Dactylosporangium darangshiense sp. nov., isolated from rock soil

Seong Hae Seot and Soon Dong Lee

Correspondence
Soon Dong Lee
sdlee@cheju.ac.kr

Department of Science Education, Cheju National University, Jeju 690-756, Republic of Korea

A novel actinomycete was isolated from soil of a rock surface collected from the peak of Darangshi Oreum (Small Mountain) in Jeju, Republic of Korea. Phylogenetic analyses based on 16S rRNA gene sequences demonstrated that strain DLS-44^T belonged to the genus *Dactylosporangium*, with the type strains of *Dactylosporangium roseum* (99.1 % sequence similarity) and *Dactylosporangium fulvum* (99.0 %) as the nearest phylogenetic relatives. Substrate mycelium was abundant, irregularly branched, twisted and vivid orange–yellow in colour. Aerial mycelium was not produced on most media tested. Finger-shaped sporangia and globose bodies were formed directly from the vegetative mycelium. The combination of morphological and chemotaxonomic characteristics supported assignment of the actinomycete to the genus *Dactylosporangium*. Strain DLS-44^T could be distinguished clearly from all type strains of the genus based on its physiological properties (utilization of methyl α -D-mannoside and glycerol, nitrate reduction and growth at 20 °C and pH 9.1) and some chemotaxonomic characteristics (absence of unsaturated fatty acids). DNA–DNA relatedness values between strain DLS-44^T and its closest phylogenetic relatives were 12.2–14.8 % with *D. roseum* DSM 43916^T and 2.5–3.6 % with *D. fulvum* IMSNU 22055^T. On the basis of phenotypic, phylogenetic and DNA–DNA hybridization data, strain DLS-44^T represents a novel species of the genus *Dactylosporangium*, for which the name *Dactylosporangium darangshiense* sp. nov. is proposed. The type strain is strain DLS-44^T (=KCTC 19560^T =DSM 45260^T).

The genus *Dactylosporangium* was first described by Thiemann *et al.* (1967) as a member of the family *Micromonosporaceae* to accommodate *Dactylosporangium aurantiacum* and *Dactylosporangium thailandense*, which produce finger-shaped sporangia and globose bodies on substrate mycelium. Subsequently, other species have been described during screening for new antibiotic producers: *Dactylosporangium fulvum* (Shomura *et al.*, 1986), *Dactylosporangium matsuzakiense* (Shomura *et al.*, 1980), *Dactylosporangium roseum* (Shomura *et al.*, 1985) and *Dactylosporangium vinaceum* (Shomura *et al.*, 1983). In this study, the taxonomy of a *Dactylosporangium* strain recovered from soil of a rock surface was studied using a polyphasic approach.

Strain DLS-44^T was isolated from soil of a rock surface collected from the peak of Darangshi Oreum (Small Mountain) in Jeju, Republic of Korea. Soil samples (1 g) were suspended in 10 ml sterilized distilled water. Aliquots (100 μ l) of the serial dilution were inoculated on starch-

casein agar (1 % soluble starch, 0.03 % casein, 0.2 % KNO₃, 0.2 % NaCl, 0.2 % KH₂PO₄, 0.002 % CaCO₃, 0.005 % MgSO₄·7H₂O, 0.001 % FeSO₄·7H₂O and 1.8 % agar; pH 7.2) and plates were incubated for 7 days at 30 °C. The isolate was maintained as a 20 % glycerol suspension at –20 and –80 °C. For phenotypic comparison, *D. aurantiacum* IMSNU 22001^T, *D. fulvum* IMSNU 22055^T, *D. matsuzakiense* IMSNU 20065^T, *D. roseum* DSM 43916^T, *D. thailandense* IMSNU 20068^T and *D. vinaceum* IMSNU 22056^T were grown on yeast extract-malt extract agar [International *Streptomyces* Project (ISP) medium 2; Shirling & Gottlieb, 1966] at 30 °C.

