**Streptomyces capparidis** sp. nov., a novel endophytic actinobacterium isolated from fruits of *Capparis spinosa* L.

Hong-Fei Wang,1,2 Qiu-Li Li,1 Min Xiao,3 Yong-Guang Zhang,2 Xing-Kui Zhou,4 Manik Prabhu Narsing Rao,3 Yan-Qing Duan4 and Wen-Jun Li2,3,*

**Abstract**

A novel endophytic actinobacterial strain, designated EGI 6500195^T, was isolated from fruits of *Capparis spinosa*. Growth occurred at 10–45 °C (optimum 30 °C), at pH 6–8 (optimum pH 7) and in the presence of 0–1 % (w/v) NaCl. Strain EGI 6500195^T shared highest 16S rRNA gene sequence similarity (97.74 %) with *Streptomyces vitaminophilus* DSM 41686^T and less than 97 % sequence similarity with other members of the genus *Streptomyces*. The diagnostic amino acid in the peptidoglycan was LL-diaminopimelic acid. Whole-cell hydrolysates contained glucose, ribose, fructose and mannose. The predominant menaquinones were MK-9(H4) and MK-9(H6). The polar lipid profile of strain EGI 6500195^T included diphosphatidylglycerol, phosphatidyldiglycerol, phosphatidylmethylethanolamine, phosphatidylinositol, phosphatidylcholine, three unknown phospholipids, an unknown aminophospholipid and an unknown aminolipid. The cellular fatty acids were anteiso-C15:0, anteiso-C17:0, iso-C15:0, iso-C16:0, anteiso-C17:0ω9c, summed feature 4 (iso-C17:0 1 and/or anteiso-C17:0 1 B) and iso-C17:0ω9c. The DNA G+C content of strain EGI 6500195^T was 74.1 mol%. The level of DNA–DNA relatedness between strain EGI 6500195^T and *Streptomyces. vitaminophilus* DSM 41686^T was 14.1±3.5 %. On the basis of the phenotypic, phylogenetic, chemotaxonomic and DNA–DNA hybridization data, strain EGI 6500195^T represents a novel species of the genus *Streptomyces*, for which the name *Streptomyces capparidis* sp. nov. is proposed. The type strain is EGI 6500195^T (=DSM 42145^T=JCM 30089^T).

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*Capparis spinosa* is a traditional medicinal shrub of the family *Capparidaceae*, which is distributed in dry regions in west or central Asia and the Mediterranean basin. It is reported to contain large amounts of bio-active components, such as phytosterols, tocopherols, carotenoids, flavonoids and glucosinolates [1, 2]. Furthermore, numerous studies have shown that it could have antioxidative [1, 3, 4], anti-fungal [5], anti-hepatotoxic [6], anti-inflammatory [7], anti-diabetic [8], anti-hypertensive [6], anti-rheumatic [9], anti-hyperlipidaemic [8], immunostimulant and anti-tumour activities [10]. Thus, *C. spinosa* has important value in the medical industry.

As a traditional medicinal plant, most of the research has focused on using plant extracts for therapy. In recent years, studies showed that a large number of phytotherapeutic compounds are actually produced by associated plant microbes or through their interaction [11]. Although extensive research has been carried out on this plant, the diversity of endophytic actinobacteria from *C. spinosa* has not yet been fully explored. During an investigation of endophytic actinobacterial diversity from *C. spinosa* collected from Urumqi city, Xinjiang, north-west China, in July 2014, a mycelial-forming strain, EGI 6500195^T, was isolated from surface-sterilized fruit of *C. spinosa*. For the isolation, plant tissues were washed and surface-sterilized as described by Qin et al. [12]. Surface-sterilized plant tissues were ground aseptically by using a commercial Joyoung blender, serially diluted with sterilized water, and spread on tap water-yeast extract (TWYE) agar (yeast 0.25 g, K2HPO4 0.5 g, agar 15.0 g, distilled water 1000 ml, pH 7.0–7.2) supplemented with 3 % (w/v) NaCl after being incubated at 30 °C for 2–6 weeks. The isolate was purified and maintained on yeast extract–malt extract agar (ISP 2) [13] slants at 4 °C and as 20 % (v/v) glycerol suspensions at −80 °C.

The reference strains *Streptomyces vitaminophilus* DSM 41686^T and *Streptomyces thermolinalus* JCM 6307^T were obtained from the German Collection of Microorganisms.

