Streptosporangium canum sp. nov., isolated from soil

Li-Ping Zhang,^{1,2} Li-Min Zhang¹ and Xiu-Min Zhang^{1,2}

Correspondence

Li-Ping Zhang
zhlping@hbu.edu.cn

¹College of Life Sciences, Hebei University, Baoding 071002, PR China

²The Key Laboratory of Microbial Diversity Research and Application of Hebei Province, Baoding 071002, PR China

An actinomycete strain, designated HBUM 170018^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^T to the genus *Streptosporangium* and were confirmed by the results of 16S rRNA gene sequence analysis. Strain HBUM 170018^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^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^T (=7177^T =DSM 45034^T =CGMCC 4.2126^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. *fukuense* (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^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^T represents a novel species of the genus *Streptosporangium*.

Strain HBUM 170018^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^T grew well on

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain HBUM 170018^T is AY996844.

Tables detailing the cultural characteristics of strain HBUM 170018^T and levels of 16S rRNA gene sequence similarity and DNA–DNA reassociation between strain HBUM 170018^T and related type strains are available as supplementary material with the online version of this paper.

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 170018^T 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 170018^T 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(H₂) and MK-9(H₄). The predominant whole-cell fatty acids were C_{16:0} (21.33 %), C_{17:0} (11.54 %), C_{17:3} (3.61 %), C_{18:0} (38.55 %) and C_{19:0} (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 170018^T 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 170018^T 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 170018^T and related strains

Strains: 1, HBUM 170018^T; 2, *S. album* DSM 43023^T; 3, *S. roseum* DSM 43021^T; 4, *S. vulgare* DSM 43802^T. All strains were positive for the utilization of cellobiose, dextrin and D-mannitol and negative for the utilization of melibiose. All strains were positive for the hydrolysis of citrate, hippurate and urea and negative for the hydrolysis of adenine, casein, cytosine, hypoxanthine, starch, L-tyrosine, uridine and xanthine. All strains required B vitamins for growth. Data are from this study.

Characteristic	1	2	3	4
Utilization of:				
L-Arabinose	+	+	–	–
Erythrose	+	–	–	–
D-Fructose	+	+	–	+
D-Galactose	–	+	–	+
Inositol	+	–	–	–
D-Mannose	+	+	–	+
Maltose	+	+	–	–
Raffinose	–	+	–	–
Rhamnose	+	+	–	–
Sorbitol	–	+	+	–
Sorbose	–	–	+	+
D-Xylose	+	–	–	+
Gelatin liquefaction	+	+	–	–
Nitrite from nitrate	+	–	+	–
Cellulose degradation	+	–	–	+
Hydrolysis of:				
Lactate	+	–	–	+
Malate	–	–	+	+
Oxalate	+	–	–	+
Salicylic acid	–	+	–	–

Fig. 1. Scanning electron micrograph of growth of strain HBUM 170018^T 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′-GAGTTTGATCCTGGCTCAG-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 170018^T (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 170018^T, *S. album* DSM 43023^T, *S. roseum* DSM 43021^T and *S. vulgare* DSM 43802^T clustered into a branch. Sequence-similarity calculations obtained after a neighbour-joining analysis indicated that the closest relatives of strain HBUM 170018^T were *S. roseum* DSM 43021^T (99.90%), *S. album* DSM 43023^T (99.90%) and *S. vulgare* DSM 43802^T (99.90%). The DNA–DNA relatedness of strain HBUM 170018^T 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 170018^T 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 170018^T and type strains of members of the genus *Streptosporangium*. On the basis of the above-mentioned phenotypic and genotypic data, therefore, strain HBUM 170018^T 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 C_{16:0}, C_{17:0}, C_{17:3}, C_{18:0} and C_{19:0}. The polar lipids consist of diphosphatidylglycerol, 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, based on almost-complete 16S rRNA gene sequences, showing the relationship between strain HBUM 170018^T and members of the genus *Streptosporangium*. Numbers at nodes indicate bootstrap percentages (based on 1000 resampled datasets); only values greater than 50% are given. The sequence of *Actinomadura madurae* NBRC 14623^T was used as the outgroup. Clades also recovered by the minimum-evolution method are labelled 'm' and clades also recovered by both the maximum-parsimony method are labelled 'p'. Bar, 0.002 substitutions per nucleotide position.

