Labedaea rhizosphaerae gen. nov., sp. nov., isolated from rhizosphere soil

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A novel actinomycete, designated strain RS-49T, was isolated from the rhizosphere soil of a cliff-associated plant (Peucedanum japonicum Thunb.) in the Republic of Korea and subjected to a polyphasic taxonomic study. The results of comparative 16S rRNA gene sequence analyses showed that the organism belonged to the family Pseudonocardiaceae, suborder Pseudonocardineae and that it was most closely related to members of the genera Kibdelosporangium (96.6–97.0 % sequence similarity), Actinokineospora (96.3–96.7 %), Streptoaelliteichus (96.2 %) and Actinophytocola (96.2 %). Substrate mycelia were well-developed and whitish or pale yellow to strong yellow. Aerial mycelia were branched and fragmented into rod-shaped elements. Single spherical spores were produced directly on the substrate mycelium. Sporangium-like structures and fragmentation of the substrate mycelium were absent. The diagnostic diamino acid in the cell-wall peptidoglycan was meso-diaminopimelic acid. The acyl type of the muramic acid residues in the peptidoglycan was N-acetylated. Whole-cell sugars were glucose, rhamnose, galactose, ribose, mannose, arabinose and xylose. The major menaquinone was MK-9(H4). Small amounts of MK-8 and MK-9(H2) were also detected. The polar lipids contained diphosphatidylglycerol, phosphatidylmethylethanolamine, phosphatidylglycerol, phosphatidylinositol, an unknown phospholipid and an unknown lipid. The predominant fatty acids were iso-C15 : 0 and iso-C16 : 0. The DNA G+C content was 64.2 mol%. The phenotypic and phylogenetic characteristics show that strain RS-49T can be differentiated from members of all genera in the suborder Pseudonocardineae and thus represents a novel species in a new genus for which the name Labedaea rhizosphaerae gen. nov., sp. nov. is proposed; the type strain of the type species is RS-49T (=KCTC 19662T=DSM 45361T).

The family Pseudonocardiaceae as emended by Stackebrandt et al. (1997) contained 12 genera: Pseudonocardia (Hessen, 1957) as the type genus, Actinopolyspora (Gochtner et al., 1975), Actinosynema (Hasegawa et al., 1978), Anycolotaxis (Lechevalier et al., 1986), Kibdelosporangium (Shearer et al., 1986), Kutneria (Stackebrandt et al., 1994), Lentzea (Yassin et al., 1995), Saccharomonospora (Nonomura & Ohara, 1971), Saccharopolyspora (Lacey & Goodfellow, 1975), Saccharothrix (Labeda et al., 1984), Streptoaelliteichus (Tomita et al., 1978) and Thermocrispum (Korn-Wendisch et al., 1995). Among them, the genera Actinosynema, Lentzea and Saccharothrix were transferred to the family Actinosynemataceae (Labeda & Kroppenstedt, 2000), but were recently reassigned to the emended family Pseudonocardiaceae Embley et al. 1989 emend. Zhi et al. 2009 (Labeda et al., 2011). The genus Actinopolyspora was recently assigned to the family Actinopolysporaceae (suborder Actinopolysporineae) in an extensive study of higher taxonomic ranks of the class Actinobacteria (Zhi et al., 2009). During the past decade, nine novel genera have been added to the family Pseudonocardiaceae: Actinoalloteichus (Tamura et al., 2000), Actinomycetospora (Jiang et al., 2008), Actinophytocola (Indananda et al., 2010), Allokutzneria (Labeda & Kroppenstedt, 2008), Crossiella (Labeda, 2001), Goodfellowiella (Labeda et al., 2008), Prauserella (Kim & Goodfellow, 1999), Sciscionella (Tian et al., 2009) and Yuhushiella (Mao et al., 2011). During a study of the bacterial diversity in rhizosphere soils, a mycelium-forming actinomycete, strain RS-49T, was isolated and was assigned to the family Pseudonocardiaceae by using 16S rRNA gene sequence comparisons. Here, identification and classification of the isolate based on data from a polyphasic taxonomic study are reported.

