Phytoactinopolyspora alkaliphila sp. nov., an alkaliphilic actinomycete isolated from a saline-alkaline soil

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An alkaliphilic, filamentous actinomycete, designated EGI 80629T, was isolated from a soil sample of Xinjiang, north-west China. Strain EGI 80629T grew at pH 6.0–11.0 (optimum pH 9.0–10.0) and in the presence of 0–13.0 % NaCl (optimum 3.0–5.0 %). The isolate formed fragmented substrate mycelia, and aerial hyphae with short spore chains with rod-like spores. Whole-cell hydrolysates of the isolate contained LL-diaminopimelic acid as the diagnostic diamino acid, and mannose and rhamnose as diagnostic sugars. The major fatty acids identified were iso-C15 : 0, iso-C16 : 0, anteiso-C15 : 0 and iso-C17 : 0. The predominant menaquinone was MK-9(H4), while the polar lipids were diphosphatidylglycerol, phosphatidylglycerol, two phosphatidylinositol mannosides, five unknown phospholipids, three unknown phosphoglycolipids, one unknown glycolipid, four unknown polar lipids and one unknown aminophospholipid. The G+C content of the genomic DNA was 67.3 mol%. Phylogenetic analysis based on 16S rRNA gene sequences showed that strain EGI 80629T clustered with the genus Phytoactinopolyspora. The 16S rRNA gene sequence similarity between strain EGI 80629T and Phytoactinopolyspora endophytica EGI 60009T was 96.8 %. Based on morphological, chemotaxonomic and phylogenetic characteristics, strain EGI 80629T represents a novel species of the genus Phytoactinopolyspora, for which the name Phytoactinopolyspora alkaliphila sp. nov. is proposed. The type strain is EGI 80629T (=CGMCC 4.7225T =KCTC 39701T).

The genus Phytoactinopolyspora was newly proposed as a member of the family Jiangellaceae by Li et al. (2015). The genus differs from the genera Jiangella and Haloactinopolyspora by the formation of short spore chains and no pseudosporangium-like, rhiziform spore aggregate at maturity, and by specific 16S rRNA gene signature nucleotides (Li et al., 2015). At the time of writing, the genus Phytoactinopolyspora consists of only one species with a validly published name, Phytoactinopolyspora endophytica. It is very interesting that members of the family Jiangellaceae are distributed in several environments, including medicinal plants (Qin et al., 2009; Li et al., 2015), an indoor environment (Kämpfer et al., 2011), a cave (Lee, 2008), a salt lake (Tang et al., 2011), desert soil (Song et al., 2005) and saline-alkaline soil (Zhang et al., 2014), despite there only being seven species in the family with validly published names (http://www.bacterio.net/).
Simultaneously, these species show different preferences for alkalinity or salts. *Haloactinopolyspora alba* is halophilic (Tang et al., 2011), *Jiangella alkaliphila* (Lee, 2008) and *Haloactinopolyspora alkaliphila* (Zhang et al., 2014) are alkaliphilic, and other species like normal cultural conditions.

During a program on diversity of alkaliphilic actinobacteria, a novel alkaliphilic strain, designated EGI 80629T, was isolated from a saline-alkaline soil of Xinjiang, north-west China. In this study, strain EGI 80629T was characterized by a polyphasic approach, and the obtained data suggest that the isolate represents a novel species of the genus *Phytoactinopolyspora*.

Strain EGI 80629T was obtained from a desert soil sample (collected from Karamay, Xinjiang, north-west China) after incubation for 3 weeks at 30 °C on International Streptomyces Project (ISP) 2 medium (Shirling & Gottlieb, 1966) modified by adjusting the pH to 10.0 with autoclaved 10 M NaOH. The same medium was also used for cultivating and maintaining the purified isolate. Strain EGI 80629T was preserved as glycerol suspensions (20 %, w/v) at −80 °C. For morphological observations, strain EGI 80629T was cultured for 14–28 days at 30 °C on ISP 2 modified with addition of 3.0 % (w/v) NaCl and adjusting the pH to 10.0 with 10 M NaOH. Morphological features were observed by light microscopy (BH-2; Olympus) and scanning electron microscopy (Quanta 200; FEI). Cultural features were determined after incubation for 2 and 4 weeks at 30 °C according to the methods of Shirling & Gottlieb (1966). The colours of the aerial and substrate mycelia were determined with the ISCC–NBS colour charts (Kelly, 1964). Carbon-source utilization tests were performed according to the methods described by Shirling & Gottlieb (1966). Nitrogen-source utilization tests were analysed as described by Williams et al. (1983). All media were adjusted to pH 10.0 with 10 M NaOH, NaCl tolerance tests were performed on R2A agar modified by the addition of various concentrations of NaCl (0–15 %, at intervals of 1 %, w/v) and adjusting pH to 10.0 with 10 M NaOH. The temperature range for growth was examined at 5–60 °C (at intervals of 5 °C) on R2A with the addition of 3.0 % (w/v) NaCl and adjusting pH to 10.0 with 10 M NaOH; cultures were incubated for 14–28 days at 30 °C. Growth at different pH (pH 4.0–12.0, at intervals of 1.0 pH unit) was examined in R2A broth supplemented with 3.0 % NaCl (w/v) using the buffer system described by Xu et al. (2005). Physiological and biochemical characteristics were examined as described previously (Goodfellow, 1971; Williams et al., 1983).

