Streptosporangium canum sp. nov., isolated from soil

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An actinomycete strain, designated HBUM 170018\textsuperscript{T}, was isolated from soil from Hebei Province, China, and subjected to a polyphasic taxonomic analysis. This study included morphological and physiological investigations and analyses concerning cell chemistry, genomic DNA G + C content, DNA–DNA hybridization and phylogeny. The data obtained were consistent with the assignment of strain HBUM 170018\textsuperscript{T} to the genus Streptosporangium and were confirmed by the results of 16S rRNA gene sequence analysis. Strain HBUM 170018\textsuperscript{T} can be differentiated from all Streptosporangium species with validly published names with reference to phenotypic characteristics, phylogenetic data and DNA–DNA hybridization results. On the basis of 16S rRNA gene sequencing, DNA–DNA hybridization and phenotypic data, strain HBUM 170018\textsuperscript{T} represents a novel species of the genus Streptosporangium, for which the name Streptosporangium canum sp. nov. is proposed. The type strain is HBUM 170018\textsuperscript{T} (=7177\textsuperscript{T} =DSM 45034\textsuperscript{T} =CGMCC 4.2126\textsuperscript{T}).

The genus Streptosporangium was first described by Couch (1955). In most species of the genus, the aerial mycelium produces spherical sporangia that enclose non-motile sporangiospores. At the time of writing, there are 14 Streptosporangium species with validly published names: Streptosporangium roseum (Couch, 1955), S. amethystogenes, S. album, S. vulgare (Nonomura & Ohara, 1960), S. longisporum (Schäfer, 1969), S. nondiastaticum, S. pseudo-vulgare (Nonomura & Ohara, 1969), S. violaceochromogenes (Kawamoto et al., 1975), S. fragile (Shearer et al., 1983), S. carneum (Mertz & Yao, 1990), S. claviforme (Petrolini et al., 1992), S. subroseum (Zhang et al., 2002), S. purpuratum and S. yunnanense (Zhang et al., 2005). S. amethystogenes has been divided into the subspecies S. amethystogenes subsp. amethystogenes (Nonomura & Ohara, 1960) and S. amethystogenes subsp. fukuiense (Iinuma et al., 1996). With the development of science and technology, many novel taxa and novel valuable secondary metabolites have been discovered and increasing numbers of scientists are undertaking studies of extreme environments and unusual actinomycetes. Strain HBUM 170018\textsuperscript{T} was isolated from a soil sample collected in Hebei Province, China, and was shown to produce spherical sporangia on the aerial mycelium. The results of this polyphasic taxonomic study indicated that this strain was distinct from known members of the genus Streptosporangium. Therefore, we consider that strain HBUM 170018\textsuperscript{T} represents a novel species of the genus Streptosporangium.

Strain HBUM 170018\textsuperscript{T} was cultured for 3, 5, 7 and 14 days on oatmeal agar (ISP3 medium; Shirling & Gottlieb, 1966) at 28 °C. Cells were cultured on glass bacteria-culture plates and microscope cover slips were inserted into the agar to examine the mycelial morphology. The strain was observed by using an Olympus light microscope (model BH-2) and an Amray scanning electron microscope (model KYKY-AMRAY 100B). Samples for scanning electron microscopy were prepared by cutting a block from an agar plate containing the strain, fixing the block in 2.5 % glutaraldehyde at room temperature for 2 h, washing it three times with 0.2 M sodium phosphate buffer (pH 7.2) for 10 min each time, fixing it once again in osmium tetroxide vapour for 4 h and then dehydrating the cells successively through 30, 50, 70, 85, 95 and 100 % ethanol (10 min each). Cultural characteristics were studied using cultures grown at 28 °C for 14 days on ISP3 medium, Bennett’s agar (Jones, 1949), yeast extract-malt extract agar (ISP2 medium), glycerol-asparagine agar (ISP5 medium) and Czapek agar (Waksman, 1967). Colour determinations were made by comparing the culture with colour strips from the standard samples of the Research Group of Actinomycetes (1970). Strain HBUM 170018\textsuperscript{T} grew well on...
various media. The strain produced aerial and vegetative hyphae of different colours on various media. The results are available in Supplementary Table S1 (available in IJSEM Online). No amethyst, diffusible pigments were produced on any of the media tested. Strain HBUM 170018T produced aerial mycelium bearing spherical sporangia (generally 3–6 μm in diameter; Fig. 1) that produced non-motile sporangiospores.

