Actinorhabdospora filicis gen. nov., sp. nov., a new member of the family Micromonosporaceae

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The actinomycete strains K12-0408T and K12-0792 were isolated on CM-cellulose agar from rhizosphere soil of a pteridophytic plant collected in Tokyo prefecture, Japan. Their taxonomic positions were determined using a polyphasic approach. Phylogenetic analysis based on 16S rRNA gene sequences revealed that strains K12-0408T and K12-0792 were positioned within the family Micromonosporaceae. The strains formed extensively branched aerial and substrate mycelia. Long chains of cylindrical spores with smooth surfaces were formed on aerial hyphae. The cell wall contained meso-diaminopimelic acid, and galactose, glucose, mannose and ribose were detected in whole-cell hydrolysates. The predominant menaquinones were MK-10(H6) and MK-10(H4). The major cellular fatty acids were anteiso-C17:0 and anteiso-C17:0 2-OH. The DNA G+C contents of strains K12-0408T and K12-0792 were 69.6 and 69.7 mol%, respectively. Based on data from the present polyphasic taxonomic study, strains K12-0408T and K12-0792 represent a novel genus, for which the name Actinorhabdospora gen. nov. is proposed, with strain K12-0408T (=NBRC 111897T=TBRC 5327T) as the type strain of the type species, Actinorhabdospora filicis sp. nov.

The family Micromonosporaceae was first described by Krasiñnikov (1938) and the description was amended by Koch et al. (1996), Stackebrandt et al. (1997) and Zhi et al. (2009) on the basis of chemotaxonomic data and 16S rRNA gene sequence analysis. During the characterization of actinomycetes isolated from rhizospheric soil of fern plants, strains K12-0408T and K12-0792 were recovered. Phylogenetic analysis based on nearly complete 16S rRNA gene sequences indicated that the two strains were distinct from genera within the family Micromonosporaceae. Herein, we report the results of phenotypic, chemotaxonomic and genomic analyses of strains K12-0408T and K12-0792, which suggested that the strains represented a novel species of a new genus.

Strains K12-0408T and K12-0792 were isolated from rhizosphere soil of ferns (pteridophyte plants) collected at Fussa and Hamura city, respectively, located in Tokyo prefecture, Japan. The CM-cellulose (CMC) medium used for the isolation procedure contained the following: 1.0 % CM-cellulose sodium salts, 0.1 % KH2PO4, 0.1 % L-asparagine monohydrate, 0.0001 % FeSO4·7H2O, 0.0001 % MnCl2·4H2O, 0.0001 % ZnSO4·7H2O and 1.5 % agar (adjusted to pH 7.0±0.2 with 1 M NaOH). The medium was supplemented with 0.025 % nalidixic acid and 0.02 % Benlate (Dupont) to minimize and inhibit growth of unwanted bacteria and fungi, respectively. The pure cultures of strains K12-0408T and K12-0792 were maintained on International Streptomyces Project (ISP) medium 2 (Shirling & Gottlieb, 1966) and in 20 % (v/v) glycerol suspension at −20 °C. Biomass for taxonomic characterization was obtained from cultures grown at 27 °C for 7 days in yeast extract-dextrose (YD) broth containing 1.0 % yeast extract and 1.0 % glucose.

The morphological characteristics of strains K12-0408T and K12-0792 were observed via a scanning electron microscope (JEOL-JSM 5600; JEOL), using cultures grown on ISP medium 4 at 27 °C for 7 days. Cultural characteristics were determined from the growth on ISP media 2, 3, 4 and 5, nutrient agar (NA; Difco), potato dextrose agar (PDA;
Difco) and Czapek’s agar (3 % sucrose, 0.3 % NaNO₃, 0.1 % K₂HPO₄, 0.05 % MgSO₄·7H₂O, 0.05 % KCl, 0.001 % FeSO₄·7H₂O, 1.5 % agar, adjusted to pH 7.0±0.2 with 1 M NaOH) after 14 days of incubation at 27 °C. The Colour Harmony Manual (Jacobson et al., 1958) was used for determining colour designations and names. Carbon-source utilization was tested using ISP medium 9 (Shirling & Gottlieb, 1966) as a basal medium, supplemented with a final concentration of 1 % (w/v) of the carbon sources. Acid production from carbon sources was examined using a basal inorganic nitrogen medium recommended by Gordon et al. (1974). Decomposition of adenine, casein, hypoxanthine, xanthine and urea and the hydrolysis of starch were determined using the methods described by Gordon et al. (1974). A gelatin hydrolysis test was performed following the method of Medina & Baresi (2007). Reduction of nitrate to nitrite was determined on media as described by Gordon & Mihm (1957). The production of hydrogen sulfide was detected on peptone-iron agar (Difco). Enzyme activities were determined using the API ZYM test kit (bioMérieux) according to the manufacturer’s instructions. Catalase and oxidase activities were determined with a 3 % (v/v) hydrogen peroxide solution and 1 % tetramethyl p-phenylenediamine solution, respectively. Growth at a range of temperatures was determined on ISP medium 2 after cultivation for 14–21 days using a gradient temperature (5–50 °C). Tolerance of NaCl (0–5 %, 1 % intervals) and pH range (3.0–11.0 at intervals of 1.0 pH unit using biological buffers: bicarbonate/carbonate buffer, citrate buffer and phosphate buffer) for growth were determined on ISP medium 2 by incubation at 27 °C for 14–21 days.

