Herbihabitans rhizosphaerae gen. nov., sp. nov., a member of the family Pseudonocardiaceae isolated from rhizosphere soil of the herb Limonium sinense (Girard)

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The taxonomic position of an actinobacterium, designated CPCC 204279T, which was isolated from a rhizosphere soil sample of the herb Limonium sinense collected from Xinjiang Province, China, was established using a polyphasic approach. Whole-cell hydrolysates of strain CPCC 204279T contained galactose and arabinose as diagnostic sugars and meso-diaminopimelic acid as the diamino acid. The muramic acid residues in the peptidoglycan were N-acetylated. The predominant menaquinone was MK-9(H4). The phospholipids consisted of diphasatidylglycerol, phosphatidylethanolamine, phosphatidylinositol and phosphatidylinositol mannosides. The major fatty acids were iso-C16:0, iso-C15:0 2-OH, C16:1ω9c, iso-C16:1 and C16:0. The genomic DNA G+C content was 73.2 mol%. Phylogenetic analysis based on 16S rRNA gene sequences revealed that strain CPCC 204279T should be placed in the family Pseudonocardiaceae, in which the strain formed a distinct lineage next to the genus Actinophytocola. Signature nucleotides in the 16S rRNA gene sequence showed that the strain contained the Pseudonocardiaceae family-specific 16S rRNA signature nucleotides and a genus-specific diagnostic nucleotide signature pattern. The combination of phylogenetic analysis and phenotypic characteristics supported the conclusion that strain CPCC 204279T represents a novel species of a new genus in the family Pseudonocardiaceae, for which the name Herbihabitans rhizosphaerae gen. nov., sp. nov. is proposed. Strain CPCC 204279T (=NBRC 111774T=DSM 101727T) is the type strain of the type species.

The family Pseudonocardiaceae was reassessed by Labeda et al. (2011) based on 16S rRNA gene sequence data embracing 25 genera with validly published names (Labeda et al., 2011). Subsequently, five other genera were reported, namely, Actinorectispora (Quadri et al., 2016), Labedaea (Lee, 2012), Longimycelium (Xia et al., 2013), Tamarsichabitus (Qin et al., 2015) and Thermotunica (Wu et al., 2014). Members of the family were discovered from various environments, including terrestrial soil, salt lake, marine sediments, plant material and manure, and clinical or veterinary samples, but all of them share a common taxon-specific 16S rRNA signature nucleotides pattern at positions 127:234 (G–C), 564 (U), 672:734 (U–G), 831:855 (U–G), 832:854 (G–Y), 833:853 (U–G), 952:1229 (U–A) and 986:1219 (U–A) (Labeda et al., 2011). Here, we report the results of a taxonomic study using a polyphasic approach on strain CPCC 204279T, which represents another member of the family Pseudonocardiaceae.

Strain CPCC 204279T was isolated from a rhizosphere soil sample of the herb Limonium sinense collected from Yili Valley (42°34′16.11″N 81°13′14.07″E, 1323 m H) in Xinjiang Province, north-west China. The isolation medium contained (g l−1): cellobiose (2), yeast extract (5), CaCO3(2), K2HPO4(1), MgSO4·7H2O (0.5) and agar (15). Aztreonam (25 mg l−1) and potassium dichromate (50 mg l−1) were added to the media to prevent the growth of Gram-stain-negative bacteria and fungi that may be present. The isolation media were adjusted to pH 7.2–7.5 using 1 M NaOH and/or 1 M HCl. After incubation at 28 °C for 3 weeks, single colonies were picked and streaked onto newly prepared ISP 2 agar plates (Shirling & Gottlieb, 1966) until the separate colonies

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain CPCC 204279T is KX128908.