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 170018^T (=7177^T =DSM 45034^T =CGMCC 4.2126^T), was isolated from soil from Hebei Province, China.

Acknowledgements

The work was supported by the National Natural Science Foundation of China (grant no. 30270002) and the Program for Basic Microorganisms Resources Arranging, Integrating and Sharing Platform Construction of National Science and Technology Board, China (grant no. 2006DKA21206).

References

- Becker, B., Gordon, R. E., Lechevalier, H. A. & Lechevalier, M. P. (1964). Rapid differentiation between *Nocardia* and *Streptomyces* by paper chromatography of whole cell hydrolysates. *Appl Microbiol* **12**, 421–423.
- Collins, M. D. (1985). Isoprenoid quinone analysis in classification and identification. In *Chemical Methods in Bacterial Systematics*, pp. 267–287. Edited by M. Goodfellow & D. E. Minnikin. London: Academic Press.
- Couch, J. N. (1955). A new genus and family of the *Actinomycetales*, with a revision of the genus *Actinoplanes*. *J Elisha Mitchell Sci Soc* **71**, 148–155.
- De Ley, J., Cattoir, H. & Reynaerts, A. (1970). The quantitative measurement of DNA hybridization from renaturation rates. *Eur J Biochem* **12**, 133–142.
- Eck, R. V. & Dayhoff, M. O. (1966). *Atlas of Protein Sequence and Structure*. Silver Springs, MD: National Biomedical Research Foundation.
- Felsenstein, J. (1993). PHYLIP (phylogenetic inference package), version 3.5c. Distributed by the author. Department of Genome Sciences, University of Washington, Seattle, USA.
- Iinuma, S., Yokota, A. & Kanamura, T. (1996). New subspecies of the genus *Streptosporangium*, *Streptosporangium amethystogenes* subsp. *fukuiense* subsp. nov. *Actinomycetologica* **10**, 35–42.
- Jones, K. L. (1949). Fresh isolates of actinomycetes in which the presence of sporogenous aerial mycelia is a fluctuating characteristic. *J Bacteriol* **57**, 141–146.
- Kawamoto, I., Takasawa, S., Okachi, R., Kohakura, M., Takahashi, I. & Nara, T. (1975). A new antibiotic victomycin (XK 49-1-B-2). I. Taxonomy and production of the producing organism. *J Antibiot (Tokyo)* **28**, 358–365.
- Kutchma, A. J., Roberts, M. A., Knaebel, D. B. & Crawford, D. L. (1998). Small-scale isolation of genomic DNA from *Streptomyces* mycelia or spores. *Biotechniques* **24**, 452–456.
- Kuykendall, L. D., Roy, M. A., O'Neill, J. & Devine, T. E. (1988). Fatty acids, antibiotic resistance, and deoxyribonucleic acid homology groups of *Bradyrhizobium japonicum*. *Int J Syst Bacteriol* **38**, 358–361.
- Lane, D. J., Pace, B., Olsen, G. J., Stahl, D. A., Sogin, M. L. & Pace, N. R. (1985). Rapid determination of 16S ribosomal RNA sequences for phylogenetic analyses. *Proc Natl Acad Sci U S A* **82**, 6955–6959.
- Lechevalier, H. A. & Lechevalier, M. P. (1980). The chemotaxonomy of actinomycetes. In *Actinomycete Taxonomy* (Special Publication 6), pp. 227–291. Edited by A. Dietz & J. Thayer. Arlington, VA: Society for Industrial Microbiology.
- Lechevalier, M. P., Stern, A. E. & Lechevalier, H. A. (1981). Phospholipids in the taxonomy of actinomycetes. In *Actinomycetes*, pp. 111–116. Edited by K. P. Schaal & G. Pulverer. New York: Gustav Fischer.
- Marmur, J. (1961). A procedure for the isolation of deoxyribonucleic acid from microorganisms. *J Mol Biol* **3**, 208–218.
- Mertz, F. P. & Yao, R. C. (1990). *Streptosporangium carneum* sp. nov. isolated from soil. *Int J Syst Bacteriol* **40**, 247–253.