The isomer of diaminopimelic acid (DAP) in the cell wall was determined using the method of Hasegawa et al. (1983). The acyl type of the muramic acid in the peptidoglycan was determined using the method of Uchida & Aida (1977). The whole-cell sugar compositions were determined following the method of Stanek & Roberts (1974). Phospholipids in the cells were extracted and detected by two-dimensional TLC, according to the methods proposed by Minnink et al. (1977). To obtain whole-cell fatty acid profiles, the strains were grown at 27 °C for 8–9 days in YD broth. Analysis of fatty acid methyl esters was performed by GLC, according to the procedures of the Sherlock Microbial Identification System (Microbial ID; MIDI, Version 6.0) using the ACTIN6 method. Menaquinones were extracted from freeze-dried biomass using the procedure of Collins et al. (1977) and subsequently analysed by LC/MS (JMS-T100LP; JEOL) with CAPCELL PAK C18 UG120 column (Shiseido) using methanol/2-propanol (7:3). Mycolic acid methyl esters were examined by TLC, according to the method of Tomiyasu (1982).

Amplification of the 16S rRNA gene and its sequencing were performed according to Také et al. (2015). The assembled sequence was compared with sequences of type strains in the EzTaxon-e server (http://eztaxon-e.ezbiocloud.net/) (Kim et al., 2012). Evolutionary trees were reconstructed using the neighbour-joining (Saitou & Nei, 1987), maximum-parsimony (Fitch, 1971) and maximum-likelihood (Felsenstein, 1993) tree-making algorithms drawn from the MEGA 6 (Tamura et al., 2013) packages; an evolutionary distance matrix for the neighbour-joining algorithm was generated using Kimura’s two parameter model (Kimura, 1980). The topologies of the resultant trees were evaluated by bootstrap analysis with 1000 replicates (Felsenstein, 1985). The DNA G+C contents were determined by HPLC according to the method of Tamaoka & Komagata (1984) on the DNA extracted according to the protocol of Saito & Miura (1963). Levels of DNA–DNA relatedness were assayed using microplate hybridization, according to the method of Ezaki et al. (1989).

Strains K12-0408T and K12-0792 formed extensively branched aerial and substrate mycelium. The substrate mycelia were fragmented into rod-shaped elements. Long spore chains (containing more than 10 spores per chain) were produced on aerial hyphae. The spores were cylindrical (approximately 0.3–0.5×1.4–1.8 μm in size) with a smooth surface (Fig. 1). Spores were non-motile. Sporangia were not observed. The colour of the substrate mycelium was light yellow to pale yellow on most media tested (except for ISP medium 5 and Czapek’s agar) and the aerial mycelium was white (Table 1). Both strains grew well on ISP media 2 and 4, NA, PDA and Czapek’s agar and showed poor growth on ISP media 3 and 5. Melanoid and soluble pigments were not produced. The physiological and biochemical characteristics of the strains are presented in the species description.

