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ORIGINAL ARTICLE

Dactylosporangium sucinum sp. nov., isolated from Thai peat swamp forest soil

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The actinomycete strain RY35-23^T was isolated from peat swamp forest soil in Thailand. The taxonomic position of this strain was determined using polyphasic approach. Strain RY35-23^T showed typical morphology and chemical properties similar to the members in the genus *Dactylosporangium*. On the basis of 16S ribosomal RNA gene analysis, this strain was closely related to *Dactylosporangium fulvum* JCM 5631^T (98.94%), *D. roseum* JCM 3364^T (98.87%) and *D. darangshiense* JCM 17441^T (98.86%). The DNA–DNA relatedness between strain RY35-23^T and its closely related species was lower than 70%, the cutoff level for assigning strains to the same species. On the basis of these results mentioned, the strain RY35-23^T could be distinguished from its closely related type strains and represents a novel species of the genus *Dactylosporangium*, for which the name *Dactylosporangium sucinum* (type strain RY35-23^T = JCM 19831^T = TISTR 2212^T = PCU 333^T) is proposed.
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INTRODUCTION

The genus *Dactylosporangium*, belongs to the family *Micromonosporaceae*, was first described by Thiemann *et al.*[1] in 1967 for the aerobic mesophilic filamentous bacteria which forms finger-shaped sporangia and globose bodies on substrate mycelium as well as motile spores.[2] In this genus, *Dactylosporangium matsuzakiense* strains have reported to produce an aminoglycoside antibiotic.[3] The members of this genus that validly published and isolated from soils comprise, *D. aurantiacum, D. fulvum, D. luridum, D. luteum, D. maewongense, D. matsuzakiense, D. roseum, D. salmoneum, D. siamense, D. thailandense, D. tropicum* and *D. vinaceum*[2–10] whereas *D. darangshiense* was isolated from rock soil.[11] In Thailand, *D. thailandense,*[1,2] *D. maewongense,*[7] *D. tropicum*[9] and *D. siamense*[10] have been reported.

In the course of our investigation for diversity of actinomycetes in peat swamp forest soil of Thailand, the strain RY35-23^T was isolated. In this study, we described the taxonomic position of the strain which exhibited the morphology similar to the members of the genus *Dactylosporangium* using polyphasic approach.

MATERIALS AND METHODS

A peat swamp soil sample was collected from a tropical moist forest at Nong Jum Rung area in Rayong province, Thailand. The sample was air dried at room temperature for 7 days and was grinded using mortar. One gram of sample was added to basic lauryl sulfate solution (0.1 g sodium lauryl sulfate, 1.75 g KH_2PO_4, 3.5 g K_2HPO_4, 1000 ml distilled water, pH 7.0) and prepared a serial dilution to 10^{-4}. 100 µl of each resultant suspension was added on humic acid–vitamin agar[12] supplemented (l^{-1}) with 25 mg of nalidixic acid and 50 mg of cycloheximide. The plates were incubated at 30 °C for 21 days. The colony of isolate RY35-23^T was selected by observing the morphology of colony and sporangia and then transferred to International *Streptomyces* project media 2 (ISP 2 medium)[13] for working culture.

The cultural characteristics of isolate RY35-23^T was observed on various media recommended by Shirling and Gottlieb.[13] The color of colony, reverse side and soluble pigment were determined using the Color Harmony Manual.[14] The morphology of sporangia and globose bodies was observed using a scanning electron microscope (JSM-7610F and JSM-5410LV, Japan) after cultivation on sucrose nitrate agar and ISP 4 agar at 30 °C for 4 weeks.

Phenotypic properties were determined using the standard methods.[15,16] The utilization and acid production of various carbon sources were determined as described by Shirling and Gottlieb[13] and Gordon *et al.*,[17] respectively. The effect of pH, temperature and NaCl tolerance for growth was observed on ISP 2 medium at 30 °C for 14–21 days.

