Actinophytocola xanthii sp. nov., an actinomycete isolated from rhizosphere soil of the plant Xanthium sibiricum

Wei Wang,1 Bin Wang,1 Haoyi Meng,1 Zhaobin Xing,2 Qiliang Lai3 and Lijie Yuan1,*

Abstract

A novel actinomycete strain, 11-183T, was isolated from the rhizosphere soil of Xanthium sibiricum, which was collected in Tangshan, Hebei, China. A phylogenetic analysis based on 16S rRNA gene sequences showed that strain 11-183T formed a clade within the genus Actinophytocola, with a maximum similarity of 98.44 % to Actinophytocola xinjiangensis QAIII60T, followed by 97.76 % similarity to Actinophytocola sediminis YIM M13705T. The average nucleotide identity and digital DNA–DNA hybridization values differed by 79.24 and 23.4 %, respectively, between strain 11-183T and Actinophytocola xinjiangensis QAIII60T. Strain 11-183T grew well on N-Z-amine agar, and it produced a scant, white aerial mycelium. The isolate formed pale yellow to brown–black colonies and a dense, non-fragmented, branched substrate mycelium, and produced aerial hyphae on which nodular spore chains formed. Growth was observed at salinities ranging from 0 to 2 %, at pH values ranging from pH 6.5 to 8.0 and at temperatures ranging from 15 to 37 °C. The cell-wall amino acids included meso-diaminopimelic acid. Whole cell hydrolysates contained galactose and glucose. The principal fatty acids were iso-C16:0, iso-C16:1H and C17:1ω6c. Diphosphatidylglycerol, phosphatidylinomethyllethanolamine and phosphatidylethanolamine were the diagnostic phospholipids. The isoprenoid quinones included MK-9(H4) and MK-10(H4). The G+C content of the genomic DNA was 71.7 mol%. Based on the genotypic and phenotypic data, we conclude that strain 11-183T belongs to a novel species of the genus Actinophytocola. The name proposed for the novel species is Actinophytocola xanthii sp. nov., with the type strain 11-183T (=KCTC 39690T= MCCC 1K02062T).

The genus Actinophytocola was established by Indananda et al. [1] as a member of the family Pseudonocardiaceae [2]. At the time of writing, the genus Actinophytocola comprised eight species, which have been described using a range of genotypic and phenotypic properties. The first proposed species of the genus Actinophytocola was Actinophytocola oryzae [1], a single strain of which was isolated from the root of the Thai glutinous rice plant (Oryza sativa L. ‘RD6’), while Actinophytocola timorenensis and Actinophytocola corallina [3], Actinophytocola burenkhanelensis [4], Actinophytocola xinjiangensis [5], Actinophytocola gilvus [6] and Actinophytocola algeriensis [7] were isolated from different types of soil samples. Actinophytocola sediminis [8] was isolated from marine sediment. Members of the genus Actinophytocola form a non-fragmented substrate mycelium, which produces an aerial mycelium that fragments into spore chains on some media. The major fatty acid and polar phospholipid are iso-C16:0 and phosphatidylethanolamine, respectively; the diagnostic menaquinone is MK-9(H4), but Actinophytocola xinjiangensis is an exception, as it contains more MK-10(H4) (76.5 %) and less MK-9(H4) (23.5 %); the cell wall of members of the genus Actinophytocola contains meso-diaminopimelic acid [1, 3–6, 8]. Here, using a polyphasic approach, we report another novel strain belonging to the genus Actinophytocola.

To investigate actinobacterial diversity from the rhizosphere soil of Xanthium sibiricum Patrin ex Widder in Fedde, strain 11-183T was isolated from a soil sample from the rhizosphere of X. sibiricum, which was collected in Tangshan, Hebei province, north China. Strain 11-183T was isolated on modified Gause’s medium (20.0 g soluble starch, 1.0 g KNO3, 0.5g K2HPO4, 3H2O, 0.5g NaCl, 0.5g MgSO4 . 7H2O, 1.0 g CaCO3, 0.5 g K2Cr2O7 and 15 g agar per litre of distilled water; pH 7.2) by the dilution plating method. After 21 days of aerobic incubation at 28 °C, the strain was...
purified and transferred onto International Streptomyces Project (ISP) 2 medium (yeast extract-malt extract agar; [9]). The purified isolate was stored on ISP 2 agar at 4 °C and as glycerol suspensions (25 %, v/v) at 80 °C.

