Sinosporangium album gen. nov., sp. nov., a new member of the suborder Streptosporangineae

Yu-Qin Zhang,1† Hong-Yu Liu,1† Li-Yan Yu,1 Jae-Chan Lee,2
Dong-Jun Park,2 Chang-Jin Kim,2 Li-Hua Xu,3 Cheng-Lin Jiang2
and Wen-Jun Li2

1Institute of Medicinal Biotechnology, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100050, PR China
2Biological Resource Center, Korea Institute of Bioscience and Biotechnology (KIRBB), Daejeon 305-806, Republic of Korea
3The Key Laboratory for Microbial Resources of the Ministry of Education, PR China, and Laboratory for Conservation and Utilization of Bio-Resources, Yunnan Institute of Microbiology, Yunnan University, Kunming 650091, PR China

A Gram-positive, aerobic, non-motile actinobacterium, designated strain 6014T, was isolated from a soil sample collected from Qinghai province, north-west China, and subjected to a polyphasic taxonomic study. The isolate formed elementary branching hyphae and abundant aerial mycelia with globose sporangia on ISP 4 and R2A media. Whole-cell hydrolysates of strain 6014T contained arabinose, galactose and ribose as diagnostic sugars and meso-diaminopimelic acid as the diagnostic diamino acid. The polar lipids consisted of phosphatidylmethylethanolamine, phosphatidylethanolamine, hydroxy-phosphatidylethanolamine, N-acetylglucosamine-containing phospholipids, two unknown phospholipids and an unknown glycolipid. The menaquinone system contained MK-9(H2) and MK-9(H4). The major fatty acids were C14:0, i-C15:0, C16:0 and 10-methyl-C16:1. The genomic DNA G+C content of the isolate was 69.4 mol%. Phylogenetic analysis based on 16S rRNA gene sequences revealed that strain 6014T fell within the radius of the suborder Streptosporangineae, in which the strain formed a distinct lineage next to genera of the family Streptosporangiaceae. Based on data from this polyphasic study, strain 6014T can be readily distinguished from previously described organisms and represents a member of a novel species within a new genus in the suborder Streptosporangineae. The name Sinosporangium album gen. nov., sp. nov. is proposed with 6014T (=DSM 45181T =KCTC 19655T) as the type strain.

The suborder Streptosporangineae was proposed by Stackebrandt et al. (1997) based on phylogenetic and signature nucleotide analysis. At the time of writing, there were only three families in this suborder, namely, Streptosporangiaceae, Nocardiopsaceae and Thermomonomosporaceae, with Streptosporangiaceae as the type family. In this paper, characterization of strain 6014T is reported, with proposals for Sinosporangium gen. nov. and Sinosporangium album sp. nov. in the suborder Streptosporangineae (Stackebrandt et al., 1997).

Strain 6014T was isolated on Czapek agar (Waksman, 1961) incubated at 28 °C for 3 weeks. The purified strain was maintained on ISP 4 agar and R2A slants at 4 °C and as glycerol suspensions (20%, v/v) at −20 °C. Biomass for molecular systematic and chemotaxonomic studies was obtained by cultivation in shake flasks using tryptic soy broth (Difco) at 28 °C for 10 days. Cultural characteristics of the isolate were determined after growth for 7–28 days at 28 °C on R2A, ISP 2, ISP 3, ISP 4 and ISP 5 (Shirling & Gottlieb, 1966), Czapek agar, nutrient agar (Difco) and potato agar (Waksman, 1961) media. The coverslip technique (Zhou et al., 1998) was employed to observe the hyphae, sporangia and spores. The sporangia and spore chain morphologies were recorded by examining gold-coated dehydrated specimens of 14 day cultures from R2A and ISP 4 agar by scanning electron microscopy (Quanta; FEI).

Growth was tested at 0, 4, 10, 15, 20, 28–37 (at intervals of 1 °C), 40, 45 and 55 °C on R2A. Other physiological and biochemical tests were performed at 28 °C. The pH range was examined at pH 4.0–11.0 (at intervals of 0.5 pH units).

†These authors contributed equally to this work.

The GenBank/EMBL/DDJB accession number for the 16S rRNA gene sequence of strain 6014T is EU438912.