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**Author affiliations:** 1College of Life Science, Liaoning Normal University, Dalian 116029, PR China; 2Key Laboratory of Biogeography and Bioresource in Arid Land, Xinjiang Institute of Ecology and Geography, Chinese Academy of Sciences, Ürümqi 830011, PR China; 3State Key Laboratory of Biocontrol and Guangdong Provincial Key Laboratory of Plant Resources, School of Life Sciences, Sun Yat-Sen University, Guangzhou 510275, PR China; 4China Tobacco Yunnan Industrial Co., Ltd, Kunming 650231, PR China.

**Keywords:** *Streptomyces capparidis* sp. nov.; Endophytic actinobacterium; *Capparis spinosa*.

The 16S rRNA gene sequence of strain EGI 6500195^T has been deposited in GenBank under the accession number KX119420.

Three supplementary figures and three supplementary tables are available with the online Supplementary Material.

**NOTE**

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Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences, showing the position of strain EGI 6500195\(^T\) in relation to recognized species of the genus *Streptomyces*. Numbers at nodes indicate the level of bootstrap support (>50 %) based on 1000 re-samplings. Asterisks indicate the corresponding nodes were recovered in the maximum-parsimony and maximum-likelihood trees. Bar, 0.005 substitutions per site.

and Zellkulturen (DSMZ) and Japan Collection of Microorganisms (JCM), respectively.

Extraction of chromosomal DNA and PCR amplification of the 16S rRNA gene was carried out as described by Li *et al.* [14]. The PCR product was purified and cloned into the pEASY-T\(_1\) simple vector (TransGen) and sequenced at Sangon Biotech (Shanghai) (http://www.sangon.com). The sequence obtained was compared with available 16S rRNA gene sequences of cultured species from the EzTaxon-e server (http://www.ezbiocloud.net/taxonomy; [15]). The almost-complete 16S rRNA gene sequence was aligned with published sequences of related representatives of the genus *Streptomyces* using CLUSTAL X 1.83 software [16]. Phylogenetic trees were reconstructed by using the neighbour-joining [17], maximum-parsimony [18] and maximum-likelihood [19] algorithms in the MEGA 5.0 software package [20]. Evolutionary distances were computed according to the algorithm of Kimura's two-parameter model [21] for the neighbour-joining method. The stability of tree topologies was evaluated by bootstrap analysis [22] based on 1000 re-sampled datasets. The G+C content of the genomic DNA was determined by HPLC [23]. DNA–DNA hybridization tests were carried out by the fluorometric micro-well method [24, 25]. The hybridization temperature was 48 °C.

The almost-complete 16S rRNA gene sequence of strain EGI 6500195\(^T\) (1532 bp) was determined. The BLAST result indicated that strain EGI 6500195\(^T\) belongs to the genus *Streptomyces*, with highest 16S rRNA gene sequence similarity to *S. vitaminophilus* DSM 41686\(^T\) (97.74 %) and below 97 % 16S rRNA gene sequence similarity to other
members of the genus *Streptomyces*. The neighbour-joining tree showed that strain EGI 6500195T was clustered with *S. vitaminophilus* DSM 41686T and *S. thermolineatus* JCM 6307T (Fig. 1). Similar topologies were also recovered in the maximum-parsimony and maximum-likelihood trees (Figs S1 S2, available in the online Supplementary Material).

The DNA–DNA hybridization value between strain EGI 6500195T and *S. vitaminophilus* DSM 41686T was 14.1 ± 3.5 % (Table S1), which was well below the cut-off point (70 %) for prokaryotic species delineation [26]. The genomic DNA G+C content of strain EGI 6500195T was 74.1 mol%.

