**Actinorugispora endophytica** gen. nov., sp. nov., an actinomycete isolated from *Daucus carota*

Min-Jiao Liu,1,2† Wen-Yong Zhu,1,3† Jie Li,1 Guo-Zhen Zhao,1 Zhi Xiong,2 Dong-Jin Park,4 Wael N. Hozzein,5 Chang-Jin Kim4 and Wen-Jun Li1,6

1Yunnan Institute of Microbiology, Yunnan University, Kunming, 650091, PR China
2Key Laboratory for Forest Resources Conservation and Use in the Southwest Mountains of China, Ministry of Education, Southwest Forestry University, Kunming 650224, PR China
3Yunnan Key Laboratory of Vaccine Research & Development on Severe Infections Diseases, Institute of Medical Biology, Chinese Academy of Medical Sciences, Kunming, 650118, PR China
4Microbial Resource Center, Korea Research Institute of Bioscience & Biotechnology, Daejeon 305-806, Republic of Korea
5Bioproducts Research Chair (BRC), College of Science, King Saud University, Riyadh 11451, Kingdom of Saudi Arabia
6State Key Laboratory of Biocontrol and Guangdong Key Laboratory of Plant Resources, College of Ecology and Evolution, Sun Yat-Sen University, Guangzhou, 510275, PR China

An actinomycete strain, designated YIM 690008T, was isolated from *Daucus carota* collected from South Korea and its taxonomic position was investigated by using a polyphasic approach. The strain grew well on most media tested and no diffusible pigment was produced. The aerial mycelium formed wrinkled single spores and short spore chains, some of which were branched. The whole-cell hydrolysates contained *meso*-diaminopimelic acid, glucose, mannose, ribose, galactose and rhamnose. The predominant menaquinones were MK-10(H4), MK-10(H6), MK-10(H8) and MK-10(H2). The polar lipids were diphosphatidylglycerol, phosphatidylcholine, phosphatidylinositol, phosphatidylinositol mannosides, some unknown phospholipids, glycolipids and polar lipids. The major fatty acids were i-C16 : 0, ai-C17 : 0 and C18 : 1v9c. The DNA G+C content of the genomic DNA was 63.1 mol%. Phylogenetic analysis indicated that the isolate belongs to the family *Nocardiopsaceae*. However, based on phenotypic, chemotaxonomic and genotypic data, it was concluded that strain YIM 690008T represents a novel genus and novel species of the family *Nocardiopsaceae*, for which the name *Actinorugispora endophytica* gen. nov., sp. nov. (type strain YIM 690008T=DSM 46770T=JCM 30099T=KCTC 29480T) is proposed.

The family *Nocardiopsaceae* was first described by Rainey et al. (1996) with *Nocardiopsis* as the type genus. So far, there are nine genera of the family *Nocardiopsaceae* with validly published names: *Nocardiopsis* (Meyer, 1976), *Thermobifida* (Zhang et al., 1998), *Streptomonomaspora* (Cui et al., 2001), *Halocactinospora* (Tang et al., 2008), *Marinactinospora* (Tian et al., 2009), *Murinocardiopsis* (Kämpfer et al., 2010), *Spinactinospora* (Chang et al., 2011), *Salinactinospora* (Chang et al., 2012) and *Allosalinactinospora* (Guo et al., 2015). According to the statistics of Chang et al. (2012), strains of the family *Nocardiopsaceae* were isolated from different environments, including hypersaline soils, marine sediments, compost, damp stored hay, manure heaps, a salt mine and salt lake. Recently, a novel strain, YIM 690008T, was isolated from *Daucus carota*, a common vegetable collected from South Korea, which was found to be closely related phylogenetically to the genus *Marinactinospora*, but with very different phenotypic

---

**Correspondence**
Wen-Jun Li
liact@hotmail.com
Chang-Jin Kim
changjin@kribb.re.kr

1These authors contributed equally to this work.

**Abbreviations:** DPG, diphosphatidylglycerol; Gal, galactose; GL, unknown glycolipids; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PIM, phosphatidylinositolmannoside; PL, unknown phospholipid; PIME, phosphatidylmethyl–ethanolamine; UL, unknown lipid; Xyl, xylose

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain YIM 690008T is KM925046.

Two supplementary figures and two supplementary tables are available with the online Supplementary Material.
and chemotaxonomic characteristics. Here, the accurate taxonomic position of strain YIM 690008\(^T\) was determined by using a polyphasic taxonomic approach.