All chemotaxonomic studies were analyzed using freeze-dried cells obtained from the culture grown in ISP2 broth at 30 °C for 6 days. Isomers of diaminopimelic acid in cell wall peptidoglycan were determined using TLC based on the method of Staneck and Roberts.[18] Whole-cell hydrolysate sugars were analyzed using HPLC following the method of Mikami and Ishida.[19] The *N*-acyl type of muramic acid was analyzed using the method of Uchida and Aida.[20] Phospholipids were extracted and analyzed using the procedure of Minnikin *et al.*[21] The isoprenoid quinones were extracted according to the method of Collins *et al.*[22] and were analyzed by HPLC. Fatty acid methyl esters were prepared according to the method described by the manufacturer's instruction (Sherlock Microbial Identification System MIDI, Inc., Newark, DE, USA)[23,24] and analyzed using gas chromatography and a HP-computer with MIDI data base (Hewlett Packard, Palo Alto, CA, USA). The presence of mycolic acids was determined using TLC based on the method of Tomiyasu.[25]

Genomic DNA was extracted from freeze-dried cells as described by Raeder and Broda.[26] The G+C content of DNA was determined using HPLC according

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to the procedure of Tamaoka and Komagata.[27] The amplification of 16S ribosomal RNA gene was carried out as described by Suriyachadkun *et al.*,[28] and the PCR products were sequenced (Macrogen Inc., Seoul, Korea) using the universal primers.[29] The sequence was determined using BLAST analysis on the EzTaxon-e database[30] and was aligned, Bioedit software (Ibis Biosciences, Carlsbad, CA, USA), against the member of the genus *Dactylosporangium*. The phylogenetic trees were constructed using MEGA5.0 software[31] based on neighbor-joining,[32] maximum-parsimony[33] and maximum-likelihood[34] methods. The confidence values of nodes were evaluated using the bootstrap resampling method with 1000 replications.[35] The DNA–DNA hybridization was performed as described by Ezaki *et al*.[36]

RESULTS AND DISCUSSION

Taxonomic properties of strain RY35-23^T

Phenotypic characteristics. Strain RY35-23^T produced branch-substrate mycelia, while aerial mycelia were not observed on agar culture. Substrate mycelia were not fragmented. The strain produced finger-shaped sporangia on short sporangiophores. The irregular rugose sporangia were 0.6–1 by 3.2–4.3 μm in size (Figure 1a). Formation of globose bodies was observed on various ISP media, nutrient agar and sucrose nitrate agar. Globose bodies were spherical to oval in shape (1–1.6 μm in size) and emerged directly from substrate mycelia (Figure 1b). The motile spores were observed after 30 min when the agar culture was flooded with yeast dextrose broth. The colony of the strain showed amber to light melon yellow on various ISP media (Table 1). The temperature for growth was 20–37 °C with the optimal temperature at 25–37 °C. No growth was observed at 15 and 45 °C. The pH range for growth was 4–9 with the optimal pH at 6–7. The phenotypic properties of strain RY35-23^T are showed in Table 2.

