Phytoactinopolyspora endophytica gen. nov., sp. nov., a halotolerant filamentous actinomycete isolated from the roots of Glycyrrhiza uralensis F.

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A novel endophytic actinomycete, designated strain EGI 60009T, was isolated from the roots of Glycyrrhiza uralensis F. collected from Xinjiang Province, north-west China. The isolate was able to grow in the presence of 0–9 % (w/v) NaCl. Strain EGI 60009T had particular morphological properties: the substrate mycelia fragmented into rod-like elements and aerial mycelia differentiated into short spore chains. LL-2, 6-Diaminopimelic acid was the cell-wall diamino acid and rhamnose, galactose and glucose were the cell-wall sugars. MK-9(H4) was the predominant menaquinone. The major fatty acids of strain EGI 60009T were iso-C15 : 0, anteiso-C15 : 0, anteiso-C17 : 0, iso-C17 : 0, iso-C17 : 1 and l/anteiso-C17 : 0. B. Mycolic acids were absent. The DNA G+C content of strain EGI 60009T was 70.4 mol%. Phylogenetic analysis based on the 16S rRNA gene sequence indicated that strain EGI 60009T belongs to the family Jiangellaceae and formed a distinct clade in the phylogenetic tree. 16S rRNA gene sequence similarities between strain EGI 60009T and other members of the genera Jiangella and Haloactinopolyspora were 96.1–96.4 and 95.7–96.0 %, respectively. Based on these results and supported by morphological, physiological and chemotaxonomic data and numerous phenotypic differences, a novel species of a new genus, Phytoactinopolyspora endophytica gen. nov., sp. nov., is proposed. The type strain of Phytoactinopolyspora endophytica is EGI 60009T (=KCTC 29657T=CPCC204078T).

The family Jiangellaceae was first described by Tang et al. (2011). It comprises, at the time of writing, two genera with validly published names: Jiangella (Song et al., 2005) and Haloactinopolyspora (Tang et al., 2011; Zhang et al., 2014), the two genera having distinctive morphological

**Abbreviations:** LL-DAP, LL-2, 6-diaminopimelic acid.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain EGI 60009T is KP271925.

One supplementary table and two supplementary figures are available with the online Supplementary Material.

Strain EGI 60009T was isolated from the healthy roots of Glycyrrhiza uralensis F. collected from Yili County, Xinjiang Province, north-west China. Morphological and/or chemotaxonomic characteristics. The genus Jiangella comprises four recognized species isolated from desert soil, cave, medicinal plant and moudly cell wall, respectively (Song et al., 2005; Lee, 2008; Qin et al., 2009; Kämpfer et al., 2011). The genus Haloactinopolyspora includes two species, Haloactinopolyspora alba (Tang et al., 2011) and Haloactinopolyspora alkaliphila (Zhang et al., 2014), isolated from a salt lake and a saline–alkali soil, respectively.
chemotaxonomic properties of strain EGI 60009T indicated that it belongs to the family *Jiangellaceae*. Phylogenetic analysis based on 16S rRNA gene sequence data showed that strain EGI 60009T formed a separate lineage within the family *Jiangellaceae*. Therefore, we propose that strain EGI 60009T should be classified as representing a novel species of a new genus in the family *Jiangellaceae*.

Glycyrrhiza uralensis F. samples were washed in running tap water to remove soil particles and were surface-sterilized first with 95 % ethanol for 5 min, then with 0.1 % (w/v) mercuric chloride for 4 min and rinsed five times with sterile water according to Vincent (1970). Then, the surface-sterilized root samples were sliced into small pieces, followed by plating on R2A agar (BD) containing nalidixic acid (25 mg l\(^{-1}\)) and nystatin (100 mg l\(^{-1}\)) to repress growth of bacteria and fungi. Plates were incubated at 28 °C for 4 weeks until the outgrowth of endophytic actinomycetes was discerned. A single colony was selected and purified by re-cultivation onto R2A plates and incubated again. One isolate, EGI 60009T, was cultured on R2A agar at 28 °C and maintained on R2A agar slants at 4 °C and as 20 % (v/v) glycerol suspensions at −80 °C.