Strain RS-49T was isolated from the rhizosphere soil of a cliff-associated plant (Peucedanum japonicum Thunb.) on Mara Island, Jeju, Republic of Korea. The procedure and medium for bacterial isolation followed the method described by Lee (2009). The isolate was maintained on ISP (International Streptomyces Project) 2 medium (Shirling & Gottlieb, 1966) and as 20 % (v/v) glycerol suspensions at

The GenBank/EMBL/DBJ accession number for the 16S rRNA gene sequence of strain RS-49T is FM998036.

A supplementary figure and a supplementary table are available with the online version of this paper.
—20 °C and —80 °C. For morphological comparisons, \( Kibdelosporangium\) aridum DSM 43828\(^T\) was grown on ISP 2 medium.

The cultural characteristics of strain RS-49\(^T\) were tested on ISP 2–7 media (Shirling & Gottlieb, 1966), oatmeal-nitrate agar (Prauser & Bergholz, 1974), soil extract agar (Thiemann et al., 1968) and water agar (15.0 g agar, 1000 ml tap water) after incubation for 3 weeks at 30 °C. Cell morphology was observed with a scanning electron microscope (model JSM-6500; JEOL), using cultures incubated for 3–6 weeks. Specimens were fixed with a vapour of 1% osmium tetroxide, dehydrated and critical-point-dried before observation. Growth at various temperatures (4, 10, 20, 25, 30, 37, 42 and 45 °C) and pH (pH 4.1–10.1 at intervals of 1.0 pH unit) and NaCl tolerance [determined in 1–9% (w/v) NaCl (at intervals of 1%)] were assessed using ISP 2 medium. Growth temperatures were recorded after 14 days of incubation. Growth pH and NaCl tolerance tests were observed after incubation for 14 days at 30 °C. Utilization of various carbohydrates was examined on ISP 9 medium (Shirling & Gottlieb, 1966) supplemented with each filter-sterilized carbon source at a final concentration of 1% (w/v) for sugars and alcohols and 0.1% (w/v) for organic acids; the results were recorded after 3 weeks incubation at 30 °C. Hydrolysis of DNA and starch was determined on DNase test agar (Difco) and starch agar (Difco), respectively. Gram staining, enzyme activities (oxidase, catalase, nitrate reductase, urease and gelatinase) and hydrolysis of aesculin and casein were tested by using the methods of MacFaddin (1980). Degradation of hypoxanthine, L-tyrosine and xanthine was determined as described by Gordon et al. (1974).

Strain RS-49\(^T\) showed moderate to good growth on all the media tested except for ISP 7 medium, on which it did not grow. Substrate mycelia were well-developed and whitish or pale yellow to strong yellow depending on the medium. Diffusible pigments were not produced on any of the media tested. The cultural characteristics of strain RS-49\(^T\) are given in Table S1 (available in IJSEM Online). Aerial mycelium was branched and abundantly to moderately produced on ISP 3, 4 and 5 media, soil extract agar and water agar and fragmented into rod-shaped elements with smooth surfaces (Fig. 1a and b). Hyphal swelling on the tips of branched aerial hyphae (Fig. 1c) and single spherical spores (1 \( \mu m \) in diameter) borne directly on the substrate mycelium (Fig. 1d) were observed after incubation for 6 weeks at 30 °C on soil extract agar. Fragmentation of the substrate mycelium was not detected. Although sporangia or naked sporangium-like structures of \( K.\) aridum DSM 43828\(^T\) were observed in this study, as described by Shearer et al. (1986), strain RS-49\(^T\) did not produce sporangium-like structures on any of the media tested. Data for physiological and biochemical properties are given in the species description.