To obtain biomass for chemotaxonomic analyses, cultures were grown in Bennett’s broth for 5–7 days at 28 °C on rotary shakers. When maximum growth was observed, the broth cultures were checked for purity, harvested by centrifugation and washed three times with distilled water. Amino acids from purified cell walls were analysed by using the methods of Lechevalier & Lechevalier (1980). Diagnostic amino acids and sugars were determined from whole-cell hydrolysates using the methods of Becker et al. (1964). Phospholipids were obtained from freeze-dried biomass (approx. 100 mg) and analysed according to Lechevalier et al. (1981). Menaquinones (from 100 mg freeze-dried biomass) were analysed as described previously by Collins (1985). Methyl esters of cellular fatty acids (from 10 mg freeze-dried biomass) were determined as described by Miller (1982) and Kuykendall et al. (1988).

Purified cell walls of strain HBUM 170018T contained meso-diaminopimelic acid. Whole-cell hydrolysates contained madurose, glucose, ribose and rhamnose. On the basis of these data, the strain was considered to have a type III/B cell wall. The major menaquinones of the strain were MK-9(H4) and MK-9(H2). The predominant whole-cell fatty acids were C16:0 (21.33 %), C17:0 (11.54 %), C17:3 (3.61 %), C18:0 (38.55 %) and C19:0 (17.49 %). The phospholipids consisted of diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol and phospholipids of unknown structure containing glucosamine and represented the type IV phospholipid pattern (Lechevalier et al., 1981).

Physiological characteristics of the strain were tested according to the Streptosporangium standard (Nonomura, 1989). B vitamin requirements were investigated using the method of Zhou & Cao (1981). The optimal growth temperature of strain HBUM 170018T was 28 °C and the optimal growth pH was 7.2. The other results are shown in Table 1 and the species description.

For 16S rRNA gene sequence analysis, determination of G+C content and DNA–DNA hybridization, chromosomal DNA was extracted using the procedures described by Marmur (1961) and Kutchma et al. (1998). The G+C content was determined by using the method of Mesbah et al. (1989). The DNA was treated with BAL-31 nuclease and alkaline phosphatase and mononucleotides were identified using HPLC. The DNA G+C content of strain HBUM 170018T was found to be 67.8 mol%. The initial reassociation-rate method (De Ley et al., 1970) and a spectrophotometer (model CE9500; Cecil Instruments) equipped with a programmable melting-temperature control unit were used for determining the degree of DNA–DNA hybridization. The analysis was repeated three times (standard deviation 0.5–0.8 %) and mean values were obtained.

### Table 1. Comparison of physiological characteristics of strain HBUM 170018T and related strains

<table>
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![bio-fig](image)

**Fig. 1.** Scanning electron micrograph of growth of strain HBUM 170018T on ISP3 medium after 14 days at 28 °C. Bar, 10 μm.
The 16S rRNA gene was amplified using a PCR with Taq DNA polymerase (Sangon) according to the methods of Zhang et al. (2008), with universal primers 27f (Escherichia coli positions 8–27; 5’-GAGTTTGTATCTTGCGCTAG-3’) and 1525r (E. coli positions 1525–1545; 5’-AGAAAGGAGGTGTACCAGCC-3’) (Lane et al., 1985). The PCR product was purified and then sequenced at the Beijing Genomics Institute, Chinese Academy of Sciences.