Cultural characteristics of strain EGI 60009T were recorded on International Streptomyces Project (ISP) media (Shirling & Gottlieb, 1966), Czapek’s agar, nutrient agar, tripticase soy agar (TSA) and R2A agar. All media were supplemented with 2 % (w/v) NaCl for growth. The colours of substrate and aerial mycelia and any soluble pigments produced were determined by comparison with chips from ISCC-NBS colour charts (Kelly, 1964). Growth was good on nutrient agar, R2A agar, Czapek’s agar, inorganic salts/starch agar (ISP 4) and oatmeal agar (ISP 3); there was poor growth on TSA and no growth on yeast extract/malt extract agar (ISP 2) or glycerol/asparagine agar (ISP 5). The aerial mycelium colour was white and that of the substrate mycelium was white–yellow. No soluble pigments were produced in all the media tested. Morphological characteristics of strain EGI 60009T were observed by light microscopy (model BH 2; Olympus) and scanning electron microscopy (JSM5600LV; JEOL) after cultivation on nutrient agar containing 2 % (w/v) NaCl at 28 °C for 2 weeks. The substrate mycelia were well developed and fragmented into rod-like elements, while the aerial mycelia had short spore chains (Fig. 1).

Growth at different temperatures (4, 10, 20, 28, 37, 40, 42, 45, 50 and 55 °C) was tested on nutrient agar plates containing 2 % (w/v) NaCl for 2 weeks. For NaCl tolerance experiments, nutrient agar medium was used as the basal medium, and salt concentrations ranging from 0 to 15 % (w/v), at intervals of 1 %, were tested at 28 °C for 2 weeks. The pH growth range was investigated between pH 4.0 and 10.0, at intervals of 1 pH unit, using the buffer system described by Tang *et al.* (2011). Anaerobic growth was determined using the GasPak Anaerobic System (BBL), according to the manufacturer’s instructions. Catalase, oxidase and gelatinase activities, starch hydrolysis, nitrate reduction, H\(_2\)S production and urease were assessed as described by Smibert & Krieg (1994). Carbon source utilization was determined as described by Williams *et al.* (1989). Enzyme activities were determined by using the API ZYM system (bioMérieux), according to the manufacturer’s instructions. Antibiotic sensitivity was explored by placing commercial antibiotic discs (HiMedia) on the modified nutrient agar plates that had been spread with the isolates and then incubated at 28 °C for 5 days.

![Fig. 1. Scanning electron micrographs of spore chains of strain EGI 60009T grown on nutrient agar with 2 % (w/v) NaCl for 1–2 weeks at 28 °C. (a) Substrate mycelium fragmented into rod-like elements; aerial mycelium was present when cultured for 7 days. (b) Short spore chains after culture for 2 weeks. Bars, 5 μm (a), 10 μm (b).](image-url)
Strain EGI 60009T was able to grow at 20–37 °C, at pH 6.0–9.0 and with 0–9 % NaCl. The isolate was sensitive to tetracycline (30 μg), gentamicin (10 μg), vancomycin (30 μg), penicillin (10 IU), chloramphenicol (30 μg), novobiocin (5 μg), tobramycin (10 μg), erythromycin (15 μg), rifampicin (5 μg), ampicillin (10 μg) and amikacin (30 μg), but resistant to norfloxacin (10 μg) and ciprofloxacin (5 μg).

The detailed physiological and biochemical characteristics of the isolate are given in the species description.

The isomer of diaminopimelic acid was analysed according to the procedure developed by Hasegawa et al. (1983). Amino acids in cell-wall hydrolysates were analysed by pre-column derivatization with o-phthalaldehyde by HPLC (Tang et al., 2009a). Cell-wall sugars were detected by pre-column derivatization with 1-phenyl-3-methyl-5-pyrazolone by HPLC (Tang et al., 2009b). Polar lipids were extracted as described by Minnikin et al. (1984) and identified by two-dimensional TLC (Collins & Jones, 1980). Menaquinones were extracted (Collins et al., 1977) and analysed by HPLC (Tamaoka et al., 1983). Mycolic acids were extracted and analysed according to the protocol of Minnikin et al. (1984). For fatty acid analysis, cells of strain EGI 60009T and the reference strains were cultured in nutrient agar (BD) containing 2 % NaCl at 28 °C for 7 days. Analysis of the cellular fatty acid pattern followed the method described by Sasser (1990), but we used version 6.0 of the Sherlock Microbial Identification System (MIDI). The genomic DNA was prepared according to the method of Marmur (1961). The G+C content of the DNA was determined by reversed-phase HPLC of nucleosides according to Mesbah et al. (1989).