Roots of Daucus carota were washed in running water to remove soil particles and sterilized by an established procedure (Li et al., 2008). After being surface-sterilized, the samples were sliced into pieces, followed by plating on TWYE agar (containing 0.25 g yeast extract, 0.5 g K\(_2\)HPO\(_4\) and 18 g agar \(\ell^{-1}\) tap water, pH 7.2) containing nalidixic acid (25 mg l\(^{-1}\)), nystatin (50 mg l\(^{-1}\)) and cycloheximide (50 mg l\(^{-1}\)) to repress growth of Gram-reaction negative bacteria and fungi. The plates were incubated at 28 °C for 4–6 weeks until the outgrowth of endophytic actinobacterial strains was discerned. Colonies originating from leaf segments were selected and pure cultures were obtained by repeated streaking on YMB medium [10 g malt extract, 4 g yeast extract, 4 g glucose, vitamin mixture (containing 0.5 mg each of thiamine-HCl, riboflavin, niacin, pyridoxine-HCl, inositol, calcium pan-thenate and \(p\)-aminobenzoic acid and 0.25 mg biotin), 20 g agar; pH 7.2] (Jiang et al., 2007). The purified strain YIM 690008\(^T\) was maintained on tryptic soy agar (TSA) slants at 4 °C and as 20 % (v/v) glycerol suspensions at −80 °C.

The extraction of genomic DNA, PCR amplification and sequencing of the 16S rRNA gene were carried out as described by Li et al. (2007). Multiple alignments with sequences of the most closely related actinobacteria were carried out using the CLUSTAL X 1.8 program (Thompson et al., 1997). Phylogenetic trees were reconstructed by the neighbour-joining (Saitou & Nei, 1987), maximum-parsimony (Fitch, 1971) and maximum-likelihood (Felsenstein, 1981) tree-making algorithms using the software packages MEGA version 5.0 (Tamura et al., 2011) and PHYLIP version 3.6. The stability of relationships was assessed by performing bootstrap analyses with 1000 resamplings (Felsenstein, 1985). The values for sequence similarity among the most closely related strains were determined using the EzTaxon-e server Database (http://eztaxon-e.ezbiocloud.net/; Kim et al., 2012). The G+C content of the genomic DNA was determined by using the HPLC method (Mesbah et al., 1989).

An almost complete 16S rRNA gene sequence (1542 bp) of strain YIM 690008\(^T\) was generated. The highest sequence similarity was found with Marinactinospora thermotolerans SCSIO 00652\(^T\) (96.7 %). Phylogenetic analysis, based on 16S rRNA gene sequences, showed that strain YIM 690008\(^T\) clustered with Marinactinospora thermotolerans SCSIO 00652\(^T\) (Figs. 1, S1 and S2, available in the online Supplementary Material). The DNA G+C content of strain YIM 690008\(^T\) was 63.1 mol%. These data supported the finding that strain YIM 690008\(^T\) should be a member of the family Nocardiopsaceae.

Gram reactivity was determined by using the standard Gram stain technique, and cell motility was established by the development of turbidity through a tube containing semi-solid medium (Leifson, 1960). The characteristics of cultures of strain YIM 690008\(^T\) were recorded from growth on ISP (International Streptomyces Project) media (Shirling & Gottlieb, 1966), Czapek’s agar, potato-glucose agar (PDA) and nutrient agar prepared as described by Dong & Cai (2001). Colony colours were determined using the ISCC–NBS colour charts (Kelly, 1964). Observations of mycelia and spores of strain YIM 690008\(^T\), which had been grown on Czapek’s agar medium for 3–7 weeks at 28 °C, were made by light microscopy (model BH 2; Olympus), scanning and electron microscopy (XL30 ESEM-TMP; Philips). Growth was tested at 4, 10, 15, 20, 28, 37, 40, 45 and 55 °C on ISP 2 medium by incubating the cultures for 35 days. The ability of the strain to grow at different pH values (pH 4, 5, 6, 7, 8, 9, 10 and 11, using the buffer system (w/v) C and as 20 % (v/v) glycerol suspensions at −80 °C.
Hasegawa et al. (1983); Lechevalier & Lechevalier (1970) and Tang et al. (2009). Polar lipids were extracted, examined by two-dimensional TLC and identified using previously described procedures (Collins & Jones, 1980; Minnikin et al., 1979). Menaquinones were isolated according to Collins et al. (1977) and separated by HPLC (Tamaoka et al., 1983). Cellular fatty acids were extracted, methylated and analysed by using the Sherlock Microbial Identification System (MIDI) according to the manufacturer’s instructions. Fatty acid methyl esters were analysed by using the Microbial Identification software package (Sherlock Version 6.1; MIDI databaseTSBA6).