For chemotaxonomic analysis, strain EGI 6500195T and related reference strains (*S. vitaminophilus* DSM 41686T and *S. thermolineatus* JCM 6307T) were cultured in tryptone soya broth (TSB; Difco) at 160 rpm and 30 °C for 7 days. Biomass was harvested by centrifugation, washed twice with distilled water, re-centrifuged and freeze-dried. Fatty acid analysis were performed by GC according to the standard protocol of the MIDI/Hewlett Packard Microbial Identification System (Sherlock Version 6.1; MIDI database: TSBA6) [27]. Menaquinones were extracted from freeze-dried biomass, according to Collins et al. [28], and then determined by reversed-phase HPLC analysis (ZORBAX Eclipse XDB-C18 250 × 4.6 mm) [29]. Polar lipids were extracted and identified by two-dimensional TLC according to the method described by Minnikin et al. [30]. The diaminopimelic acid in the cell wall was analysed according the method of Lechevalier and Lechevalier [31]. Whole-cell sugars were identified using HPLC after perchloric acid derivatization with 1-phenyl-3-methyl-5-pyrazoleone as described by Tang et al. [32]. The major cellular fatty acids of strain EGI 6500195T were anteiso-C15:0 (19.4 %), anteiso-C17:0 (18.7 %), iso-C15:0 (15.6 %), iso-C16:0 (15.1 %), anteiso-C17:1ω9c (7.4 %), summed feature 4 (7.2 %) and iso-C17:1ω9c (5.2 %). The detailed fatty acid compositions of strain EGI 6500195T and reference strains are shown in Table S2. The predominant menaquinones were MK-9(H6) (66.7 %) and MK-9(H8) (31.8 %). The polar lipids detected were diphasphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, phosphatidylcholine, phosphatidylmethylethanolamine, three unknown phospholipids, an unknown aminophospholipid and an unknown aminolipid (Fig. S3). Strain EGI 6500195T contained LL-diaminopimelic acid as the diamino acid. The whole-cell hydrolysates contained fructose, ribose, glucose and mannose.

Gram staining was carried out by using the standard Gram reaction. The cultural properties of strain EGI 6500195T were determined on ISP 2, oatmeal agar (ISP 3), inorganic salts-starch agar (ISP 4), glycerol asparagines agar (ISP 5) [13], nutrient agar (NA), potato-glucose agar (PDA) and Czapek’s agar at 30 °C for 15 days. Morphological features of strain EGI 6500195T were observed using light microscopy (BH-2; Olympus) and scanning electron microscopy (QUANTA200; FEI). Colours of substrate mycelium, aerial mycelium and soluble pigments were determined by using colour chips from the ISCC-NBS colour charts standard [33]. Growth at different temperatures (0, 5, 10, 15, 20, 25, 30, 37, 40, 45, 50 and 55 °C) and different NaCl concentrations (0–10 %, w/v, at intervals of 1 %) was tested on ISP 2 medium at 30 °C for 15 days. The pH range (pH 4.0–12.0 at intervals of 1.0 pH unit) for growth was tested by using ISP 2 using the buffer system as described by Xu et al. [34]. Catalase and oxidase activities were determined in 3 % (v/v) H2O2 and 1 % (w/v) tetramethyl-p-phenylenediamine, respectively. Carbon source utilization was tested by using ISP 2 medium [13] supplemented with 0.5 % carbon sources. The utilization of nitrogen sources was performed as described by Williams et al. [35]. Other physiological and biochemical characteristics were performed as described by Gonzalez et al. [36].

Strain EGI 6500195T was Gram-stain-positive. The mycelium of strain EGI 6500195T was observed on ISP 2, ISP 3, NA and PDA medium, and moderately on ISP 4, ISP 5 and Czapek’s medium. No diffusible pigment was detected using any of the test media and spores (white) were produced only on PDA medium. The colour of the substrate and aerial mycelium varied on the test media (Table S3). Morphological observation of a 21-day-old culture grown on PDA medium revealed an extensively well-developed substrate mycelium that did not fragment. Aerial hyphae were smooth-surfaced and spores were long straight to flexuous chains (Fig. 2). The temperature for growth ranged from 10 to 45 °C, with an optimum at 30 °C. Strain EGI 6500195T was able to grow in the presence of 0–1 % (w/v) NaCl. The pH for growth ranged from 6.0 to 8.0, with optimum at pH 7.0. The detailed physiological characteristics of strain EGI 6500195T are given in the species description and comparative features with its related reference type strains are shown in Table 1.