The results indicated that strain EGI 60009T contained LL-2, 6-diaminopimelic acid (LL-DAP), alanine, aspartate and glutamic acid as cell-wall amino acids. Glucose, galactose, mannose and rhamnose were the major cell-wall sugars. The polar lipids were diphostatidyglycerol, phosphatidylglycerol, phosphatidylinositol mannosides, three unknown phosphoglycolipids and four unknown phospholipids (Fig. S1, available in the online Supplementary Material). The predominant menaquinone was MK-9(H4) (99.2 %), with a minor amount of MK-9(H6) (0.8 %) detected. Strain EGI 60009T had a cellular fatty acid profile that contained major amounts of branched fatty acids and minor amounts of saturated and unsaturated fatty acids. The major fatty acids were anteiso-C15 : 0 (23.1 %), anteiso-C17 : 0 (16.9 %), iso-C17 : 0 (11.4 %), iso-C15 : 0 (11.1 %) and iso-C17 : 1I (anteiso-C17 : 0 B (11.1 %); a moderate amount of iso-C16 : 0 (8.0 %) was found, and the minor fatty acids were C14 : 0 (1.2 %), C16 : 0 (2.7 %), C17 : 0 (1.1 %), C18 : 0 (1.1 %), iso-C16 : 0 (8.0 %), anteiso-C17 : 0 A (0.8 %), C17 : 0 109c (1.5 %), C18 : 109c (1.0 %), iso-C14 : 0 (0.9 %), iso-C14 : 0 3-OH (0.4 %), C15 : 0 2-OH (0.3 %), iso-C16 : 0 3-OH (0.9 %), C17 : 0 2-OH (1.8 %) and C16 : 107c/C16 : 109c (2.2 %). Mycolic acids were absent (Fig. S2). The G+C content of the DNA was 70.4 mol%.

The extraction of genomic DNA and PCR amplification of the 16S rRNA gene were done as described by Li et al. (2007). Multiple alignments with sequences of the suborder Propionibacterineae and calculations of levels of sequence similarity were carried out using EzBio Cloud (http://www.eztaxon.org; Kim et al., 2012). Phylogenetic analyses were performed using three tree-making algorithms: the neighbour-joining (Saitou & Nei, 1987), maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Fitch, 1971) methods. A phylogenetic tree was reconstructed using the neighbour-joining method of Saitou & Nei (1987) from K1 values (Kimura, 1980) using MEGA version 6.0 Tamura et al. (2013). The topology of the phylogenetic tree was evaluated by the bootstrap resampling method of Felsenstein (1985) with 1000 replicates.