Strain EGI 80629T formed well-differentiated substrate mycelium, which fragmented into short or long rods at the early growth stage. The isolate also produced white aerial hyphae on yeast extract-malt extract agar (ISP 2), potato-dextrose-agar (PDA) and nutrient agar; the hyphae fragmented into rod-like-spore chains, and the spore chains did not aggregate at maturity (Fig. 1). Strain EGI 80629T grew well on ISP 2 and PDA, moderately on nutrient agar and oatmeal agar (ISP 3), and weakly on inorganic starch agar (ISP 4), glycerol-asparagine agar (ISP 5), Czapek’s agar and Gauze No. 1 agar. No soluble pigments were observed on any of the media tested. The cultural characteristics of the strain are depicted in Table S1 (available in the online Supplementary Material).

Strain EGI 80629T could grow at pH 6.0–11.0 and 15–45 °C, with optimum growth at pH 9.0–10.0 and 30 °C. The isolate could tolerate up to 13.0 % (w/v) NaCl and grow optimally in the presence of 3.0–5.0 % (w/v) NaCl. These results indicated that strain EGI 80629T is moderately alkaliphilic and halotolerant. The reactions for catalase and gelatin liquefaction were positive, but negative for oxidase activity, nitrate reduction, coagulation and peptonization of skimmed milk and production of

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**Fig. 1.** Scanning electron micrograph of spore chains of strain EGI 80629T grown for 4 weeks at 30 °C on ISP 2 agar modified with 3 % (w/v) NaCl added and pH adjusted to 10.0 with 10 M NaOH. (a), Fragmented substrate mycelium; (b), aerial hyphae with rod-like spore chains. Bars, 5 μm.
H₂S. Strain EGI 80629ᵀ could hydrolyse Tweens 20, 40, 60 and 80, but not cellulose or starch.

Biomass for chemical and molecular studies was obtained by cultivation at 30 °C for 6 days in shake flasks (about 150 r.p.m.) containing ISP 2 broth adjusted to pH 10.0 with NaOH. Diaminopimelic acid isomers of whole-cell hydrolysates were analysed by TLC as described previously (Staneck & Roberts, 1974). Whole-cell sugars were detected by HPLC after pre-column derivatization with 1-phenyl-3-methyl-5-pyrazolone (PMP) (Tang et al., 2009). For identification of the mycolic acids present in the cell wall, one-dimensional TLC was carried out following the standard procedure described by Minnikin et al. (1975). Polar lipids were extracted by two-dimensional TLC and identified following the method of Minnikin et al. (1984). Menaquinones were extracted and purified according to Collins et al. (1977) and analysed by HPLC (Groth et al., 1996). For fatty acid analyses, strain EGI 80629ᵀ was cultured for 4 days at 30 °C on TSB medium modified by the addition of 0.5% (w/v) NaCl and adjusting pH to 10.0 with NaOH. Cellular fatty acids analysis was performed as described by Sasser (1990) according to the standard protocol of the MIDI/Hewlett Packard Microbial Identification System. For determination of G+C content, the genomic DNA of strain EGI 80629ᵀ was prepared according to Marmur (1961), and the G+C content was determined by the HPLC method (Mesbah et al., 1989).

Strain EGI 80629ᵀ contained LL-diaminopimelic acid as diagnostic cell-wall diamino acid. Whole-cell hydrolysates contained mannose and rhamnose as the diagnostic sugars, as well as three unknown sugars as minor sugars. Mycolic acids were absent in the cell wall of strain EGI 80629ᵀ. The predominant menaquinone detected was MK-9(H₄). The polar lipids were diphosphatidylglycerol, two phosphatidyl-inositol mannosides, phosphatidylglycerol, three unknown phosphoglycolipids, one unknown glycolipid, five unknown phospholipids, four unknown polar lipids and one unknown aminophospholipid (Fig. S1). Strain EGI 80629ᵀ contained branched saturated fatty acids as major fatty acids (>70%). The main fatty acids (>10%) were iso-C₁₅:₀, iso-C₁₆:₀, anteiso-C₁₅:₀ and iso-C₁₇:₀, and the other fatty acids are given in Table S2. The DNA G+C content of strain EGI 80629ᵀ was 67.3 mol%.