- Mesbah, M., Premachandran, U. & Whitman, W. B. (1989). Precise measurement of the G+C content of deoxyribonucleic acid by high-performance liquid chromatography. *Int J Syst Bacteriol* **39**, 159–167.
- Miller, L. T. (1982). Single derivatization method for routine analysis of bacterial whole-cell fatty acid methyl esters, including hydroxy acids. *J Clin Microbiol* **16**, 584–586.
- Nonomura, H. (1989). Genus *Streptosporangium* Couch 1955, 148^{AL}. In *Bergey's Manual of Systematic Bacteriology*, vol. 4, pp. 2545–2551. Edited by S. T. Williams, M. E. Sharpe & J. G. Holt. Baltimore: Williams & Wilkins.
- Nonomura, H. & Ohara, Y. (1960). Distribution of the actinomycetes in soil. V. The isolation and classification of the genus *Streptosporangium*. *J Ferment Technol* **38**, 405–409.
- Nonomura, H. & Ohara, Y. (1969). Distribution of the actinomycetes in soil. VII. A culture method effective for both preferential isolation and enumeration of *Microbispora* and *Streptosporangium* strains in soil. Part 2. Classification of the isolates. *J Ferment Technol* **47**, 701–709.
- Petrolini, B., Quaroni, S., Sardi, P., Saracchi, M. & Andriollo, N. (1992). A sporangiate actinomycete with unusual morphological features: *Streptosporangium claviforme* sp. nov. *Actinomycetes* **3**, 45–50.
- Research Group of Actinomycetes (1970). *Manual of Determinative Actinomycetes*. Beijing: Science Press.
- Rzhetsky, A. & Nei, M. (1992). A simple method for estimating and testing minimum evolution trees. *Mol Biol Evol* **9**, 945–967.
- Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.
- Schäfer, D. (1969). Eine neue *Streptosporangium*-Art aus türkischer Steppenrde. *Arch Mikrobiol* **66**, 365–373 (in German).
- Shearer, M. C., Colman, P. M. & Nash, C. H., III (1983). *Streptosporangium fragile* sp. nov. *Int J Syst Bacteriol* **33**, 364–368.
- Shirling, E. B. & Gottlieb, D. (1966). Methods for characterization of *Streptomyces* species. *Int J Syst Bacteriol* **16**, 313–340.
- Tamura, K., Dudley, J., Nei, M. & Kumar, S. (2007). MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. *Mol Biol Evol* **24**, 1596–1599.
- Thompson, J. D., Higgins, D. G. & Gibson, T. J. (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res* **22**, 4673–4680.
- Waksman, S. A. (1967). *The Actinomycetes. A Summary of Current Knowledge*. New York: Ronald Press.
- Wayne, L. G., Brenner, D. J., Colwell, R. R., Grimont, P. A. D., Kandler, O., Krichevsky, M. I., Moore, L. H., Moore, W. E. C., Murray, R. G. E. & other authors (1987). International Committee on Systematic Bacteriology. Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int J Syst Bacteriol* **37**, 463–464.

Zhang, L., Jiang, C. & Chen, W. (2002). *Streptosporangium subroseum* sp. nov., an actinomycete with an unusual phospholipid pattern. *Int J Syst Evol Microbiol* **52**, 1235–1238.

Zhang, L.-P., Jiang, C.-L. & Chen, W.-X. (2005). *Streptosporangium yunnanense* sp. nov. and *Streptosporangium purpuratum* sp. nov., from soil in China. *Int J Syst Evol Microbiol* **55**, 719–724.

Zhang, X., Zhang, L.-P., Yang, R., Shi, N., Lu, Z., Chen, W. X., Jiang, C.-L. & Xu, L.-H. (2008). *Nocardiopsis ganjiahuensis* sp. nov., isolated from a soil of Ganjiahu in China. *Int J Syst Evol Microbiol* **58**, 195–199.

Zhou, J. C. & Cao, Y. Z. (1981). Nutrient requirements of the fast-growing rhizobia. *J Huazhong Agric Coll* **3**, 44–56 (in Chinese).