Growth was investigated on various culture media: ISP 2, oatmeal agar (ISP 3), inorganic salts-starch agar (ISP 4), glycerol-asparagine agar (ISP 5), peptone-yeast extract-iron agar (ISP 6), tyrosine agar (ISP 7) (Shirling & Gottlieb, 1966) and sucrose-nitrate agar (Waksman, 1961). Cultural characteristics were recorded after incubation for 21 days at 30 °C. For electron microscopy, cells grown on various media for 21 days were fixed with 1 % osmium tetroxide and gradually dehydrated with ethanol and a mixture of ethanol and isoamyl acetate. Specimens were critical-point dried before being viewed by scanning electron microscopy (model JSM-6500; JEOL) as described previously (Lee, 2006).

†Deceased.

Abbreviation: ISP, International *Streptomyces* Project.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain DLS-44^T is FM882231.

Strain DLS-44^T grew well on ISP 2, 3, 4 and 5 (moderate growth), but poor growth was observed on ISP 6 and 7 and sucrose-nitrate agar. The reverse colour of colonies was vivid orange–yellow on most media, but pale yellow on ISP 4 and 5. Diffusible pigments were not produced on any of the media tested. Vegetative mycelium was irregularly branched, twisted and 0.1–0.2 µm in diameter. Aerial hyphae were not produced except for traces of rudimentary mycelium on ISP 5. Globose bodies (1.0–1.1 µm in diameter) were produced abundantly on ISP 3, 4 and 5, but moderate production was observed on ISP 2. Finger-shaped sporangia (0.6–0.9 × 1.7 µm), which emerged directly from the vegetative mycelium, were arranged singly or in clusters on ISP 4. A detailed depiction of cell morphology of strain DLS-44^T is given in Fig. 1.

Chromosomal DNA was extracted and purified according to the method of Hopwood *et al.* (1985). Amplification and purification of the 16S rRNA gene was performed as described previously (Lee *et al.*, 2000; Lee, 2006).

Fig. 1. Cell morphology of strain DLS-44^T grown for 3 weeks at 30 °C, showing globose bodies on ISP 3 (a) and finger-shaped sporangium on ISP 4 (b). Bars, 1 µm.

Sequencing of the 16S rRNA gene was carried out using an ABI PRISM BigDye Terminator cycle sequencing kit (Applied Biosystems) and an automatic DNA sequencer (model 3730xl; Applied Biosystems). Sequences of strain DLS-44^T and members of the genus *Dactylosporangium* were aligned by using the CLUSTAL_X algorithm (Thompson *et al.*, 1997). Evolutionary distances between pairs of micro-organisms were determined with the correction of Jukes & Cantor (1969) and a tree was constructed by using the neighbour-joining method (Saitou & Nei, 1987). Trees were also constructed using the maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Fitch, 1971) methods. Bootstrap analysis was performed using the programs SEQBOOT, DNADIST, NEIGHBOR and CONSENSE (Felsenstein, 1985), with 1000 neighbour-joining datasets.

A neighbour-joining tree (Fig. 2) based on 16S rRNA gene sequences showed that strain DLS-44^T formed a sub-branch at the foot of a *D. fulvum*/*D. roseum* cluster within the radiation of the genus *Dactylosporangium*, with a bootstrap value of 84 %. The branching pattern was also supported in trees constructed using the maximum-parsimony and maximum-likelihood methods. The closest relatives were the type strains of *D. roseum* and *D. fulvum* (99.1 and 99.0 % 16S rRNA gene sequence similarities, respectively). Levels of 16S rRNA gene sequence similarity between strain DLS-44^T and strains of other recognized species of the genus *Dactylosporangium* ranged from 98.1 to 98.3 %.

For phenotypic characterization, strain DLS-44^T was cultivated routinely at 30 °C on ISP 2. Growth was tested over a range of temperatures (4, 10, 25, 30, 37 and 42 °C) and pH (4.1–12.1). Tolerance to NaCl was examined on ISP 2 supplemented with 1–9 % (w/v) NaCl. All tests were performed at 30 °C and recorded after 2 weeks. Gram staining and oxidase and catalase activities were examined

Fig. 2. Neighbour-joining tree showing the position of strain DLS-44^T within the radiation of the genus *Dactylosporangium* based on 1418 aligned positions present in the 16S rRNA gene sequences of all taxa. Asterisks represent branches supported by both maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Fitch, 1971) methods. Bootstrap values >50 % are shown on nodes. Bar, 0.01 substitutions per nucleotide position.