The almost-complete 16S rRNA gene sequence of strain EGI 60009T comprised 1514 bp. Phylogenetic analysis based on 16S rRNA gene sequences revealed that the organism clustered with members of the genera Jiangella and Haloactinopolyspora (Fig. 2). Levels of 16S rRNA gene sequence similarity between strain EGI 60009T and the type strains of Jiangella alba, Jiangella gansuensis, Jiangella muralis, Jiangella alkaliphila, H. alba and H. alkaliphila were 96.4, 96.2, 96.2, 96.1, 96.0 and 95.7 %, respectively. In the phylogenetic tree based on the neighbour-joining algorithm, strain EGI 60009T and members of the genera Jiangella and Haloactinopolyspora clustered in a distinct clade supported by a high bootstrap value (100 %). This relationship was supported by all the tree-making methods used in this study. Although strain EGI 60009T was similar to members of the genera Jiangella and Haloactinopolyspora with MK-9(H4) as the predominant menaquinone and LL-DAP as the cell-wall amino acid, it differed based on morphological properties (Fig. 1). Moreover, strain EGI 60009T could be distinguished from members of the genus Jiangella in having short spore chains. In addition, strain EGI 60009T exhibited chemotaxonomic differences from the genus Jiangella. Strain EGI 60009T contained glucose, galactose, mannose and rhamnose (no ribose) as major cell-wall sugars, anteiso-C15 : 0, anteiso-C17 : 0, iso-C17 : 0, iso-C15 : 0 and iso-C17 : 1I as major fatty acids, and diphostatidyglycerol, phosphatidylglycerol, three unknown phosphoglycolipids, four unknown phospholipids and phosphatidylinositol mannosides as polar lipids (Fig. S1). Members of the genus Jiangella contain glucose and ribose as cell-wall sugars (rhamnose, mannose or galactose may be present), anteiso-C15 : 0, iso-C16 : 0 and iso-C17 : 1I as major fatty acids (iso-C14 : 0 or iso-C15 : 0 or iso-C16 : 0 may also be present as major fatty acids), and phosphatidylinositol as the predominant polar lipid (phosphatidy choline may also be present) (Table 1; see also Table S1 and Fig. S1). The isolated strain could also be distinguished from members of the genus Haloactinopolyspora (Table 1). The latter contain glucosamine, glucose, galactose, mannose and arabinose as cell-wall sugars, anteiso-C15 : 0, iso-C16 : 0 and anteiso-C17 : 0 as major fatty acids, and phosphatidylinositol and
an unknown glycolipid as the predominant polar lipids (Tang et al., 2011; Zhang et al., 2014). In particular, strain EGI 60009T was morphologically distinct from members of the genus Haloactinopolyspora (Fig. 1): strain EGI 60009T did not form pseudosporangium-like, rhiziform spore aggregates at maturity.

The 16S rRNA gene sequences of strain EGI 60009T and all members of the family Jiangellaceae were scanned for signature nucleotides; strain EGI 60009T and the type strains of members of the two related genera Jiangella and Haloactinospora had many unique 16S rRNA gene signature nucleotides, but also showed two specific positions at 127:234 (G-C) and 833:853 (G-C). Therefore, on the basis of differences in phenotypic, chemotaxonomic and phylogenetic characteristics between the new isolate and its closest neighbours, the genera Jiangella and Haloactinospora could be distinguished from related genera.

**Fig. 2.** Phylogenetic relationships between strain EGI 60009T and members of the family Jiangellaceae and the type strains of related species on the All-Species Living Tree based on the 16S rRNA gene sequences. The branching pattern was generated by the neighbour-joining method. Bootstrap values (expressed as percentages of 1000 replications) above 50% are shown at branch points. Bar, 0.02 substitutions per nucleotide position.
Table 1. Differential phenotypic characteristics between strain EGI 60009<sup>T</sup> and related members of the genera *Jiangella* and *Haloactinopolyspora*

Strains: 1, EGI 60009<sup>T</sup>; 2, *J. gansuensis* YIM 002<sup>T</sup>; 3, *J. alkaliphila* DSM 45079<sup>T</sup>; 4, *J. murlais* YIM 61503<sup>T</sup>; 5, *J. alba* YIM 61503<sup>T</sup> and *J. muralis* DSM 45357<sup>T</sup>; 6, *H. alkaliphila* EGI 80088<sup>T</sup> (Zhang et al., 2014); 7, *H. alba* YIM 93246<sup>T</sup> (Tang et al., 2011). All data for members of the genus *Jiangella* are from the present study except aerial mycelium characteristics, cell-wall sugars, polar lipids and genomic DNA G+C contents for *J. gansuensis* YIM 002<sup>T</sup>, *J. alkaliphila* DSM 45079<sup>T</sup>, *J. alba* YIM 61503<sup>T</sup> and *J. muralis* DSM 45357<sup>T</sup>, which are from Song et al. (2005), Lee (2008), Qin et al. (2009) and Kämpfer et al. (2011), respectively. The reference type strains were cultivated at their optimum culture conditions. +, Positive/utilized; +W, weakly positive; –, negative/not utilized; ND, not detected; Ara, arabinose; Gal, galactose; GlcN, glucosamine; Glu, glucose; Man, mannose; Rha, rhamnose; Rib, ribose; DPG, diphosphatidylglycerol; PC, phosphatidylcholine; PG, phosphatidylglycerol; GL, unknown glycolipid; PGL, unknown phosphoglycolipid; PI, phosphatidylinositol; PIM, phosphatidylinositol mannoside; PL, unknown phospholipid; UL, unknown polar lipid.