Three supplementary figures and one supplementary table are available with the online Supplementary Material.
Growth conditions of strain CPCC 204279T were tested using trypticase soy agar (TSA; Difco) and ISP 2 media, incubating the cultures for 14 days. Different temperatures (at 4, 10, 15, 20, 28, 30, 32, 35, 37, 40, 45 and 48°C) for growth were tested. NaCl tolerance was tested after 14-day incubation in ISP 2 broth supplemented with 0, 1, 3, 5, 7 and 10% (w/v) NaCl. The pH range (pH 4.0–11.0, at intervals of 1.0 pH unit) for growth was observed in ISP 2 broth (28°C, 14–28 days) using the buffer system described by Xu et al. (2005). Cultural characteristics were determined by observing growth of the strain at 28°C for 4–6 weeks on ISP 2, ISP 3, ISP 4 and ISP 5 agars (Shirling & Gottlieb, 1966), R2A medium (Difco), nutrient agar (Waksman, 1961) and potato agar (Waksman, 1961). The morphology of hyphae was examined using the coverslip technique (Zhou et al., 1998) and then observed by light microscopy and scanning electron microscopy (Quanta 200; FEI) using gold-coated dehydrated specimens of 28-day-cultures from ISP 4 and ISP 5 agar. Motility of spores was tested by light microscopic observation of cells suspended in phosphate buffer (pH 7.0, 1 mM) for approximately 2 h at 28°C. Metabolic characters were examined by Biolog GEN III MicroPlates, and API 50CH and API ZYM test kits (bioMerieux) according to the manufacturers’ instructions. Results were evaluated after incubation at 28°C for 48–96 h. Oxidase activity was detected using API oxidase reagent (bioMerieux) according to the manufacturer’s instructions. Catalase activity was determined by evaluating the production of bubbles with addition of a drop of 3% (v/v) H₂O₂. Other physiological tests of the strain were examined according to previously described procedures (Yuan et al., 2008).

Growth of strain CPCC 204279T was observed at 20–40°C, pH 5.0–9.0, on TSA and ISP 2 media with the presence of 0–3% (w/v) NaCl. Optimum growth occurred at 28°C, pH 7.0, with the presence of 1% (w/v) NaCl. Convex-shaped colonies with plicate surface formed on ISP 2 media were ivory white to light carnation, and 0.8–1.1 mm in diameter after incubation at 28°C for 7 days. No diffusible pigments were produced on any tested media. Abundant yellowish-brown to brown substrate mycelium was formed on R2A, ISP 2, ISP 4, ISP 5 and ISP 7 agar, while moderate growth of substrate mycelium occurred on ISP 3, potato agar and nutrient agar media. White aerial hyphae occurred only on ISP 2, ISP 4, ISP 5 and ISP 7 after 4 weeks. Mature aerial hyphae were branched and fragment into rod-shaped elements (Fig. 1). Non-motile rod-shaped spores were observed.

Strain CPCC 204279T was positive for hydrolysis of gelatin, but negative for hydrolysis of starch and urea, peptonization of milk, and H₂S and indole production. Detailed physiological and biochemical characteristics of strain CPCC 204279T are given in Table 1 and the species description.

Biomass for molecular systematic and chemotaxonomic studies was obtained by cultivation of strain CPCC 204279T to logarithmic phase in shake flasks on a rotary shaker (150 r.p.m.) using trypticase soy broth (Difco) at 28°C. The sugar pattern and isomer of diaminopimelic acid in whole-cell hydrolysates were determined using TLC as described by Lechevalier & Lechevalier (1965, 1980). Purified peptidoglycan preparations were prepared by the method of Schleifer & Kandler (1972). Amino acids and peptides in cell-wall hydrolysates were analysed by two-dimensional ascending TLC on cellulose plates, using the solvent systems of Schleifer & Kandler (1972). Analysis of the N-acyl type of the muramic acid residues was performed as described by Uchida et al. (1999). Polar lipids were extracted, examined by two-dimensional TLC and identified using previously described procedures (Minnikin et al., 1984). Menaquinones were extracted according to Collins et al. (1977), analysed by HPLC (Groth et al., 1997) and then confirmed by LC/MS as described previously (Du et al., 2013). The extraction, derivatization and analysis of mycolic acids were determined with Sherlock Mycobacteria Identification System (MIDI) (Kellogg et al., 2001). Cellular fatty acids were extracted, methylated and analysed using the Sherlock Microbial Identification System (MIDI) according to the manufacturer’s instructions (Kroppenstedt, 1985; Meier et al., 1993). MIDI Sherlock Version 6.0 and ACTIN1 database were employed in this analysis.

Galactose and arabinose were detected as diagnostic sugars and meso-diaminopimelic acid was detected as the diamino acid in whole-cell hydrolysates. The muramic acid residues in the peptidoglycan were detected as N-acetylated. The phospholipids consisted of large amounts of diphosphatidylglycerol and phosphatidylethanolamine, as well as small amounts of phosphatidylinositol and phosphatidylglycerol mannosides (Fig. S1, available in the online Supplementary Material). In the menaquinones extraction, the ratio of 9:4:6 for MK-9(H₄) : MK-10(H₄) were determined. Mycolic acids were not detected. The major components in the cellular fatty acids profile were iso-C₁₆:₀ (20.0%), iso-C₁₆:₁ω₉c (12.2%), iso-C₁₆:₁ (10.7%) and C₁₆:₀ (10.6%) (Table S1).