using the method of MacFaddin (1980). Utilization of carbohydrates was determined as described previously (Lee, 2007). Urease activity was investigated by means of a colour change in Bacto urea broth (Difco). Nitrate reduction, gelatin liquefaction and aesculin hydrolysis were assessed using the methods of MacFaddin (1980). Decomposition of hypoxanthine, DL-tyrosine and xanthine was tested as described previously (Gordon *et al.*, 1974). Degradation of casein [1% (w/v) skimmed milk], was examined using ISP 2 as basal medium. Hydrolysis of DNA and starch was determined by using DNase test agar (Difco) and starch agar (Difco), respectively. Data for physiological and biochemical properties are given in the species description and Table 1.

For chemotaxonomic analyses, strain DLS-44^T was grown in a shake flask containing YMG (0.4% yeast extract, 1.0% malt extract and 0.4% glucose, pH 7.2) broth for 3 days at 30 °C. Analysis of the diaminopimelic acid isomer was performed as described previously (Staneck & Roberts, 1974). Whole-cell sugars were analysed by GC as described previously (Saddler *et al.*, 1991). Isoprenoid quinones were extracted by using the method of Minnikin *et al.* (1984) and identified by HPLC as described by Kroppenstedt (1985). Analysis of polar lipids by TLC was performed as described previously (Minnikin *et al.*, 1977). The DNA G+C content was determined by HPLC according to the method of Mesbah *et al.* (1989). The cell wall contained *meso*-diaminopimelic acid and glycine. Whole-cell sugars contained glucose (56%), mannose (15%), xylose (10%) and rhamnose (8%), together with trace amounts of galactose, arabinose and ribose. The menaquinone profile comprised MK-9(H₈) (78%) and MK-9(H₆) (22%). Polar lipids were diphosphatidylglycerol, phosphatidylethanolamine and phosphatidylinositol. The DNA G+C content of strain DLS-44^T was 69.8 mol%. For cellular fatty acid analyses, strain DLS-44^T and the type strains of species of the genus *Dactylosporangium* were grown on ISP 2 for 7 days at 30 °C. Fatty acid methyl esters were prepared and analysed according to the instructions for the Sherlock Microbial Identification System (version 6; MIDI). The fatty acid profile of DLS-44^T contained major amounts of iso- and anteiso-branched acids. Small amounts of straight-chain saturated fatty acids were detected, but unsaturated fatty acids were not found. The predominant fatty acids were iso-C_{16:0} (35.6%), iso-C_{15:0} (24.7%) and anteiso-C_{17:0} (13.9%). Strain DLS-44^T differed from all type strains of species of the genus *Dactylosporangium* by the absence of unsaturated fatty acids. The cellular fatty acid profiles of strain DLS-44^T and members of the genus *Dactylosporangium* are given in Table 2.

The combination of morphological and chemotaxonomic characteristics supports assignment of strain DLS-44^T to the genus *Dactylosporangium*. However, strain DLS-44^T can be readily differentiated from all type strains of *Dactylosporangium* species in that it utilizes methyl α -D-mannoside and glycerol as sole carbon and energy sources, it does not reduce nitrate to nitrite and it does not grow at

Table 1. Differential characteristics of strain DLS-44^T and type strains of the genus *Dactylosporangium*

Strains: 1, DLS-44^T; 2, *D. aurantiacum* IMSNU 22001^T; 3, *D. fulvum* IMSNU 22055^T; 4, *D. matsuzakiense* IMSNU 20065^T; 5, *D. roseum* DSM 43916^T; 6, *D. thailandense* IMSNU 20068^T; 7, *D. vinaceum* IMSNU 22056^T. All data are from this study, except for DNA G+C contents for *D. aurantiacum* and *D. thailandense*, which were taken from Vobis (1989). All of the strains utilized dextrin, D-fructose, D-glucose, maltose, D-mannose, raffinose, sucrose, trehalose and D-xylose as sole carbon sources, but not methyl α -D-glucoside, L-ribose, L-sorbose, D-dulcitol, *myo*-inositol, benzoate, formate or DL-tartrate.

