td>–</td>
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</table>

Table 1. Differential characteristics of strain I10A-01402T and its nearest phylogenetic neighbours in the genus Nocardioides

Strains: 1, I10A-01402T; 2, N. koreensis KCTC 19272T; 3, N. ginsengisegetis KCTC 19469T; 4, N. alkalitolerans KCTC 19037T. +, Positive; –, negative; w, weakly positive. All data were obtained in this study.
xanthine. Negative for H₂S and indole production. In the API ZYM strip, positive for acid phosphatase, alkaline phosphatase, cystine arylamidase, esterase (C4), esterase lipase (C8), α-glucosidase, β-glucuronidase, leucine arylamidase, naphthol-AS-BI-phosphohydrolase and valine arylamidase. In the Biolog GEN III MicroPlate, acetic acid, α-ketoglutaric acid, L-lactic acid, D-lactic acid methyl ester, D-malic acid, glycerol and phosphatidylinositol. MK-8(H₄) is the predominant menaquinone. The major fatty acids (>10%) are C₁₂:0 3-OH, C₁₈:0 3-OH, C₁₇:0 H₂, iso-C₁₆:0 and iso-C₁₆:0 3-OH. Other fatty acids detected are C₁₅:0 H₂, C₁₈:0 3-OH summed feature 8 (C₁₈:1 ω7c and/or C₁₈:1 ω6c), C₁₆:0 10c, C₁₄:0 anteiso-C₁₇:0 H₂, iso-C₁₇:0 H₂, iso-C₁₄:0 H₂, iso-C₁₅:0 H₂, C₁₇:0 3-OH, iso-C₁₈:0 3-OH and C₁₃:0. The DNA G+C content of the type strain is 70.4 mol%.

The type strain is I10A-01402ᵀ (= CPCC 203382ᵀ = DSM 24552ᵀ = KCTC 29022ᵀ), which was isolated from a surface-sterilized root of Perilla frutescens (L.) Britt. var. frutescens, a medicinal plant collected from a suburb of Beijing, China.

Acknowledgements

We thank the KCTC for kindly providing us with the type strains N. ginsengisoli KCTC 19469ᵀ, N. koreensis KCTC 19272ᵀ and N. alkalitololerans KCTC 19037ᵀ. This research was supported by the National Infrastructure of Microbial Resources (NIMR), the National Natural Science Foundation of China (NSFC) (nos 30970008, 30870026 and 81173026), the National S&T Major Special Project on Major New Drug Innovation (nos 2010ZX09040, 2010ZX09042 and 2010ZX09043) and the Special Fund for Health-scientific Research in the Public Interest (no. 201002021).

Fig. 1. Neighbour-joining tree derived from aligned 16S rRNA gene sequences (1454 nt), showing the position of strain I10A-01402ᵀ among its phylogenetic neighbours. Numbers at nodes indicate percentage levels of bootstrap support based on a neighbour-joining analysis of 1000 resampled datasets; only values >50% are given. Bar, 0.01 substitutions per nucleotide position.

Nocardioides perillae sp. nov.

References