Extraction of genomic DNA and PCR amplification of the 16S rRNA gene were performed using the method described by Li et al. [10]. The sequence obtained was compared with available 16S rRNA gene sequences from the GenBank database using the Basic Local Alignment Search Tool program (http://eztaxon-e.ezbiocloud.net/; [11]). The results of the comparisons were used to determine the approximate phylogenetic affiliation of strain 11-183 T. The almost complete 16S rRNA gene sequences were aligned manually with corresponding sequences representing species with validly published names using CLUSTAL X 1.83 [12]. Algorithms for reconstructing phylogenetic trees were run using the software package MEGA version 5.0 [13]. Phylogenetic trees were inferred using the maximum-likelihood [14], neighbour-joining [15] and maximum-parsimony methods [16]. The topologies of the evolutionary trees were evaluated by a bootstrap analysis based upon 1000 replicates, as described by Felsenstein [17].

We determined the almost complete 16S rRNA gene sequence (1515 bp) of strain 11-183 T. A Basic Local Alignment Search Tool search using the 16S rRNA gene sequence of this strain indicated that the isolate had the highest similarities to members of the genus Actinophytocola in the family Pseudonocardiaceae. Strain 11-183 T shared the highest sequence similarity (98.44 %) with Actinophytocola xinjiangensis QAIII60 T, followed by Actinophytocola sediminis YIM M13705 T (97.76 %). Strain 11-183 T showed less than 97.0 % 16S rRNA gene sequence similarity to the type strains of all other species of the genus Actinophytocola. As shown in the neighbour-joining tree (Fig. 1), strain 11-183 T formed a clade with Actinophytocola xinjiangensis and Actinophytocola sediminis, which had 99 % bootstrap support. The close relationship was supported solidly by the maximum-likelihood and maximum-parsimony trees (Figs S1 and S2, available in the online Supplementary Material), respectively. Strain 11-183 T represents a novel member of the genus Actinophytocola, because its 16S rRNA gene sequence similarities to all other type strains were less than 98.65 %, which was the cut-off point proposed by Kim et al. [18] for the threshold of differentiating two species.