Whole-cell hydrolysates of strains K12-0408T and K12-0792 contained the amino acid meso-DAP with the sugars galactose, glucose, mannose and ribose. This result suggests that the whole-cell sugar profile was pattern C of Lechevalier & Lechevalier (1970). The N-acyl type of the cell-wall muramic acid was glycolyl. The predominant menaquinones were MK-10(H₄) (48.7 %) and MK-10(H₄) (35.4 %) and small amounts of MK-9(H₄), MK-9(H₀) and MK-10(H₄) were also present. Phosphatidyethanolamine, diphosphatidylglycerol, hydroxy...
Table 1. Growth and cultural characteristics of strains K12-0408<sup>T</sup> and K12-0792

<table>
<thead>
<tr>
<th>Medium</th>
<th>Strain K12-0408&lt;sup&gt;T&lt;/sup&gt;</th>
<th>Strain K12-0792</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth</td>
<td>Aerial mycelium (colour)</td>
<td>Reverse colour</td>
</tr>
<tr>
<td>ISP medium 2</td>
<td>Good</td>
<td>Moderate (white)</td>
</tr>
<tr>
<td>ISP medium 3</td>
<td>Poor</td>
<td>Moderate (white)</td>
</tr>
<tr>
<td>ISP medium 4</td>
<td>Moderate</td>
<td>Good (white)</td>
</tr>
<tr>
<td>ISP medium 5</td>
<td>Poor</td>
<td>Sparse (white)</td>
</tr>
<tr>
<td>NA</td>
<td>Good</td>
<td>Moderate (white)</td>
</tr>
<tr>
<td>PDA</td>
<td>Poor</td>
<td>Sparse (white)</td>
</tr>
<tr>
<td>Czapek’s agar</td>
<td>Moderate</td>
<td>Moderate (white)</td>
</tr>
</tbody>
</table>

The 16S rRNA gene sequence of strain K12-0408<sup>T</sup> showed 100% similarity to that of strain K12-0792. The DNA–DNA relatedness values between strains K12-0408<sup>T</sup> and K12-0792 were higher than 90%, suggesting that they are members of the same species. The DNA G+C contents for strains K12-0408<sup>T</sup> and K12-0792 were 69.6 and 69.7 mol%, respectively. A phylogenetic tree of 16S rRNA gene sequences was constructed by the neighbour-joining method indicated that strains K12-0408<sup>T</sup> and K12-0792 were positioned within the family Micromonosporaceae. The two strains formed a clade with *Phytomonospora endophytica* YIM 65646<sup>T</sup> (type species of the genus, similarity levels 97.2–97.3%; Li et al., 2011) and *P. cypria* KT1403 (97.4–97.5%; Sahin et al., 2015), and separate from other related genera belonging to the family *Micromonosporaceae* in the neighbour-joining (Fig. 2), maximum-likelihood and maximum parsimony trees (Figs S2 and S3). The level of DNA–DNA relatedness between *P. endophytica* YIM 65646<sup>T</sup> and strain K12-0408<sup>T</sup> was 14.5%. Strains K12-0408<sup>T</sup> and K12-0792 were distinguished from members of the genus *Phytomonospora* in their cellular fatty acid profile, menaquinone composition and phospholipid pattern (Table 3). Furthermore, strains K12-0408<sup>T</sup> and K12-0792 produced aerial mycelium with long spore chains while members of the genus *Phytomonospora* produce single spores on substrate mycelium and do not produce aerial hyphae.

The morphological features of strains K12-0408<sup>T</sup> and K12-0792 were similar to those of 16 genera in the family *Micromonosporaceae* in terms of spore-chain production, but both strains could be clearly distinguished from the genera *Asanoa*, *Catenuloplanes*, *Couphiococcus*, *Krasilnikovia*, *Longispora*, *Phytobacillus*, *Polymorphosphora*, *Rhizocola*, *Spirilliplanes*, *Wangella* and *Xiangella* on the basis of fatty acid profile compositions, and distinguished from the genera *Actinocatenispora*, *Catellatospora*, and *Hama*dae on the basis of predominant menaquinones. In addition, they could be distinguished from the genera *Allocatelliglobosispora* and *Catelliglobosispora* based on differences in diamino acids type in the cell wall (Table 3). On the basis of their morphological features, chemotaxonomic characteristics and phylogenetic analysis, strains K12-0408<sup>T</sup> and K12-0792 are readily distinguishable from members of all established genera in the family