On the basis of phenotypic, phylogenetic, chemotaxonomic and DNA–DNA hybridization data, we conclude that strain EGI 6500195T represents a novel species of the genus...
Table 1. Comparison of the phenotypic characteristics of strain EGI 6500195T with closely related species of the genus Streptomyces

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
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<tbody>
<tr>
<td>Isolation source</td>
<td>Plant</td>
<td>Soil</td>
<td>Compost</td>
</tr>
<tr>
<td>Growth on ISP 4, ISP 5 and Czapek’s media</td>
<td>Moderate</td>
<td>None</td>
<td>Good</td>
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<tr>
<td>Growth on ISP 3 medium</td>
<td>Good</td>
<td>None</td>
<td>Good</td>
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<tr>
<td>Aerial spore mass colour on PDA medium</td>
<td>White</td>
<td>None</td>
<td>Light greenish</td>
</tr>
<tr>
<td>Aerial spore mass colour on ISP 2 and NA medium</td>
<td>None</td>
<td>None</td>
<td>Grey yellow green</td>
</tr>
<tr>
<td>Temperature range for growth (°C)</td>
<td>10–45</td>
<td>15–40</td>
<td>5–45</td>
</tr>
<tr>
<td>NaCl range for growth (% w/v)</td>
<td>0–1</td>
<td>0–2</td>
<td>0–6</td>
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<tr>
<td>Optimal NaCl for growth (% w/v)</td>
<td>0</td>
<td>0–1</td>
<td>3–4</td>
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<tr>
<td>pH range for growth</td>
<td>6–8</td>
<td>6–8</td>
<td>6–9</td>
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<tr>
<td>Milk coagulation</td>
<td>+</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Milk peptonization</td>
<td>+</td>
<td>–</td>
<td>–</td>
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<td>Growth on sole carbon source:</td>
<td></td>
<td></td>
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<tr>
<td>D-Sorbitol, D-fructose</td>
<td>+</td>
<td>–</td>
<td>+</td>
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<tr>
<td>Raffinose pentahydrate, trehalose (anhydrous),</td>
<td>–</td>
<td>+</td>
<td>+</td>
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<td>D-arabinose, xylitol, D-xylitol</td>
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<tr>
<td>L-Rhamnose, mannosone, melibiose monohydrate</td>
<td>–</td>
<td>+</td>
<td>+</td>
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<td>Growth on sole nitrogen source:</td>
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<td>L-Serine, L-phenylalanine, glycine, L-histidine,</td>
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<tr>
<td>L-threonine, L-valine, L-aspartic acid</td>
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<tr>
<td>L-Arginine, L-tryptophan, L-lysine, L-glutamic acid</td>
<td></td>
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<tr>
<td>Whole-cell hydrolysates</td>
<td>Fructose/ribose/</td>
<td>Fructose/mannose/</td>
<td>Fructose/glucose</td>
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<tr>
<td></td>
<td>glucose/mannose</td>
<td>ribose/glucose</td>
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<tr>
<td>Predominant menaquinones</td>
<td>MK-9 (H₈₅),</td>
<td>MK-9 (H₈₅),</td>
<td>MK-9 (H₈₅),</td>
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<tr>
<td></td>
<td>MK-9 (H₈₄)</td>
<td>MK-9 (H₈₄)</td>
<td>MK-10 (H₈₅)</td>
</tr>
</tbody>
</table>

Streptomyces, for which the name *Streptomyces capparidis* sp. nov. is proposed.

**DESCRIPTION OF STREPTOMYCES CAPPARIDIS SP. NOV.**

*Streptomyces capparidis* (cap.pa’ri.dis. N.L. gen. n. *capparidis* of *Capparis*, referring to the isolation of the type strain from *Capparis spinosa* L.).

Grain-stain-positive actinobacterium with extensive non-fragmenting substrate and aerial mycelia. Spores have a smooth surface and occur in straight or flexuous chains. No soluble pigment is produced on any of the test media. Growth occurs at 10–45 °C and pH 6.0–8.0. Growth occurs with 0–1 % (w/v) NaCl. Positive for catalase reaction, nitrate reduction, and milk coagulation and peptonization. Negative for methyl red test and H₂S production. Hydrolysates contain glucose, ribose, fructose and mannose. The predominant menaquinones are MK-9 (H₈₅) and MK-9 (H₈₄). Polar lipids are diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, phosphatidylcholine, phosphatidylmethylethanolamine, three unknown phospholipids, an unknown aminophospholipid and an unknown aminolipid. The major cellular fatty acids (>10 %) are anteiso-C₁₅:₀, anteiso-C₁₇:₀, iso-C₁₅:₀ and iso-C₁₆:₀.

The type strain, EGI 6500195T (=DSM 42145T=JCM 30089T), was isolated from *Capparis spinosa* L., collected from Urumqi city, Xinjiang, China. The G+C content of the genomic DNA of the type strain is 74.1 mol%.

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

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