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**Fig. 1.** Scanning electron micrograph of strain CPCC 204279T grown on ISP 4 agar for 28 days at 28°C. Bar, 10 μm.
**Table 1.** Differentiating characteristics of strain CPCC 204279T and phylogenetically related genera of the family Pseudonocardiaceae

Genera: 1, Herbithabits gen. nov. (strain CPCC 204279T, data from this study); 2, Actinophytocola (Indananda et al., 2010; Ara et al., 2011; Guo et al., 2011; Otoguro et al., 2011); 3, Labedaea (Lee, 2012); 4, Saccharopolyspora (Korn-Wendisch et al., 1989; Zhang et al., 2008; Cheng et al., 2013).

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<th>Characters</th>
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<td>Whole-cell sugars*</td>
<td>Ara, Gal</td>
<td>Ara, Gal, Man, Rib, Glu† Rha† (Xyl) †</td>
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<td>PE, DPG, OH-PE†, NPG†, NPL†, PL†</td>
<td>DPG, PDE, PG, PI, PL, L</td>
<td>DPG, PG, PI, PC†, PE†</td>
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<td>MK-9(H4)</td>
<td>MK-9(H4), MK-10(H2)†</td>
<td>MK-9(H4), MK-9(H2)†</td>
<td>MK-9(H4), MK-9(H2)†</td>
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<td>Major fatty acids</td>
<td>iso-C15:0, iso-C16:0, 2-OH, C16:1ω9c, C18:0, iso-C16:1</td>
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<td>iso-C15:0, iso-C16:0</td>
<td>iso-C15:0, iso-C17:0, anteiso-C15:0, iso-C18:1ω8c †, anteiso-C17:0 †</td>
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<td>U–A</td>
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* Ara, Arabinose; Gal, galactose; Glc, glucose; Man, mannosone; Rha, rhamnose; Rib, ribose; Xyl, xylose.
† Variable depending on species.
‡ DPG, Diphosphatidylglycerol; PC, phosphatidylcholine; PE, phosphatidylethanolamine; OH-PE, PE with hydroxyl fatty acid; PG, phosphatidylglycerol; PI, phosphatidylinositol; PIM, phosphatidylinositol mannoside; PDE, phosphatidylmethylethanolamine; NPL, ninhydrin positive phospholipid; L, unknown lipid; PL, unknown phospholipid.

Genomic DNA was extracted and PCR amplification of the 16S rRNA gene was performed from strain CPCC 204279T as described by Li et al. (2007), using the universal 16S rRNA gene-targeting primers 8F (5′-GAGTTTGATCCTGCTTCAG-3′) and 1492R (5′-ACGGCTACCTTGTTACGACTT-3′) and 1492R (5′-ACGGCTACCTTGTTACGACTT-3′). Purified PCR products were sequenced with an ABI PRISM automatic sequencer. The obtained sequence was compared with available 16S rRNA gene sequences from the GenBank database using the BLAST program and the EzTaxon-e server (http://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 MEGA software package version 5.0 (Tamura et al., 2011). Phylogenetic trees were reconstructed using the neighbour-joining method (Saitou & Nei, 1987) with Kmax values (Kimura, 1980, 1983) and complete deletion gaps, and the maximum-parsimony (Kluge & Farris, 1969) and maximum-likelihood (Felsenstein, 1981) methods. 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 Streptomyces griseus ATCC 23345T as a reference) using a UV-1700 spectrophotometer (SHIMADZU) equipped with a DCW-2008 thermo bath.

The G+C content in the genomic DNA of strain CPCC 204279T was detected as 73.2 mol%. The almost-complete 16S rRNA gene sequence (1496 bp) of strain CPCC 204279T was obtained. BLAST search results showed that the novel isolate exhibited the highest similarities with members of the family Pseudonocardiaceae, such as Actinophytocola timorensis ID05-A0653T (95.7 % 16S rRNA gene sequence similarity), Actinokineospora enzanensis DSM 44649T (95.3 %), Labedaea rhizosphaerae RS-49T (95.2 %) and 95.1–93.2 % with other members of the family Pseudonocardiaceae. In the phylogenetic tree based on 16S rRNA gene sequences of all genera within the family Pseudonocardiaceae, the strain CPCC 204279T formed a distinct lineage next to the genus Actinophytocola (Figs 2, S2 and S3), standing for a genus position, which indicated that strain CPCC 204279T could not be placed into any known genus.
Fig. 2. Neighbour-joining tree based on 16S rRNA gene sequences showing the relationship of strain CPCC 204279T with representatives of the family Pseudonocardiaceae. Numbers at nodes are bootstrap values shown as percentages of 1000 replicates; only values >50% are shown. Filled circles indicate that the corresponding nodes were also recovered in the trees generated with the maximum-likelihood and maximum-parsimony methods. Bar, 1 nt substitution per 100 nt.
which was defined for the family Pseudonocardiaceae (Labeleda et al., 2011). Additionally, strain CPCC 204279T possessed its own signature nucleotides pattern distinguished from other recognized genera at positions 69:99 (A–U), 100 (U), 184:193 (U–C), 185:192 (U–G), 200:217 (G–G), 201:216 (U–C), 258:268 (C–G), 1030 (G) and 1440:1461 (C–G) (Table 1).