The peptidoglycan of isolate YIM 690008\(^\text{T}\) contained meso-diaminopimelic acid as the diagnostic diamino acid of the peptidoglycan, whereas the whole-cell sugars detected were glucose, mannose, ribose, galactose and rhamnose. The predominant menaquinones were MK-10(H\(_2\)) (35.6 %), MK-10(H\(_4\)) (34.3 %), MK-10(H\(_6\)) (11.2 %) and MK-10(H\(_8\)) (6.1 %), with MK-11(H\(_3\)) (3.7 %), MK-9(H\(_4\)) (2.8 %), MK-11(H\(_4\)) (2.6 %), MK-9(H\(_6\)) (2.4 %) and MK-11(H\(_6\)) (1.3 %) as minor components. Diphosphatidylglycerol, phosphatidylcholine, phosphatidylinositol, phosphatidylinositol mannosides, unknown phospholipids, glycolipids and polar lipids were detected (Fig. S3). The major fatty acids were i-C\(_{16}:0\) (43.9 %), ai-C\(_{17}:0\) (11.0 %) and C\(_{18}:1\)\(^{\omega}9\)c (10.9 %) (Table 1, Table S2).

On the basis of the phenotypic, genotypic and phylogenetic data presented here, strain YIM 690008\(^\text{T}\) differed from its closest relative, \textit{M. thermotolerans} SCSIO 00652\(^\text{T}\), and the other genera of the family \textit{Nocardiopsaceae}. Strain YIM 690008\(^\text{T}\) represents a novel genus, for which the name \textit{Actinorugispora} gen. nov. is proposed. The type species of the genus is \textit{Actinorugispora endophytica} sp. nov.

**Description of \textit{Actinorugispora} gen. nov.**

\textit{Actinorugispora} (Ac.ti.no.ru.gi.spo’ra. Gr. n. actis, actinos a ray; L. fem. n. ruga a wrinkle; Gr. n. spora a seed; N. L. fem. n. \textit{Actinorugispora} an organism with wrinkled spores). Aerobic, non-motile, Gram-stain-positive actinomycete that forms well-developed substrate mycelium. The aerial

![Fig. 1. Neighbour-joining tree based on 16S rRNA gene sequences showing the relationships between strain YIM 690008\(^\text{T}\) and related members of the family \textit{Nocardiopsaceae}. Bootstrap values ( > 50 %) based on 1000 replicates are shown at the branch nodes. Asterisks indicate that the corresponding branches were also recovered in trees generated with the maximum-parsimony and maximum-likelihood methods. \textit{Actinopolyspora halophila} DSM 43834\(^\text{T}\) was used as the outgroup. Bar, 0.02 substitutions per nucleotide position.](image-url)
mycelium forms wrinkled single spores and short spore chains, some of which are branched. No diffusible pigments are produced. The whole-cell hydrolysates contain meso-diaminopimelic acid, glucose, mannose, ribose, galactose and rhamnose. The predominant menaquinones are MK-10(H_{4}), MK-10(H_{6}), MK-10(H_{8}) and MK-10(H_{2}). The polar lipids are diphosphatidylglycerol, phosphatidylcholine, phosphatidylinositol, phosphatidylinositol mannosides, unknown phospholipids, glycolipids and polar lipids. The major fatty acids are i-C_{16 : 0}, ai-C_{17 : 0} and C_{18 : 1 \alpha 9c}.

The type species is *Actinorugispora endophytica*. The DNA G+C content of the genomic DNA of the type strain of the type species is 63.1 mol%.

**Description of Actinorugispora endophytica sp. nov.**

*Actinorugispora endophytica* (en.do.phy’ti.ca. Gr. pref. endo within; Gr. n. phyton plant; L. fem. suff. -ica adjectival suffix used with the sense of belonging to; N.L. fem. adj. *endophytica* within plant, endophytic).

Displays the following properties in addition to those described for the genus. Good growth on ISP 1, ISP 2, ISP 6, ISP 7 and PDA, with moderate growth on ISP 4, nutrient agar and Czapek’s agar and poor growth on ISP 5 medium. Colonies are yellow-white on ISP 1, ISP 2, ISP 5, ISP 6, ISP 7 and PDA media, and deep yellow on ISP 4, nutrient agar and Czapek’s agar media. Wrinkled single spores and short spore chains are found on aerial hyphae after incubation for 42 days on Czapek’s agar medium. Grows at 10–40 °C, at pH 7–8 and in the presence of 0–5.5% (w/v) NaCl, but not in 6% (w/v) NaCl. Negative for the production of gelatinase and H_{2}S. Positive for the production of catalase, oxidase and urease. Negative for milk coagulation and peptonization. The hydrolysis of starch, Tweens 20, 40 and 80, and nitrate reduction are positive. Tween 60 and cellulose are not hydrolysed.