The genome sequence of strain 11-183 T and its closest type strain Actinophytocola xinjiangensis QAIII60 T (=CGMCC 4.4663 T), were determined by Shanghai Majorbio Bio-pharm Technology (Shanghai, China) using Solexa paired-end (500 bp library) sequencing technology. The G+C content of the chromosomal DNA was determined from the draft genome sequence. The average nucleotide identity (ANI) between strain 11-183 T and Actinophytocola xinjiangensis QAIII60 T was calculated using the algorithm of Goris et al. [19] using the EZGenome web service. Estimates of DNA–DNA hybridization (DDH) values for these two strains were analysed using the genome-to-genome distance calculator (GGDC2.0) [20–22]. A total of 1000 Mbp of clean data for strain 11-183 T and Actinophytocola xinjiangensis QAIII60 T were generated, reaching an approximately 100-fold depth of coverage, using an Illumina/Solexa Genome Analyzer IIx. The clean data were assembled by SOAPdenovo2 [23]. The draft genome sequence demonstrated that the chromosomal DNA G+C content of strain 11-183 T was 71.7 mol%, which is close to that of other species of the genus Actinophytocola (Table 1). The ANI value between strains 11-183 T and Actinophytocola xinjiangensis QAIII60 T was 79.24 %, which is below standard ANI criteria for species identity (95–96 %) [24]. The estimated difference in the digital DDH value between strain 11-183 T and Actinophytocola xinjiangensis QAIII60 T was 23.4 %, which is far below the standard criterion (70 %) for delineating prokaryotic species [25]. In addition, two close type strains (Actinophytocola xinjiangensis QAIII60 T and Actinophytocola sediminis YIM M13705 T) shared 98.71 % 16S rRNA gene sequence similarity, but their DNA–DNA relatedness was only 46.3 % [8]. These results confirm that strain 11-183 T represents a novel species.
The characteristics of strain 11-183\(^{T}\) were recorded after incubation for 28 days at 28 °C on various media [ISP 2, ISP 3, ISP 4, ISP 5, ISP 7, ISP 9, potato extract agar (200 g potato, 10 g glucose and 20 g agar per litre; pH 7.2–7.4), Czapek’s agar, glucose-asparagine agar, nutrient agar and trypticase soy agar (TSA; Difco)], according to the methods described by Shirling and Gottlieb [9]. Spore motility was tested by light microscopic observation of cells suspended in phosphate buffer (pH 7.0, 1 mM) for approximately 2 h at 28 °C. The morphology of hyphae was examined using the coverslip technique [26], followed by observation with a light microscope (BX-53; Olympus) and a scanning electron microscope (model SU8010; Hitachi) using specimens of 49-day-old cultures from N-Z-amine agar. Growth at different temperatures (4, 10, 15, 23, 28, 37 and 45 °C) was examined on TSA. The pH range for testing growth was pH 5.0–11.5 (at intervals of 0.5 pH units), and the pH was adjusted using different buffers (pH 5.0–5.5, 0.1 M citric acid/0.1 M sodium citrate; pH 6.0–8.0, 10 mM HEPES/0.5 M NaOH; and pH 8.5–11.5, 0.1 M NaHCO\(_3/0.1\) M Na\(_2\)CO\(_3\)). NaCl tolerance [0, 1, 2, 3, 4, 5, 6, 7, 10, 15 and 20 % (w/v)] was assessed on TSA. Catalase activity was determined using 3 % H\(_2\)O\(_2\), and the production of gas was deemed a positive reaction [27]. Oxidase activity was determined by the oxidation of tetramethyl-p-phenylenediamine. Cellulose degradation was tested using the basal medium recommended by Pridham and Gottlieb [28]. Hydrolyses of casein, gelatin and starch were examined using previously described methods [29]. Utilization of carbohydrates as sole carbon sources was tested using ISP 4 medium without soluble starch, because strain 11-183\(^{T}\) did not grow on carbon utilization medium (ISP 9). Nitrogen source utilization was detected using the basal medium recommended by Williams et al. [30] (supplemented with a final concentration of 0.1 % of the nitrogen sources tested). Other physiological tests of this novel strain were examined according to previously described methods [27-30].

### Table 1. Differential phenotypic and chemotaxonomic characteristics of strain 11-183\(^{T}\) and other members of the genus Actinophytocola

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Strain 1</th>
<th>11-183</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
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<th>9</th>
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</thead>
<tbody>
<tr>
<td>Isolation source</td>
<td>Rhizosphere soil</td>
<td>Forest soil</td>
<td>Marine sediment</td>
<td>Soil</td>
<td>Soil</td>
<td>Soil</td>
<td>Soil</td>
<td>Soil</td>
<td>Soil</td>
<td>Desert soil crust</td>
<td>Saharan soil</td>
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<tr>
<td>Colony colour on ISP 2 medium</td>
<td>Brown-black</td>
<td>Light yellow</td>
<td>Olive black</td>
<td>Pale yellow</td>
<td>Melon yellow</td>
<td>Rod-shaped</td>
<td>Cylindrical</td>
<td>Rod-shaped</td>
<td>White-yellow rod-shaped</td>
<td>Grey</td>
<td>White</td>
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<tr>
<td>Spore morphology</td>
<td>Cylindrical</td>
<td>Cylindrical</td>
<td>Irregular round</td>
<td>ND</td>
<td>≤7</td>
<td>≤2</td>
<td>≤3</td>
<td>≤5</td>
<td>≤3</td>
<td>ND</td>
<td>ND</td>
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<tr>
<td>NaCl for growth (%, w/v)</td>
<td>≤2</td>
<td>≤4</td>
<td>≤6</td>
<td>≤3</td>
<td>ND</td>
<td>+</td>
<td>ND</td>
<td>+</td>
<td>ND</td>
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<tr>
<td>pH for growth</td>
<td>6.5–8.0</td>
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<td>4.0–11.0</td>
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<td>Oxidase</td>
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<td>W</td>
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<td>H(_2)S</td>
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<td>Melanin</td>
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<td>Starch</td>
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<td>+</td>
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<td>ND</td>
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<tr>
<td>Gelatin</td>
<td>+</td>
<td>+</td>
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<td>Urea</td>
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<td>Nitrate reductase</td>
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<td>+</td>
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<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
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<tr>
<td>Predominant menaquinone(s)</td>
<td>MK-9(H4), MK-10(H4)</td>
<td>MK-9(H4), MK-10(H4)</td>
<td>MK-9(H4), MK-10(H4)</td>
<td>MK-9(H4)</td>
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<td>MK-9 (H4)</td>
<td>MK-9 (H4)</td>
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<tr>
<td>Major phospholipids</td>
<td>DPG, PE, PME, NPG</td>
<td>DPG, PE, PME, NPG</td>
<td>DPG, PE, PME, NPG</td>
<td>DPG, PE, PME, NPG</td>
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<td>DPG, PE, PME, NPG</td>
<td>DPG, PE, PME, NPG</td>
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<tr>
<td>DNA G+C content (mol%)</td>
<td>71.7</td>
<td>72.5</td>
<td>68.2</td>
<td>70.6</td>
<td>71.2</td>
<td>71.1</td>
<td>69.7</td>
<td>70.1</td>
<td>ND</td>
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</table>
described procedures [31]. The type strain Actinophytoncola xinjiangensis CGMCC 4.4663T (=QAIII60T), which was obtained from the CGMCC, was used as a reference in the physiological and biochemical tests.