In the cellular fatty acids profile, besides the fact that strain CPCC 204279T shared the common major fatty acid of iso-C\(_{16:0}\) with its close phylogenetic neighbours, iso-C\(_{16:0}\) 2-OH, C\(_{16:1}\) \(\omega\)9c, iso-C\(_{16:1}\) and C\(_{16:0}\) were also detected as the main components. MK-10(H\(_4\)) was detected in the menaquinones system and phosphatidylinositol mannosides was found in the polar lipids extracts. These data supported to differentiate strain CPCC 204279T from other genera in the family Pseudonocardiaceae.

On the basis of the phenotypic and phylogenetic distinctiveness presented above, strain CPCC 204279T represents a novel species of a new genus in the family Pseudonocardiaceae, for which the name Herbihabitans rhizosphaerae gen. nov., sp. nov. is proposed.

**Description of Herbihabitans gen. nov.**


Gram-positive, aerobic actinobacterium that produces branched substrate mycelia and sparse aerial mycelia on ISP 2, ISP 4, ISP 5 and ISP 7 media. Rod-shaped spores are non-motile. The genus is characterized by containing meso-diaminopimelic acid as the diamino acid, galactose and arabinose as diagnostic sugars, and diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol and phosphatidylinositol mannosides in the polar lipids profile. Mycolic acids are absent. The predominant menaquinone is MK-9(H\(_4\)). Major fatty acids are iso-C\(_{16:0}\), iso-C\(_{15:0}\) 2-OH, C\(_{16:1}\) \(\omega\)9c, iso-C\(_{16:1}\) and C\(_{16:0}\). Possesses the genus-specific signature nucleotides pattern at the following sites, 69:99 (A–U), 100 (U), 184:193 (U–C), 185:192 (U–G), 200:217 (G–G), 201:216 (U–C), 258:268 (C–G), 1030 (G) and 1440:1461 (C–G). The G+C content of the genomic DNA is about 73 mol%.

The type species is *Herbihabitans rhizosphaerae*.

**Description of Herbihabitans rhizosphaerae sp. nov.**

*Herbihabitans rhizosphaerae* (rhi.zo.spha’er.ae. Gr. fem. n. rhiza a root; L. fem. n. sphaera ball, sphere; N.L. gen. fem. n. *rhizosphaerae* of the rhizosphere, referring to the site from which the type strain was isolated).

In addition to the characteristics given in the genus description, the properties of the species are detailed as follows. The pH, NaCl concentration and temperature ranges for growth are pH 5.0–9.0, 0–3 % NaCl (w/v) and 20–40 °C, with optimum growth at pH 7.0, with 1 % NaCl (w/v) and at 28 °C, respectively. Colonies are convex-shaped with plicate surface on ISP 2, ISP 4, ISP 5 and ISP 7 agar. No diffusible pigments are produced. Positive for catalase and oxidase activities, and hydrolysis of gelatin and Tween 40, but negative for hydrolysis of starch and urea, peptonization of milk, H\(_2\)S and indole production, and nitrate reduction. Can utilize D-arabinose, cellobiose, dextrin, D-fucose, D-galactose, D-gluconic acid, D-glucuronic acid, D-mannitol, D-mannose, D-salicin, turanose, glucuronamide, glycerol, L-fucose and \(\alpha\)-D-glucose as sole carbon sources. Produces acid from D-arabinose, cellobiose and glycerol. Positive for activities of alkaline phosphatase, chymotrypsin, cystine arylamidase, esterase (C4), esterase lipase (C8), \(\beta\)-glucosidase and lipase (C14).

The type strain is CPCC 204279T (=NBRC 111774=DSM 101727\(^T\)), which was isolated a rhizosphere soil sample of the herb Limonium sinense collected from Xinjiang Province, China. The DNA G+C content of the type strain is 73.2 mol%.

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**References**