Genomic DNA preparation, PCR amplification and sequencing of the 16S rRNA gene were carried out using procedures described by Li et al. (2007). Multiple alignments with sequences of the members of the order Jiangelales, and calculations of levels of sequence similarity were carried out using the EzTaxon-e server (http://eztaxon-e.ezbiocloud.net/; Kim et al., 2012) on the basis of 16S rRNA gene sequence data. Phylogenetic analysis was performed using three tree-making algorithms, neighbour-joining (Saitou & Nei, 1987), maximum-
Description of Phytoactinopolyspora alkaliphila sp. nov.

Phytoactinopolyspora alkaliphila (al.ka.li’phi.la. Arabic article al the; Arabic n. qalty ashes of saltwort; N.L. n. alkalii alkali; Gr. adj. philos loving; N.L. fem. adj. alkaliphila loving alkaline conditions).

Gram-stain-positive, aerobic actinomycete. Well-developed substrate mycelium which exhibits fragmentation into...
short or elongated rods at early stage of growth; fragmented aerial mycelium with short chains of spores, and spore chains do not aggregate at maturity. Grows well on ISP 2 and PDA, moderately on nutrient agar and ISP 3, and weakly on ISP 4, ISP 5, Czapek’s agar and Gauze No. 1 modified with pH adjusted to 10.0 with autoclaved 10 M NaOH. Diffusible pigment is not observed on any of the media tested. Colours of aerial mycelium and substrate mycelium on ISP2 agar medium are white and pale yellow, respectively. Growth occurs at pH 6.0–11.0, with 0–13.0% (w/v) NaCl and at 15–45 °C; optimum growth occurs at pH 9.0–10.0, with 3.0–5.0% NaCl and at 30 °C. D- Arabinose, D-fructose, D-galactose, D-glucose, glycerol, D-inositol, lactose, DL-malic acid, maltose, D-mannose, raffinose, L-rhamnose, D-sorbitol, L-sorbose, sucrose, trehalose, D-xylitol, D-xyitol, sodium citrate and sodium pyruvate can be utilized as sole carbon sources for growth, but not D-ribose. DL-Alanine, L-asparagine, L-aspartic acid, L-cystine, L-glutamic acid, L-histidine, L-isoleucine, L-lysine, L-methionine, L-phenylalanine, L-serine, L-threonine, L-tryptophan and L-valine can be used as sole nitrogen sources for growth, but not L-arginine or L-tyrosine. Positive reactions for catalase and gelatin liquefaction, but negative reactions for oxidase, coagulation and peptonization of skimmed milk, nitrate reduction and liquefaction, but negative reactions for oxidase, coagulation and peptonization of skimmed milk, nitrate reduction and liquefaction, but negative reactions for oxidase, coagulation and peptonization of skimmed milk, nitrate reduction and liquefaction. DL-Alanine, L-asparagine, L-aspartic acid, L-cystine, L-glutamic acid, L-histidine, L-isoleucine, L-lysine, L-methionine, L-phenylalanine, L-serine, L-threonine, L-tryptophan and L-valine can be used as sole nitrogen sources for growth, but not L-arginine or L-tyrosine. Positive reactions for catalase and gelatin liquefaction, but negative reactions for oxidase, coagulation and peptonization of skimmed milk, nitrate reduction and H₂S production. Hydrolyses Tweens 20, 40, 60 and 80, but not cellulose or starch. Whole-cell hydrolysates contain L- diaminopimelic acid as the diagnostic cell-wall amino acid, and mannose and rhamnose as diagnostic sugars. Mycolic acids are absent. The predominant menaquinone detected is MK-9(H₄). The polar lipids are diphosphatidylglycerol, two phosphtidylinositol mannosides, phosphatidylglycerol, three unknown phosphoglycolipids, one unknown glycolipid, five unknown phospholipids, four unknown polar lipids and one unknown aminophospholipid. The major fatty acids are iso-C₁₅:₀, anteiso-C₁₅:₀ and iso-C₁₇:₀.

The type strain is EGI 80629T (=CGMCC 4.7225T =KCTC 39701T) isolated from a soil sample collected in Karamay, Xinjiang, north-west China. The G+C content of the DNA of the type strain is 67.3 mol%.

Acknowledgements

This research was supported by the joint fund of the National Natural Science Foundation of China (NSFC) (nos U1403101 and 31400099), the West Light Foundation of Chinese Academy of Sciences (RCYP201203) and Xinjiang Uygur Autonomous Region Natural Science Foundation (2014211A074). W.-J. L. was also supported by the ‘One Hundred Talents Program’ of the Chinese Academy of Sciences, the High-level Talents Program of Xinjiang Autonomous Region and Guangdong Province Higher Vocational Colleges & Schools Pearl River Scholar Funded Scheme (2014).

References


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