For chemotaxonomic studies of strain RS-49\(^T\), freeze-dried biomass was obtained from cells grown in trypticase soy broth (Difco) for 3 days at 30 °C in a shaking incubator. Polar lipids were extracted and examined by two-dimensional TLC as described by Minnikin et al. (1977). Menaquinones
were analysed by HPLC (Kroppenstedt, 1985). Extraction and analysis of mycolic acids followed the procedure described by Minnikin et al. (1980). The dianaminopimelic acid isomer was determined according to the method of Stanek & Roberts (1974). Analysis of the N-acyl type of the muramic acid residues was performed as described by Uchida & Aida (1984). Sugar composition in whole-cell hydrolysates was determined by GC (Saddler et al., 1991). Cellular fatty acid methyl esters were prepared and analysed by GC according to the instructions of the Microbial Identification System (version 2.11; MIDI), using the AEROBE package including the TSBA (version 3.9), CLIN (version 3.9) and M17H10 (version 3.8) databases for the identification of fatty acids. For analysis of cellular fatty acids, cells were grown on trypticase soy agar at 30 °C for 5 days. The DNA G+C content of strain RS-49T was determined according to the procedure of Mesbah et al. (1989); genomic DNA was extracted by using the method of Hopwood et al. (1985).

meso-Diaminopimelic acid was the diagnostic diamino acid in the cell-wall peptidoglycan. The muramic acid residues were N-acetylated. Whole-cell sugars were glucose, rhamnose, galactose, ribose, mannose, arabinose and xylose in decreasing amounts. The polar lipid profile of strain RS-49T contained diphosphatidylglycerol, phosphatidylglycerol, phosphatidylcholine, phospholipid, and an unknown phospholipid and an unknown lipid (see Fig. S1). The menaquinones of strain RS-49T were MK-9(H2), MK-8 and MK-9(H2) in the ratio of 69:28:3.
The predominant fatty acids were iso-branched fatty acids, iso-C15:0 (27.5 %) and iso-C16:0 (24.1 %). The other components (> 1 % of the total fatty acids) were anteiso-C17:0 (6.2 %), iso-C16:1 H (6.0 %), anteiso-C15:0 (4.8 %), C17:1ω6c (4.2 %), iso-C17:0 (3.4 %), iso-C14:0 (3.1 %), C15:0 (3.0 %), iso-C17:1ω9c (2.2 %), C15:1ω6c (2.0 %), anteiso-C17:1ω9c (1.3 %) and C16:1ω7c and or iso-C15:0 2-OH (7.4 %). The DNA G+C content of strain RS-49T was 64.2 mol%.

Amplification of the 16S rRNA gene by PCR was performed according to the procedure described by Lee (2006). The PCR product was purified using the Wizard PCR preps DNA Purification System (Promega) and subjected to direct sequencing using the ABI PRISM BigDye Terminator cycle sequencing kit (Applied Biosystems) and an automatic DNA sequencer (model 3730xl; Applied Biosystems). A partial 16S rRNA gene sequence (1424 nt) of strain RS-49T was subjected to a preliminary BLAST search (Altschul et al., 1997) against the GenBank database, revealing that the isolate belongs to the suborder Pseudonocardiineae. Multiple alignments of the sequences of strain RS-49T and related taxa were performed by using the program CLUSTAL_X (Thompson et al., 1997) and manually optimized by comparison with the secondary structure of the Escherichia coli sequence (Brosius et al., 1978). Phylogenetic analyses were performed by using the neighbour-joining (Saitou & Nei, 1987), maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Fitch, 1971) treeing algorithms. A phylogenetic tree was constructed by using the neighbour-joining method and evolutionary distances were generated by using the model of Jukes & Cantor (1969) and displayed using the program TreeView (Page, 1996). Bootstrap analysis (Felsenstein, 1985) was performed by using 1000 neighbour-joining datasets.