Characteristic	1	2	3	4	5	6	7
Growth at/in:							
20 °C	–	+	+	+	+	+	+
42 °C	–	–	+	+	–	+	+
1% NaCl	–	+	+	+	–	+	+
pH 6.1	+	–	+	–	+	+	+
pH 9.1	–	+	+	+	+	+	+
Nitrate reduction	–	+	+	+	+	+	+
H ₂ S production	–	+	+	–	+	–	+
Urea hydrolysis	–	–	+	+	–	–	–
Gelatin liquefaction	–	–	–	+	–	–	+
Decomposition of:							
Casein	–	–	+	+	+	+	+
DNA	–	+	+	+	–	+	+
Starch	–	+	+	+	–	+	+
DL-Tyrosine	–	–	–	–	–	–	+
Utilization of:							
D-Arabinose	+	–	–	–	–	+	+
L-Arabinose	+	+	+	+	–	+	+
Cellobiose	+	+	+	–	+	+	+
D-Galactose	–	+	+	–	–	+	+
D-Lactose	+	+	+	–	+	+	+
Melezitose	+	+	–	+	–	–	+
Melibiose	+	+	+	–	–	–	+
Methyl α -D-mannoside	+	–	–	–	–	–	–
L-Rhamnose	+	+	–	+	–	+	–
Salicin	+	–	+	+	–	–	+
Adonitol	–	–	–	+	+	–	–
<i>meso</i> -Erythritol	–	–	–	+	–	–	–
Glycerol	+	–	–	–	–	–	–
D-Mannitol	+	+	+	+	–	–	+
D-Sorbitol	–	–	+	–	–	–	+
D-Xylitol	–	–	–	+	–	–	–
Acetate	–	–	–	–	+	–	+
Citrate	–	–	–	+	–	+	+
Malate	–	–	–	+	+	–	–
Succinate	–	–	–	+	–	–	–
DNA G+C content (mol%)	69.8	73	72.2	68.5	69.8	71	71.7

20 °C and pH 9.1 (Table 1) and by the absence of unsaturated components in the fatty acid profile (Table 2). DNA–DNA hybridization experiments were performed by the initial renaturation method (De Ley *et al.*, 1970; Huß *et al.*, 1983). DNA relatedness values between strain

Table 2. Cellular fatty acid compositions (%) of strain DLS-44^T and type strains of the genus *Dactylosporangium*

Strains: 1, DLS-44^T; 2, *D. aurantiacum* IMSNU 22001^T; 3, *D. fulvum* IMSNU 22055^T; 4, *D. matsuzakiense* IMSNU 20065^T; 5, *D. roseum* DSM 43916^T; 6, *D. thailandense* IMSNU 20068^T; 7, *D. vinaceum* IMSNU 22056^T. Data were obtained in this study. All strains were grown on ISP 2 for 7 days at 30 °C. Values less than 1 % of the total fatty acids were not shown. —, Not detected; TBSA, tuberculostearic acid.

Fatty acid	1	2*	3	4†	5‡	6	7
Saturated							
C _{12:0}	1.2	—	—	—	—	—	—
C _{14:0}	—	1.0	—	—	1.2	—	—
C _{15:0}	—	—	—	1.2	1.7	—	—
C _{16:0}	1.5	2.6	1.0	1.8	4.6	1.1	2.9
C _{17:0}	—	—	1.6	5.7	2.3	1.3	1.0
C _{18:0}	1.7	1.2	1.6	1.6	1.6	1.6	3.8
Unsaturated							
C _{17:1ω8c}	—	1.2	5.0	4.8	3.0	3.9	1.4
C _{18:1ω9c}	—	3.6	4.5	2.2	3.5	4.4	6.4
iso-C _{16:1} H	—	—	—	—	—	1.1	—
iso-C _{17:1ω9c}	—	—	6.6	—	—	6.9	6.2
anteiso-C _{17:1} A	—	—	—	2.4	—	—	—
anteiso-C _{17:1ω9c}	—	—	1.0	—	—	1.2	—
Branched							
iso-C _{14:0}	1.0	6.3	1.7	—	6.0	1.8	2.7
iso-C _{15:0}	24.7	27.7	15.7	15.2	30.5	15.2	12.8
iso-C _{16:0}	35.6	29.7	24.8	12.4	28.6	25.6	27.2
iso-C _{17:0}	4.7	2.4	6.2	6.6	2.2	5.4	8.0
iso-C _{18:0}	—	—	1.4	—	—	1.6	1.9
anteiso-C _{15:0}	5.9	9.6	6.7	8.7	4.0	6.6	4.4
anteiso-C _{17:0}	13.9	5.8	9.7	16.7	3.4	10.0	8.4
Methyl-branched							
9-Methyl C _{16:0}	—	—	—	5.8	—	—	—
10-Methyl C _{16:0}	1.0	1.7	—	—	1.6	—	—
10-Methyl C _{17:0}	1.1	1.5	4.6	5.4	1.3	5.3	4.3
10-Methyl C _{18:0}	—	1.3	1.1	2.3	—	1.4	1.9
(TBSA)							