Strain 11-183T grew well on ISP 2 medium, glucose-asparagine agar and potato extract agar, and it formed black colonies on ISP 2 medium. It exhibited moderate growth on ISP 3, ISP 4 and ISP 5 media, and TSA, as well as nutrient agar medium. Strain11-183T did not grow on carbon utilization medium (ISP 9) [9]. A small aerial mycelium was produced on ISP 2 medium within 49 days. A pale yellow pigment was observed on ISP 3 and ISP 5 media, as well as glucose-asparagine agar, while a pale brown, diffusible pigment was observed on potato extract agar. The colour of the substrate mycelium depended on the medium tested. Detailed culture characteristics are shown in Table S1. Investigations of the 49-day-old culture revealed the characteristics of the mycelium on a coverslip. An abundant, compact, branched substrate mycelium formed close to the agar medium, while scant aerial hyphae formed with nodular spore chains (Fig. 2). Strain 11-183T grew at 15–37°C (optimum 28°C), and it grew well on TSA at pH 6.5–8.0 (optimum pH 8.0). However, scant growth was visible at pH 6.5 and with 0–2.0 % (w/v) NaCl. Strain 11-183T degraded starch, gelatin and cellulose, and it produced catalase. Nitrate reduction was negative. The detailed physiological and biochemical characteristics of strain 11-183T are summarized in Table 1. Strain 11-183T could be differentiated from the closest species of the genus Actinophytoncola by the colour of its substrate mycelium, and its production of an aerial mycelium and a diffusible pigment on the media tested (Table S1). Moreover, its range of temperatures and pH for growth, and NaCl tolerance, as well as oxidase, urease and nitrate reductase activities, differed from those of other species of the genus Actinophytoncola (Table 1).

Biomass for analyses of cellular fatty acids was obtained by cultivation in tryptic soy broth (Difco) for 14 days, while that for other chemotaxonomic characteristics was obtained by cultivation in ISP 2 medium for 28 days. All cultures were incubated in flasks, with shaking at 180 r.p.m. at 28°C, and they were harvested by centrifugation at 2219 g for 10 min and washed twice with a physiological saline solution. According to the methods of Lechevalier and Lechevalier [32], isomers of dianimonopellic acid and sugars in whole-cell hydrolysates were prepared and analysed using TLC. Polar lipids were extracted using a chloroform/methanol/0.3 % aqueous NaCl mixture [1 : 2 : 0.8 (v/v)] [33], separated by two-dimensional TLC and then analysed as described by Tindall et al. [34]. Menaquinones were extracted [35] and analysed by HPLC [36]. According to the manufacturer’s instructions, cellular fatty acids were extracted, methylated and analysed using the Sherlock Microbial Identification System (version 6.0B; MIDI). Fatty acid methyl esters were then analysed by gas chromatography (6850 gas chromatograph; Agilent Technologies) and identified using the TSB6A database of the Microbial Identification System [37]. Mycolic acids were detected by TLC using the method of Tomiyasu [38].