### Table 2. Cellular fatty acid profiles of strains K12-0408<sup>T</sup> and K12-0792

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Strain K12-0408&lt;sup&gt;T&lt;/sup&gt;</th>
<th>Strain K12-0792</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saturated</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C&lt;sub&gt;17:0&lt;/sub&gt;</td>
<td>5.4</td>
<td>5.7</td>
</tr>
<tr>
<td>Branched</td>
<td></td>
<td></td>
</tr>
<tr>
<td>iso-C&lt;sub&gt;15:0&lt;/sub&gt;</td>
<td>9.8</td>
<td>11.2</td>
</tr>
<tr>
<td>anteiso-C&lt;sub&gt;15:0&lt;/sub&gt;</td>
<td>6.5</td>
<td>6.4</td>
</tr>
<tr>
<td>iso-C&lt;sub&gt;16:0&lt;/sub&gt;</td>
<td>5.1</td>
<td>2.7</td>
</tr>
<tr>
<td>iso-C&lt;sub&gt;16:0&lt;/sub&gt; 2-OH</td>
<td>3.1</td>
<td>1.7</td>
</tr>
<tr>
<td>iso-C&lt;sub&gt;17:0&lt;/sub&gt;</td>
<td>5.8</td>
<td>7.7</td>
</tr>
<tr>
<td>iso-C&lt;sub&gt;17:0&lt;/sub&gt; 2-OH</td>
<td>2.0</td>
<td>2.1</td>
</tr>
<tr>
<td>anteiso-C&lt;sub&gt;17:0&lt;/sub&gt;</td>
<td>23.3</td>
<td>26.0</td>
</tr>
<tr>
<td>anteiso-C&lt;sub&gt;17:0&lt;/sub&gt; 2-OH</td>
<td>17.9</td>
<td>18.4</td>
</tr>
<tr>
<td>10-methylated</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10-methyl C&lt;sub&gt;17:0&lt;/sub&gt;</td>
<td>5.75</td>
<td>3.6</td>
</tr>
<tr>
<td>10-methyl C&lt;sub&gt;18:0&lt;/sub&gt; (TBSA)*</td>
<td>2.19</td>
<td>2.2</td>
</tr>
</tbody>
</table>

*TBSA, tuberculostearic acid.*
**Description of Actinorhabdospora filicis gen. nov.**

*Actinorhabdospora* (Ac.ti.no.rhab.do.sp'o'ra. Gr. n. *actis*, actinos ray; Gr. n. *rhabdo* rod shape; Gr. n. *spora* a seed, and in biology, a spore; N.L. fem. n. *Actinorhabdospora* actinomycetes forming rod-shaped spores).

Aerobic and Gram-stain-positive. Aerial mycelium is well developed, white and forms long chains of cylindrical spores (more than 10 spores per chain) with smooth surfaces. Spores are non-motile and sporangia are not observed. Fragments of substrate mycelia are observed. Cell wall contains meso-DAP. Whole-cell hydrolysates contain galactose, glucose, mannose and ribose. Phosphatidylethanolamine is detected (phospholipid pattern type PI). The major fatty acids are anteiso-C<sub>17:0</sub>3-OH and anteiso-C<sub>17:0</sub> 2-OH. The predominant menaquinones are MK-10(H<sub>4</sub>) and MK-10(H<sub>6</sub>). Mycolic acids are not detected. The type species is *Actinorhabdospora filicis* sp. nov.

**Description of Actinorhabdospora filicis sp. nov.**

*Actinorhabdospora filicis* (fi.l'i.cis. L. gen. n. *filicis* of a fern plant, referring to the source of the rhizosphere soil from which the type strain was isolated).

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*Micromonosporaceae* (Table 3). Therefore, we suggest that strains K12-0408<sup>T</sup> and K12-0792 should be classified in a novel species of a new genus, for which the name *Actinorhabdospora filicis* gen. nov., sp. nov. is proposed.