A supplementary figure and a supplementary table are available with the online version of this paper.
Tolerance to sodium chloride [0, 1, 3 and 5–10 % (w/v), at intervals of 0.5 %] was examined using ISP 4 as basal medium. Carbon source utilization was tested as described by Shirling & Gottlieb (1966) and also using Biolog GEN III (MicroPlate) according to the manufacturer’s instructions. Qualitative enzyme tests and acid production from carbohydrates were determined by using the API ZYM and API 50CH systems (bioMerieux) according to the manufacturer’s instructions. Other physiological tests and antimicrobial activities of the strain were examined according to previously described procedures (Yuan et al., 2008).

Strain 6014\(^\top\) grew well on R2A, ISP 4, ISP 5, Czapek solution agar, nutrient agar and potato agar media at 28–32 °C and pH 7.0–7.5. White to buff branching vegetative hyphae developed well and abundant white aerial hyphae were produced on the above media, whereas the strain grew very slowly with few aerial hyphae on ISP 2 and ISP 3 media. Diffusible pigments were not observed on any test media. Globose sporangia formed singly from the aerial hyphae (Fig. 1a). The sporangia were a mean size of 2.8–3.0 × 3.4–4.2 μm (Fig. 1b) and contained coiled spore chains (Fig. 1a). The non-motile, smooth-surfaced, cylindrical spores were about 0.5–0.6 × 0.6–1.2 μm. Gelatin, starch, urea and aesculin were not hydrolysed. Milk was not coagulated or peptonized and H₂S was not produced. The isolate could use most of the carbon sources listed in Biolog GEN III as sole carbon sources (Supplementary Table S1, available in IJSEM Online) for energy and growth except α-lactose, D-galactose and myo-inositol. Detailed physiological and biochemical characteristics of strain 6014\(^\top\) are given in the species description.

The whole-cell sugar pattern and diagnostic diaminopimelic acid isomers were determined by TLC (Lechevalier & Lechevalier, 1965, 1980). Polar lipids were extracted and examined by two-dimensional TLC and identified using procedures described by Minnikin et al. (1984). Menaquinones were isolated using the method of Collins et al. (1977) and analysed by HPLC (Groth et al., 1997). Analysis of the whole-cell fatty acid pattern followed described methods using the MIDI system (Microbial ID) (Kroppenstedt, 1985; Meier et al., 1993). Extraction of genomic DNA and PCR amplification of the 16S rRNA gene were done as described by Li et al. (2007). The DNA G+C content was determined by reverse-phase HPLC of nucleosides according to Mesbah et al. (1989). 16S rRNA gene multiple alignments with sequences of most closely related taxa and calculations of sequence similarity levels were carried out using CLUSTAL X (Thompson et al., 1997). A phylogenetic tree and distance matrix were reconstructed using the neighbour-joining method of Saitou & Nei (1987) from \(K_{\text{muc}}\) values (Kimura, 1980, 1983) using MEGA version 4.0 (Tamura et al., 2007). A maximum-likelihood (Felsenstein, 1981) tree (not shown) was generated using the treeing algorithm contained in the PHYLIP package (Felsenstein, 1993). Topology of the phylogenetic tree was evaluated by the bootstrap resampling method of Felsenstein (1985) with 1000 replicates.

Whole-cell hydrolysates of strain 6014\(^\top\) contained ribose, galactose and arabinose. The diagnostic diamin acid was meso-diaminopimelic acid. The polar lipids comprised phosphatidylmethylethanolamine, phosphatidylethanolamine, hydroxy-phosphatidylethanolamine, N-acetylgalactosamine-containing phospholipids, two unknown phospholipids and an unknown glycolipid (Supplementary Fig. S1). The menaquinone system contained MK-9(H₂) (53.1 %) and MK-9(H₄) (46.9 %). The major fatty acids detected were saturated, iso- and 10-methyl branched fatty acids. The detailed cellular fatty acid profile is as follows: C₁₂:0 (1.0 %), C₁₃:0 (0.6 %), C₁₄:0 (10.4 %), C₁₅:0 (2.1 %), C₁₆:0 (20.9 %), C₁₇:0 (0.6 %), C₁₈:0 (1.0 %), iso-C₁₄:0 (1.7 %), iso-C₁₅:0 (10.2 %), iso-C₁₆:0 (8.3 %), iso-C₁₇:0 (2.0 %), anteiso-C₁₇:0 (0.7 %), 10-methyl-C₁₆:0 (10.1 %), 10-methyl-C₁₇:0 (4.7 %), 10-methyl-C₁₈:0 (7.4 %), C₁₆:1ω7c (8.1 %), C₁₇:1ω8c (2.2 %), C₁₉:1ω9c (5.9 %). The genomic DNA G+C content was 69.4 mol%.