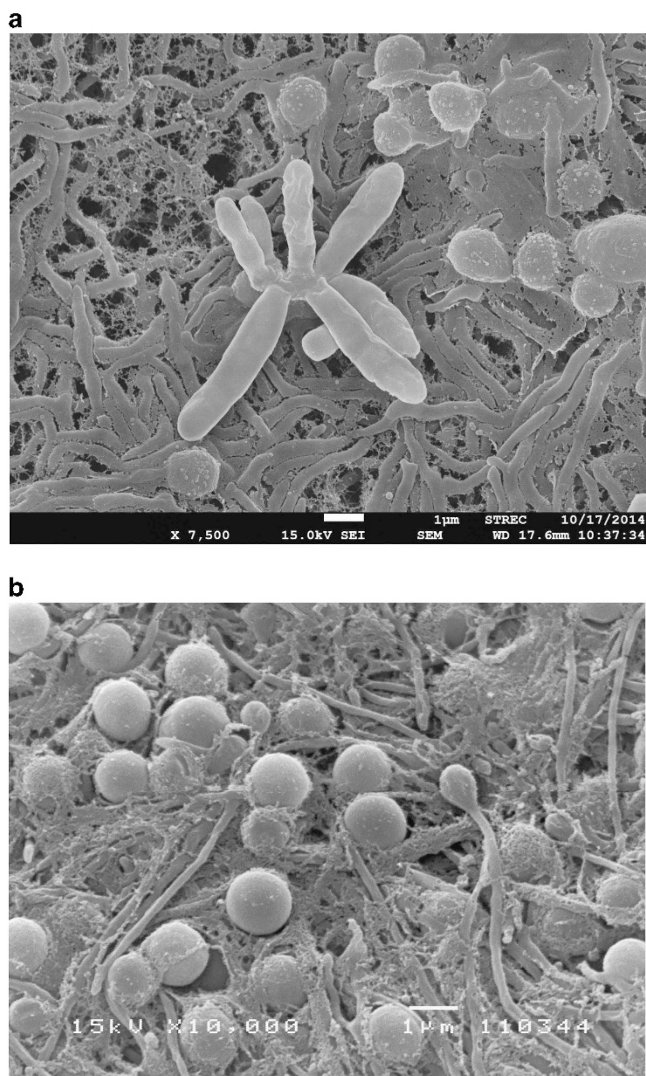

Figure 1 Scanning electron micrograph of sporangia (**a**) and globose bodies (**b**) of strain RY35-23^T grown on sucrose nitrate agar and ISP 4 at 30 °C for 4 weeks, respectively. ISP, International *Streptomyces* project.

Chemotaxonomic characteristics. Cell wall peptidoglycan of strain RY35-23^T contained 3-hydroxy-diaminopimelic acid and *meso*-diaminopimelic acid as the major and minor diaminopimelic acids, respectively. The *N*-acyl muramic acid was glycolyl type. Whole-cell hydrolysate contained rhamnose, ribose, galactose, mannose, glucose, arabinose and xylose. The two latter sugars, arabinose and xylose, are diagnostic sugars that are classified as whole-cell sugar type D according to the classification of Lechevalier and Lechevalier.[37] The major phospholipids were diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylinositol and phosphatidylinositol mannosides (Figure 2). This polar lipid pattern corresponds to polar lipid type II, which showed one nitrogenous phospholipid, phosphatidylethanolamine, as diagnostic polar lipid.[38] The major isoprenoid quinones were MK-9(H_8; 75%) and MK-9(H_6; 25%), the same as *D. darangshiense*, the closest species.[11] The major cellular fatty acids were $C_{17:0}$, $C_{18:0}$, $C_{18:1}\omega9c$, anteiso-$C_{15:0}$, iso-$C_{15:0}$, iso-$C_{16:0}$, iso-$C_{17:0}$ and anteiso-$C_{17:0}$ as shown in Table 3. This fatty acid profile of strain RY35-23^T was similar to the other closely related *Dactylosporangium* type strains but was different in the percentage and some minor fatty acids. The mycolic acid was absent. The G+C content was 72.5 mol%. These chemical analysis results showed that the strain RY35-23^T exhibited typical chemotaxonomic characteristics of the genus *Dactylosporangium*.[39]

Phylogenetic analysis. BLAST analysis revealed that the strain RY35-23^T showed 16S ribosomal RNA gene similar to *D. fulvum* JCM 5631^T (98.94%), *D. roseum* JCM 3364^T (98.87%) and *D. darangshiense* JCM 17441^T (98.86%). The phylogenetic tree based on neighbor-joining analysis revealed that strain RY35-23^T shared monophyletic clade with those three closest *Dactylosporangium* species as showed in Figure 3.

DNA–DNA hybridization. The levels of DNA–DNA relatedness among strain RY35-23^T, *D. fulvum* JCM 5631^T (27.4 ± 3.1 to 36.1 ± 2.9%), *D. roseum* JCM 3364^T (37.1 ± 3.7 to 39.5 ± 1.4%) and *D. darangshiense* JCM 17441^T (25.0 ± 2.9 to 32.9 ± 3.5%) were lower than 70% (Table 4), the cutoff level for assigning strains to the same species.[40] This can be indicated that strain RY35-23^T represents a genomic distinct from those *Dactylosporangium* species.