**Fig. 2.** Neighbour-joining phylogenetic tree, based on almost-complete 16S rRNA gene sequences, showing the relationships between strains K12-0408<sup>T</sup> and K12-0792 and related genera in the family *Micromonosporaceae*. Numbers at nodes indicate levels of bootstrap support (%) based on a neighbour-joining analysis of 1000 resampled datasets. Asterisks indicate the clades that were conserved when the neighbour-joining, minimum-parsimony and maximum-likelihood methods were used in reconstructing phylogenetic trees. Bar, 0.01 substitutions per nucleotide position.
Morphological, chemotaxonomic and general characteristics are given above for the genus description. Abundant, white aerial mycelium is well developed on most media tested. Substrate mycelia are light yellow to pale yellow. No diffusible pigment is produced. Melanin pigments are not produced on peptone-yeast extract-iron or tyrosine agars. Good growth occurs between 22 and 30 °C, and from pH 6.0 to 8.0. Maximum NaCl concentration for growth is 1 % (w/v). Nitrate is not reduced to nitrite; casein, gelatin and Tween 80 are degraded but not adenine, cellulose, hypoxanthine, starch or xanthine. H₂ Tween 80 are degraded but not adenine, cellulose, hypoxanthine, starch or xanthine. H₂, Tween 80 are degraded but not adenine, cellulose, hypoxanthine, starch or xanthine. H₂, Tween 80 are degraded but not adenine, cellulose, hypoxanthine, starch or xanthine. H₂, Tween 80 are degraded but not adenine, cellulose, hypoxanthine, starch or xanthine. H₂, Tween 80 are degraded but not adenine, cellulose, hypoxanthine, starch or xanthine. H₂, Tween 80 are degraded but not adenine, cellulose, hypoxanthine, starch or xanthine. H₂, Tween 80 are degraded but not adenine, cellulose, hypoxanthine, starch or xanthine. H₂, Tween 80 are degraded but not adenine, cellulose, hypoxanthine, starch or xanthine. H₂, Tween 80 are degraded but not adenine, cellulose, hypoxanthine, starch or xanthine. H₂, Tween 80 are degraded but not adenine, cellulose, hypoxanthine, starch or xanthine. H₂, Tween 80 are degraded but not adenine, cellulose, hypoxanthine, starch or xanthine. H₂, Tween 80 are degraded but not adenine, cellulose, hypoxanthine, starch or xanthine. H₂, Tween 80 are degraded but not adenine, cellulose, hypoxanthine, starch or xanthine. H₂, Tween 80 are degraded but not adenine, cellulose, hypoxanthine, starch or xanthine. H₂, Tween 80 are degraded but not adenine, cellulose, hypoxanthine, starch or xanthine. H₂, Tween 80 are degraded but not adenine, cellulose, hypoxanthine, starch or xanthine. H₂, Tween 80 are degraded but not adenine, cellulose, hypoxanthine, starch or xanthine. H₂, Tween 80 are degraded but not adenine, cellulose, hypoxanthine, starch or xanthine. H₂, Tween 80 are degraded but not adenine, cellulose, hypoxanthine, starch or xanthine. H₂, Tween 80 are degraded but not adenine, cellulose, hypoxanthine, starch or xanthine. H₂, Tween 80 are degraded but not adenine, cellulose, hypoxanthine, starch or xanthine. H₂, Tween 80 are degraded but not ad
L(+)-arabinose, myo-inositol, D(-)-mannitol, melibiose, raffinose, D(-)-threOSE, saccharose, sorbose, sucrose, xylitol and xylose are not used. Acid is formed from D(+)-cellobiose, glucose, maltose and D(+)-mannose. In the API ZYM system, acid phosphatase, esterase (C4), esterase lipase (C8), α-fucosidase, α-galactosidase, β-galactosidase, α-glucosidase, β-glucosidase, leucine aminopeptidase, phosphorylase, trypsin and valine aminopeptidase are positive; alkaline phosphatase, α-chymotrypsin, cysteine aminopeptidase, N-acetyl-β-glucosaminidase, β-glucuronidase, lipase (C14) and α-mannosidase are negative. The predominant menaquinones are MK-10(3OH) and MK-10(4OH) and small amounts of MK-9(3OH), MK-9(4OH) and MK-10(4OH) are also present. Polar lipids are phosphatidylethanolamine, diphosphatidylglycerol, hydroxy phosphatidylethanolamine, phosphatidylglycerol, phosphatidylinositol, phosphatidylitol mannoside and an unknown phosphoamino lipid. The major fatty acids are anteiso-C15:0 and anteiso-C17:0 2-0H. Other cellular fatty acids detected as minor components are iso-C15:0, anteiso-C15:0, iso-C17:0 10-methyl C17:0 and iso-C16:0.

The type strain, K12-0408T (=NRBC 111897T =TBRCC 5327T), was isolated from rhizosphere soils of fern (pterido-phyte) plants. The G+C content of the DNA of the type strain is 69.6 mol%.

Acknowledgements

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References


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