BLAST search results using the 16S rRNA gene sequence of strain 6014\(^\top\) showed that the novel isolate exhibited highest similarities with members of the suborder Streptosporangiineae, such as Streptosporangium violaceochromogenes DSM 43849\(^\top\) (96.1 %). However, in the phylogenetic tree...
Fig. 2. Neighbour-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the position of strain 6014T among its phylogenetically nearest neighbours. Bootstrap percentages (based on 1000 replications) >50% are shown at branch points. Filled circles indicate that the corresponding nodes were also recovered in the tree generated with the maximum-likelihood method. Bar, 0.01 substitutions per nucleotide position.

Table 1. Signature nucleotide patterns of the genus *Sinosporangium* and related genera

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Table 2. Morphological and chemotaxonomic characteristics of strain 6014T and members of genera in the family Streptosporangiaceae

Taxa: 1, 6014T; 2, Streptosporangium; 3, Acrocarpospora; 4, Herbidospora; 5, Microbispora; 6, Microtetraspora; 7, Nonomuraea; 8, Planobispora; 9, Planomonospora; 10, Planotetraspora; 11, Sphaerisporangium; 12, Thermopolyspora. Data were taken from this and previous studies (Greiner-Mai et al., 1987; Goodfellow et al., 1990; Kudo et al., 1993; Tamura et al., 2000; Stackebrandt et al., 2001; Tamura & Sakane, 2004; Ara & Kudo, 2007; Goodfellow et al., 2005; Cao et al., 2009). For all taxa, the fatty acid type [saturated fatty acids, unsaturated fatty acids, iso-fatty acids, variable and methyl-branched fatty acids (Kroppenstedt, 1985)] was 3c. +, Present; −, absent.

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| Diagnostic phospholipids: PII, phosphatidylethanolamine; PIV, glucosamine (with phosphatidylethanolamine and phosphatidylymethyllethanolamine variable) (Lechevalier et al., 1977).

*Whole-cell sugar patterns of actinomycetes containing meso-diaminopimelic acid: A, arabinose and galactose; B, madurose; C, no diagnostic sugar; D, arabinose and xylose (Lechevalier & Lechevalier, 1970).

†Diagnostic phospholipids: PII, phosphatidylethanolamine; PIV, glucosamine (with phosphatidylethanolamine and phosphatidylyl methyllethanolamine variable) (Lechevalier et al., 1977).
Based on its phylogenetic position (Fig. 2) and chemotaxonomic data (Table 2), it is proposed that strain 6014T represents a novel species in a new genus, *Sinosporangium* *album* gen. nov., sp. nov.

**Description of Sinosporangium gen. nov.**

*Sinosporangium* [Si.no.spo.ran’gi.um. M.L. n. *sina* China; N.L. n. *sporangium* from Gr. n. *spora* a seed and, in biology, a spore and Gr. n. *angeion* (Latin transliteration *angium*) vessel, sporangium; N.L. neut. n. *Sinosporangium* an organism isolated in China bearing sporangia].

Cells are Gram-positive and form branching hyphae. Globose sporangia are borne on aerial mycelia. Coiled spore chains are contained in the sporangia. Smooth-surfaced spores are non-motile. Grows at pH 6.5–8.5 and 10–37 °C. Catalase-positive, oxidase-negative. The diagnostc amino acid of the peptidoglycan is meso-diaminopimelic acid. Whole-cell hydrolysates contain arabinose, galactose and ribose. Phospholipids consist of phosphatidylethanolamine, meso-diaminopimelate, glycerol, acid can be produced from D-adonitol, D-glucose, fructose, lactose, melibiose and myo-inositol. Acid can be produced from D-adenosine, D-glucose, trehalose and potassium 5-ketoglucuronate.

The type strain is 6014T (=DSM 45181T =KCTC 19655T), isolated from soil collected in Qinghai, China. The genomic DNA G+C content of the type strain is 69.4 mol%.

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