**D. aurantiacum* IMSNU 22001^T also contained C_{16:1ω7c} and/or iso-C_{15:0} 2-OH (1.1 %).

†*D. matsuzakiense* IMSNU 20065^T also contained C_{16:1} 2-OH (1.1 %).

‡*D. roseum* DSM 43916^T also contained C_{19:1ω11c} and/or unknown ECL 18.756 (1.6 %).

DLS-44^T and its closest relatives were 12.2–14.8 % with *D. roseum* DSM 43916^T and 2.5–3.6 % with *D. fulvum* IMSNU 22055^T, which are distinctly lower than the threshold value (70 %) recommended for delineating bacterial species (Wayne *et al.*, 1987).

On the basis of the phenotypic and DNA relatedness data presented here, strain DLS-44^T is considered to represent a novel species of the genus *Dactylosporangium*, for which the name *Dactylosporangiumarangshiense* sp. nov. is proposed.

Description of *Dactylosporangiumarangshiense* sp. nov.

Dactylosporangiumarangshiense (da.rang.shi.en'se. N.L. neut. adj. *arangshiense* pertaining to Darangshi, referring to Darangshi Oreum in Jeju, Republic of Korea, the site from which the type strain was isolated).

Aerobic, Gram-stain-positive and catalase- and oxidase-positive. Vegetative mycelium is well-developed and orange-yellow in colour on ISP 2. Globose bodies (1.0–1.1 µm in diameter) are formed directly from substrate mycelium. Finger-shaped sporangia (0.6–0.9 × 1.7 µm) emerge directly from the vegetative mycelium and are arranged singly or in clusters. Soluble pigments are not produced on any media tested. Growth occurs at 30–37 °C (optimum 37 °C) and pH 6.1–8.1 (optimum pH 7.1). Growth does not occur in the presence of 1 % NaCl. Aesculin is hydrolysed, but casein, DNA, CM-cellulose, gelatin, starch and urea are not. Hypoxanthine, DL-tyrosine and xanthine are not decomposed. Nitrate reduction and H₂S production are not observed. D-Arabinose, L-arabinose, cellobiose, dextran, D-fructose, D-glucose, D-lactose, maltose, D-mannose, melezitose, melibiose, methyl α-D-mannoside, raffinose, L-rhamnose, salicin, sucrose, trehalose, D-xylose, glycerol and D-mannitol are utilized as sole carbon sources, but D-galactose, methyl α-D-glucoside, L-ribose, L-sorbose, adonitol, dulcitol, *meso*-erythritol, *myo*-inositol, D-sorbitol, acetate, benzoate, citrate, formate, malate, succinate and DL-tartrate are not. The polar lipids are diphosphatidylglycerol, phosphatidylethanolamine and phosphatidylinositol. The predominant fatty acids are iso-C_{16:0}, iso-C_{15:0} and anteiso-C_{17:0}.

The type strain is DLS-44^T (=KCTC 19560^T =DSM 45260^T), isolated from rock soil collected from the peak of Darangshi Oreum in Jeju, Republic of Korea. The DNA G+C content of the type strain is 69.8 mol%.

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