Utilizes dulcitol, D-galactose, lactose, maltose, sucrose,
Table 1. Differential phenotypic and chemotaxonomic characteristics of strain YIM 690008\(^T\) and other genera of the family Nocardiopsaceae

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spore surface</td>
<td>Wrinkled</td>
<td>Wrinkled</td>
<td>Spiny</td>
<td>No aerial mycelium</td>
<td>Smooth</td>
<td>Smooth</td>
<td>Smooth</td>
<td>Wrinkled</td>
<td>Smooth</td>
<td>None</td>
</tr>
<tr>
<td>pH range for growth</td>
<td>7–8</td>
<td>6–9</td>
<td>6.0–10.5</td>
<td>5–9</td>
<td>7–9</td>
<td>6–14</td>
<td>6–9</td>
<td>5–9</td>
<td>6–9</td>
<td>6–9</td>
</tr>
<tr>
<td>NaCl concentration range for growth (% w/v)</td>
<td>0–5.5</td>
<td>0–5</td>
<td>1–15</td>
<td>0–11</td>
<td>0–5</td>
<td>0–20</td>
<td>9–21</td>
<td>5–25</td>
<td>1–23</td>
<td>0–10</td>
</tr>
<tr>
<td>Diagnostic sugars</td>
<td>Gal</td>
<td>None</td>
<td>MK-10 (H8)</td>
<td>None</td>
<td>None</td>
<td>MK-10 (H4)</td>
<td>None</td>
<td>Gal</td>
<td>MK-10 (H2, H6, H8) or MK-9 (H4, H6)</td>
<td>None</td>
</tr>
<tr>
<td>Predominant menaquinones</td>
<td>MK-10 (H9)</td>
<td>MK-11 (H8)</td>
<td>MK-9 (H8)</td>
<td>MK-10 (H4)</td>
<td>MK-10 (H9)</td>
<td>MK-10 (H8)</td>
<td>MK-10 (H4)</td>
<td>MK-10 (H6)</td>
<td>MK-10 (H6)</td>
<td>MK-10 (H8)</td>
</tr>
<tr>
<td>Phospholipids</td>
<td>DPG, PI, PC, PL, PGs, GLs, ULs</td>
<td>DPG, PC, PG, PI, PMs, PLs, GLs, ULS</td>
<td>PC, PG, PI, DPG, PI, GLs</td>
<td>PC, PG, PI, DPG, PLs, GLs</td>
<td>PC, PG, PI, DPG, PLs, GLs</td>
<td>PC, PME</td>
<td>DPG, PME, PC, PI, DPG, PLs, GLs</td>
<td>DPG, PC, PI, DPG, PME</td>
<td>DPG, PC, PI, DPG, PME</td>
<td>DPG, PC, PLs, GLs, ULS</td>
</tr>
<tr>
<td>Major fatty acids (&gt;10 %)</td>
<td>i-C(_{16}:0)</td>
<td>i-C(_{16}:0)</td>
<td>i-C(_{16}:0)</td>
<td>i-C(_{16}:0)</td>
<td>i-C(_{16}:0)</td>
<td>i-C(_{16}:0)</td>
<td>i-C(_{16}:0)</td>
<td>i-C(_{16}:0)</td>
<td>i-C(_{16}:0)</td>
<td>i-C(_{16}:0)</td>
</tr>
<tr>
<td>DNA G + C content (mol%)</td>
<td>63.1</td>
<td>72</td>
<td>i-C(_{16}:0)</td>
<td>71.1</td>
<td>ND</td>
<td>i-C(_{16}:0)</td>
<td>66–72</td>
<td>64–76</td>
<td>68</td>
<td>72 – 75</td>
</tr>
</tbody>
</table>
starch and xylitol as sole carbon sources. D-Fructose, inositol, mannitol, raffinose, sorbitol and trehalose are not utilized. L-Arginine, L-glutamine, L-lysine and L-phenylalanine are used as sole nitrogen sources, but not L-alanine, L-asparagine, L-cystine, DL-α-methionine, L-serine, L-threonine, L-tyrosine, L-valine or xanthine.

The type strain, YIM 690008T (=DSM 46770T = JCM 30099T = KCTC 29480T), was isolated from Daucus carota, collected from South Korea. The DNA G+C content of the type strain is 63.1 mol%.

Acknowledgements

This research was supported by a grant (NRF-2013M3A9A5076601) funded by Ministry of Science, ICT and Future Planning of Korean government and the Deanship of Scientific Research at King Saud University for funding this work through the research group no RGP-205. W-J. L was also supported by Guangdong Province Higher Vocational Colleges & Schools Pearl River Scholar Funded Scheme (2014).

References