Whole-cell hydrolysates of strain 11-183T contained meso-diaminopellic acid, galactose and glucose, respectively. Major cellular fatty acids (>10 %) were iso-C16 : 0 (28.55 %), iso-C16 : 1H (15.58 %) and C17 : 1ω6c (14.5 %); data for the specific contents are provided in Table S2. The menaquinones were MK-9(H4) (87.1 %) and MK-10(H4) (12.9 %). The phospholipids profile, as shown in Fig. S3, mainly contained diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylmonomethylethanolamine, two ninhydrin-positive phosphoglycolipids, three ninhydrin-positive glycolipids and some unknown phospholipids. This profile was very similar to those of recognized species of the genus Actinophytoncola. No mycolic acids were detected. Strain 11-183T contained minor amounts of MK-10(H4), which has not been reported in existing species of the genus Actinophytoncola, although MK-10(H2) has been found in two closely related type strains (Actinophytoncola xinjiangensis QAII60T and Actinophytoncola sediminis YIM M13705T). These chemical characteristics of strain 11-183T easily distinguish it from those strains classified in the genus Actinophytoncola.

Based on phenotypic, chemotaxonomic and phylogenetic analyses, 16S rRNA gene similarities, and low ANI and DDH values, it is clear that strain 11-183T represents a novel member of the genus Actinophytoncola, for which the name Actinophytoncola xanthii sp. nov. is proposed.

**DESCRIPTION OF ACTINOPHYTOCOLA XANTHII SP. NOV.**

Actinophytoncola xanthii (xan’thi.i. N.L. gen. n. xanthii of Xanthium, the source of the soil sample collected from the rhizosphere of Xanthium sibiricum Patrin ex Widder).

Gram-stain-positive and non-motile, and forms compact, branched substrate mycelium during aerobic cultivation. Good growth occurs on ISP 2 medium, glucose-asparagine agar and potato extract agar, but grows poorly on Czapek’s agar. The colour of the substrate mycelium depends on the medium, and a diffusible pigment is produced on ISP 3 and

![Fig. 2. Scanning electron micrographs of mycelium of strain 11-183T.](image-url)
ISP 5 media, glucose-asparagine agar and potato extract agar. A scant white aerial mycelium is only produced on ISP 2 medium. At maturity, nodular spor chains are borne on aerial hyphae. Growth occurs at pH 6.5–8.0 (optimum pH 8.0), 15–37 °C (optimum 28 °C) and with 0–2.0 % (w/v) NaCl (optimum 0–1%). Produces catalase, cellulase and amylase, and hydrolyses gelatin, but does not produce H₂S, melanin, oxidase or urease, and does not reduce nitrate. Utilizes l-arabinose, cellobiose, d-fructose, glucose, lactose, maltose, l-rhamnose, D-sorbitol, sucrose and D-xyllose as sole carbon sources. Utilizes l-alanine, DL-α-alanine, DL-aspartic amide, L-glutamate, l-histidine, L-lysine, L-methionine, L-phenylalanine, L-serine, l-threonine and L-tryptophan as sole nitrogen sources, but not L-cysteine, glycine, hypoxanthine or L-tyrosine. Contains meso-diaminopimelic acid, galactose and glucose in whole-cell hydrolysates. The major cellular fatty acids are iso-C₁₆:0, iso-C₁₅:0 3-OH and C₁₇:1ω₅c; diphosphatidylglycerol, phosphatidylmonomethylethanolamine and phosphatidylethanolamine are the diagnostic phospholipids. The menaquinones are MK-9(H₄) (87.1 %) and MK-10(H₄) (12.9 %).

The type strain, 11–183T (=KCTC 39690=MCCC 1K02062T), was isolated from a soil sample that was collected from the rhizosphere of Xanthium sibiricum growing in Tangshan, Hebei, China. The G+C content of its genomic DNA is 71.7 mol%.

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**Conflicts of interest**
The authors declare that there are no conflicts of interest.

**References**


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