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and Haloactinopolyspora, we suggest that strain EGI 60009T represents a novel species of a new genus, for which the name Phytoactinopolyspora endophytica gen. nov., sp. nov. is proposed.

Description of Phytoactinopolyspora gen. nov.

Phytoactinopolyspora (Phy.to.ac.ti.no.po.ly.spo’ra. Gr. n. phyton plant; Gr. n. actis actinos a ray; Gr. adj. poly many; Gr. n. spora a seed and, in biology, a spore; N.L. fem. n. Phytoactinopolyspora many-spored ray isolated from plant tissues).

Gram-reaction-positive, strictly aerobic, moderately halotolerant filamentous actinomycetes. The substrate mycelium fragments into rod-like elements, and the aerial mycelium has long or short spore chains. The cell-wall hydrolysates contain LL-DAP, alanine, aspartate and glutamic acid as the cell-wall amino acids; glucose, galactose, mannose and rhamnose are major cell-wall sugars. The polar lipids are diposphatidylglycerol, phosphatidylinositol, phosphatidylinositol mannosides, three unknown phosphoglycolipids and four unknown phospholipids. The predominant menaquinone is MK-9 (H4). The major fatty acids are anteiso-C15:0, anteiso-C17:0, iso-C17:0, iso-C15:0 and iso-C17:1 I/anteiso-C17:0 B. Mycolic acids are absent. The G+C content of the DNA is about 70 mol%. The type species is Phytoactinopolyspora endophytica.

Description of Phytoactinopolyspora endophytica sp. nov.

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

In addition to the morphological, chemotaxonomic and general characteristics described for the genus, colonies of the isolate develop well on ISP 3, ISP 4, Czapek’s agar, R2A agar and nutrient agar media. Poor growth occurs on TSA and no growth occurs on ISP 2 and ISP 5 agar. Soluble pigment is not produced on tested media. The colour of colonies is white on the tested media. Growth occurs at 20–37 °C and pH 6.0–9.0. Tolerates up to 9 % (w/v) NaCl. Optimal temperature and pH for growth are 28 °C and pH 7.0. Positive for catalase, oxidase, gelatin liquefaction, nitrate reduction and hydrolysis of Tweens 40, 60 and 80. Negative for urease, milk coagulation, milk peptonization, H2S production, and hydrolysis of Tween 20, cellulose and starch. D-Mannitol, D-galactose, L-arabinose, D-lactose, melibiose, raffinose, melezitose, D-rhamnose, D-xylitol, D-mannose, D-glucose, sucrose and glycerol can be used as sole carbon sources for growth, but not D-sorbitol, maltose, cellobiose, turanose, L-sorbitose, inositol, D-fructose, malate or citrate. L-Alanine, L-asparagine, L-tyrosine, hypoxanthine, xanthine and adenine can be used as sole nitrogen sources, but not glutamine, threonine, aspartic acid or glycine. In the API ZYM system, alkaline phosphatase, acid phosphatase,
esterase (C4), esterase lipase (C8), lipase (C14), leucine ary-
lamidase, valine arylamidase, cystine arylamidase, trypsin,
\(\alpha\)-chymotrypsin, naphthol-AS-BI-phosphohydrolase, \(\beta\)-
galactosidase, \(\alpha\)-glucosidase, \(\beta\)-glucosidase, \(\alpha\)-mannosidase
and \(\alpha\)-fucosidase are positive; \(\alpha\)-galactosidase, \(\beta\)-glucoronidi-
ase and \(N\)-acetyl-\(\beta\)-glucosaminidase are negative.

The type strain, EGI 60009\(^T\) (=KCTC 29657\(^T\)=CPCC 204078\(^T\)),
was isolated from surface-sterilized roots of *Glycyrrhiza uralesis* F. collected from Yili County, Xinjiang Province,
north-west China. The DNA G+C content of the type
strain is 70.4 mol%.

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