A neighbour-joining tree (Fig. 2) based on 16S rRNA gene sequences showed that strain RS-49T belonged to the family Pseudonocardiaeeae and formed a monophyletic line between the genus Kibdelosporangium and the Pseudonocardia–Actinomycetospora cluster, albeit with a moderate bootstrap value of 49 %. This branching pattern was also found in the maximum-likelihood tree, whereas strain RS-49T formed a monophyletic line at the base of a Kibdelosporangium–Pseudonocardia–Actinomycetospora cluster in a maximum-parsimony tree (data not shown). The highest levels of 16S rRNA gene sequence similarity were recorded between strain RS-49T and members of genera in the suborder Pseudonocardiineae: Kibdelosporangium (96.6–97.0 %), Actinophytocola (96.2 %), Streptotaleichus (96.2 %), Actinokineospora riparia (96.2 %) and Pseudonocardia (94.7–96.0 %). The 16S rRNA gene sequence similarity values between strain RS-49T and the other type strains of the suborder Pseudonocardiineae were less than 96.0 %. The genera Streptotaleichus and Actinokineospora occupied phylogenetic positions that were separate from strain RS-49T and formed a coherent cluster, together with the genera Actinalloteichus, Allokutzneria, Crossiella, Goodfellowiella and Kutzneria and members of the family Actinosynmataceae (Kim & Goodfellow, 1997; Labeda & Kroppenstedt, 2000). These relationships were supported by a high bootstrap value of 81 % and by all the treeing algorithms applied.

The differential characteristics of strain RS-49T and phylogenetically related genera of the family Pseudonocardiaeeae are given in Table 1. Strain RS-49T can be morphologically differentiated from the closest phylogenetic genus, Kibdelosporangium, in that it does not produce sporangium-like structures. Furthermore, strain RS-49T does not have phosphate(yl)ethanolamine or phosphatidylinositol mannoside as polar lipids and madurose as a whole-cell sugar, in contrast to members of the genus Kibdelosporangium. The genera Actinophytocola and Pseudonocardia are morphologically similar to strain RS-49T in that they produce cylindrical spores on aerial mycelium without the formation of sporangium-like structures. However, they differ from strain RS-49T mainly in their DNA G+C contents and polar lipid profiles. Strain RS-49T can also be distinguished from members of the genus Pseudonocardia by the major menaquinone and fatty acid profiles.

On the basis of the phenotypic and phylogenetic distinctiveness presented here, strain RS-49T is considered to represent a novel species of a new genus in the family Pseudonocardiaeeae, for which the name Labedae rhizosphaeræ gen. nov., sp. nov. is proposed.
Description of Labedaea gen. nov.

Labedaea (La.be.da’e.a. N.L. fem. n. Labedaea named after David P. Labeda, a microbiologist who has contributed significantly to the systematics of actinomycetes).

Aerobic, Gram-positive, non-acid-fast, non-motile actinomycete. Aerial hyphae are branched and fragment into rod-shaped elements. Hyphal swelling occurs on the tips of branched aerial hyphae and single spherical spores (1 μm in diameter) are observed on the substrate mycelium on soil extract agar. Fragmentation of the substrate mycelium does not occur and sporangium-like structures are not produced. meso-Diaminopimelic acid is the diagnostic diamino acid in the cell-wall peptidoglycan. The acyl type of the muramic acid residues is N-acetylated. Whole-cell sugars are glucose, rhamnose, galactose, ribose, mannose, arabinose and xylose. The predominant menaquinone is MK-9(H4); small amounts of MK-8 and MK-9(H2) are also detected. The polar lipid profile contains diphosphatidylglycerol, phosphatidylglycerol, phosphatidylmethylethanolamine, phosphatidylglycerol, phosphatidylinositol, an unknown phospholipid and an unknown lipid. The predominant fatty acids are detected. The type species is Labedaea rhizosphaerae.
### Table 1. Morphological and chemotaxonomic characteristics of strain RS-49T and phylogenetically related genera of the family Pseudonocardiaceae