The almost-complete 16S rRNA gene sequence determined for strain HBUM 170018T (1432 nt) was aligned with the corresponding sequences of representative reference strains from the genus Streptosporangium (retrieved from the GenBank database) using CLUSTAL W, version 1.4b (Thompson et al., 1994). The evolutionary tree was inferred using the neighbour-joining method (Saitou & Nei, 1987). Evolutionary distance matrices were generated according to the algorithms of the neighbour-joining, maximum-parsimony (Eck & Dayhoff, 1966) and minimum-evolution (Rzhetsky & Nei, 1992) methods and the robustness of the tree topology from the neighbour-joining data was evaluated by means of bootstrap analysis (Felsenstein, 1993) (based on 1000 resamplings) using the MEGA4 package (Tamura et al., 2007). It was evident from the phylogenetic tree (Fig. 2) that strain HBUM 170018T, S. album DSM 43023T, S. roseum DSM 43021T and S. vulgare DSM 43802T clustered into a branch. Sequence-similarity calculations obtained after a neighbour-joining analysis indicated that the closest relatives of strain HBUM 170018T were S. roseum DSM 43021T (99.90 %), S. album DSM 43023T (99.90 %) and S. vulgare DSM 43802T (99.90 %). The DNA–DNA relatedness of strain HBUM 170018T with respect to related strains was found to range from 23.28 to 64.26 % (Supplementary Table S2). These values were below the value of 70 % recommended by Wayne et al. (1987) for the assignment of strains to the same species.

In summary, phenotypic and genotypic characteristics showed that HBUM 170018T is a member of the genus Streptosporangium. However, morphological, physiological and chemotaxonomic characteristics serve to differentiate this strain from recognized species of the genus. DNA–DNA hybridization is the standard used for species designation: the cut-off point indicating members of the same species is at ≥70 % DNA–DNA relatedness. Low DNA–DNA relatedness values (<70 %) were determined between strain HBUM 170018T and type strains of members of the genus Streptosporangium. On the basis of the above-mentioned phenotypic and genotypic data, therefore, strain HBUM 170018T represents a novel species of the genus Streptosporangium, for which the name Streptosporangium canum sp. nov. is proposed.

**Description of Streptosporangium canum sp. nov.**

*Streptosporangium canum* (ca’num. L. neut. adj. canum whitish grey).

Gram-positive-staining. The colour of the aerial mycelium is whitish grey on ISP3 medium, ISP5 medium and Czapek agar. Grows well on various media. No amethyst, diffusible pigments are produced. The sporangia are spherical and 3–6 μm in diameter. Spores are rod-shaped (0.5–1.0 μm long) and non-motile. Optimal growth at 28 °C and pH 7.2. Cell walls contain meso-diaminopimelic acid. Whole-cell hydrolysates contain madurose, glucose, rhamnose and ribose (type III/B). The menaquinones consist mainly of MK-9(H4) and MK-9(H2). The major cellular fatty acids are C16:0, C17:0, C17:1ω7c, C18:1ω7c and C19ω7c. The polar lipids consist of diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, phosphatidylethanolamine and phospholipids of unknown structure containing glucosamine (type IV phospholipid pattern). The DNA G + C content of the type strain is 67.8 mol%. L-Arabinose, cellobiose, D-fructose, D-mannose, D-mannitol, D-xylose, erythrose, rhamnose, maltose, inositol and dextrin are utilized, but D-galactose, sorbitol, sorbose, melibiose and raffinose are not utilized. Positive for cellulose degradation and negative for starch.

![Fig. 2. Neighbour-joining phylogenetic tree](http://ijs.sgmjournals.org)

http://ijs.sgmjournals.org
degradation. Urea, citrate, hippurate, lactate and oxalate are hydrolysed. Malate, salicylic acid, adenine, xanthine, cytosine, hypoxanthine, casein, l-tyrosine and uridine are not hydrolysed. Positive for reduction of nitrate and liquefaction of gelatin. B vitamins are required for growth.

The type strain, strain HBUM 170018T (=7177T = DSM 45034T = CGMCC 4.2126T), was isolated from soil from Hebei Province, China.

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References