<table>
<thead>
<tr>
<th>Characters</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
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<tbody>
<tr>
<td>Morphology</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Aerial mycelium</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>v</td>
</tr>
<tr>
<td>Fragmented mycelia</td>
<td>+</td>
<td>v</td>
<td>+</td>
<td>v</td>
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<tr>
<td>Sporangium-like structures</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Whole-cell sugars*</td>
<td>Glc, Rha, Gal, Rib, Man, Ara, Xyl</td>
<td>Ara, Gal, Man, Rib, Glc+, Rha+, (Xyl)†</td>
<td>Ara, Gal, (Mad)</td>
<td>Ara, Gal</td>
</tr>
<tr>
<td>Polar lipids‡</td>
<td>DPG, PDE, PG, PI, PL, L</td>
<td>PE, DPG, OH-PE†, NPG†, NPL†, PI+</td>
<td>PE, PI, PME, PG, DPG, PIM</td>
<td>PE or PG, PME, PI, PG, DPG, GlcNu</td>
</tr>
<tr>
<td>Major menaquinone(s)</td>
<td>MK-9(H4) i-C15:0, i-C16:0</td>
<td>MK-9(H4), MK-10(H2)†, i-C16:0, i-C14:0, i-C15:0, i-C14:1 H4, C17:0 O6†</td>
<td>MK-9(H4) i-C15:0, i-C16:0, ai-C17:0</td>
<td>MK-9(H4) i-C16:0, C16:0</td>
</tr>
<tr>
<td>Major fatty acids§</td>
<td></td>
<td></td>
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<tr>
<td>DNA G+C content (mol%)</td>
<td>64.2</td>
<td>69.7–71.2</td>
<td>66</td>
<td>68–79</td>
</tr>
</tbody>
</table>

* Ara, Arabinose; Gal, galactose; Glc, glucose; Mad, madurose; Man, mannose; Rha, rhamnose; Rib, ribose; Xyl, xylose.
† Variable depending on species.
‡ DPG, Diphosphatidylglycerol; PC, phosphatidylycholine; PE, phosphatidylethanolamine; OH-PE, PE with hydroxyl fatty acid; PG, phosphatidylglycerol; PI, phosphatidylinositol; PIM, phosphatidylinositol mannoside; DPE, phosphatidylmethylglycerolphosphatidylethanolamine; PGE, phosphatidylglycerolphosphatidylethanolamine; GlcNu, N-acetylglucosamine-containing phospholipids; NPG, ninhydrin-positive glycosphosphatidylethanolamine; NPL, ninhydrin-positive phospholipid; L, unknown lipid; PL, unknown phospholipid.
§ i, iso; ai, anteiso.

**Description of Labedaea rhizosphaerae sp. nov.**


The morphological and chemotaxonomic characteristics are given in the genus description. Grows well on ISP 2, 3, 4 and 5 media and moderately on ISP 6, oatmeal-nitrate agar, soil extract agar and water agar. Substrate mycelia are well-developed and whitish or pale yellow to strong yellow depending on the medium. No diffusible pigments are produced. Aerial mycelium is produced abundantly on ISP 2, 3, 4 and 5 media and soil extract agar and moderately on water agar. Grows at 25–42 °C (optimum at 30–37 °C) and pH 5.1–10.1 (optimum at pH 6.1–9.1). Growth does not occur below 20 °C or at 45 °C. Growth occurs in the presence of up to 3.0 % (w/v) NaCl, but not in 4.0 % (w/v) NaCl. Hydrolyses casein, DNA, aesculin and gelatin, but not carboxymethyl-cellulose, starch or urea. Nitrate reduction and H₂S production do not occur. Decomposes hypoxanthine and D,L-tyrosine, but not xanthine. Utilizes cellobiose, dextran, D-fructose, D-glucose, lactose, maltose, melezitose, melibiose, raffinose, L-rhamnose, sucrose, trehalose, D-xylene and D-mannitol as sole carbon and energy sources. Does not utilize D-arabinose, L-arabinose, D-galactose, D-mannose, methyl D-glucoside, methyl D-mannoside, L-ribose, salicin, L-sorbose, adonitol, D- dulcitol, meso-erythritol, glycerol, myo-inositol, D-sorbitol or D-xylitol.

Citrate is assimilated, but acetate, benzoate, formate, DL-malate, succinate and L-tartrate are not.

The type strain, RS-49T (=KCTC 19662T =DSM 45361T), was isolated from the rhizosphere soil of a cliff-associated plant (_Peucedanum japonicum_ Thunb.) on Mara Island, Jeju, Republic of Korea. The DNA G+C content of the type strain is 64.2 mol%.

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**References**