CONCLUSION

Based on the results of polyphasic approach, the strain RY35-23^T showed typical morphology and chemical characteristics similar to the members of genus *Dactylosporangium*. The taxonomic position of strain RY35-23^T was confirmed by phylogenetic tree analysis, which revealed that our strain belonged to the genus *Dactylosporangium* (Figure 2). The strain RY35-23^T was compared with its closest *Dactylosporangium* species and could be distinguished using

Table 1 Cultural characteristics of strain RY35-23^T and closely related type strains

Medium	RY35-23^T	D. fulvum JCM 5631^T	D. roseum JCM 3364^T	D. darangshiense JCM 17441^T
Yeast extract–malt extract agar (ISP medium 2)				
Growth	Good	Good	Good	Good
Sporangia	None	Moderate	Poor	None
Color of colony	Amber (3lc)	Maple (4le)	Coral (6½ lc)	Light maize (2ea)
Reverse color	Orange (4la)	Maple (4le)	Red wood (6ne), Chili (6lc)	Melon yellow (3ga)
Soluble pigment	Light wheat (2ea)	Tan (3ie)	Chili (6lc)	None
Oat meal agar (ISP medium 3)				
Growth	Moderate	Moderate	Poor	Moderate
Sporangia	None	Poor	None	None
Color of colony	Amber (3lc)	Light brown (3lg)	Light melon yellow (3ea)	Bright melon yellow (3ia)
Reverse color	Bright melon yellow (3ia)	Olive (1½ni)	Light wheat (2ea), colorless	Light orange (4ia)
Soluble pigment	None	None	None	None
Inorganic salt starch agar (ISP medium 4)				
Growth	Moderate	Moderate	Moderate	Moderate
Sporangia	Poor	Moderate	Abundant	None
Color of colony	Apricot (3ga)	Luggage tan (4ne)	Pale pink (6ca)	Melon yellow (3ga), Light melon yellow (3ea)
Reverse color	Bright melon yellow (3ia)	Maple (4le)	Light Apricot (4ea), Dusty coral (6gc)	Light melon yellow (3ea)
Soluble pigment	Light wheat (2ea)	Turf tan (3le)	Pastel orange (4ic)	None
Glycerol-asparagine agar (ISP medium 5)				
Growth	Poor	Poor	Poor	Poor
Sporangia	Moderate	None	Abundant	None
Color of colony	Light melon yellow (3ea), colorless	Ivory (2db), colorless	Colorless	Light melon yellow (3ea), colorless
Reverse color	Colorless	Colorless	Colorless	Melon yellow (3ga)
Soluble pigment	None	None	None	None
Peptone-yeast extract iron agar (ISP medium 6)				
Growth	Moderate	Moderate	Moderate	Poor
Sporangia	None	None	None	None
Color of colony	Amber (3lc)	Bamboo	Light gold (2ic)	Light amber (3ic)
Reverse color	Bright melon yellow (3ia), Melon yellow (3ga)	Colonial yellow (2ga)	Bright gold (2nc)	Amber (3nc)
Soluble pigment	None	None	Honey gold (2ic)	None
Tyrosine agar (ISP medium 7)				
Growth	Good	Poor	Poor	Poor
Sporangia	None	None	Abundant	Moderate
Color of colony	Orange (4la)	Ivory (2db), Colorless	Light melon yellow (3ea)	Light melon yellow (3ea)
Reverse color	Light orange (4ia)	Colorless	Light melon yellow (3ea), colorless	Light apricot (4ea), Light orange (4ia)
Soluble pigment	Light wheat (2ea)	None	Bamboo (2fb)	None
Nutrient agar				
Growth	Moderate	Good	Moderate	Moderate
Sporangia	None	Poor	None	None
Color of colony	Light melon yellow (3ea)	Bamboo (2fb)	Bamboo (2fb)	Ivory (2db)
Reverse color	Light wheat (2ea)	Colorless	Light wheat (2ea), colorless	Light maize (2ea)
Soluble pigment	None	None	None	None

Abbreviation: ISP, International *Streptomyces* project.
Numbers and letters in parentheses referred to the color based on the Color Harmony Manual.[14]

phenotypic properties, especially the cultural characteristics on ISP 2 medium, whose strain RY35-23^T showed the amber colony and light maize pigment, whereas *D. fulvum* JCM 5631^T, *D. roseum* JCM 3364^T and *D. darangshiense* JCM 17441^T showed maple, coral and light maize colony, and tan pigment, chili pigment and no pigment, respectively. Moreover, other phenotypic properties can also use for distinguishing between species as showed in Table 2. Therefore, on the basis of phenotypic properties, chemotypic properties, 16S ribosomal RNA gene analysis and DNA–DNA relatedness, strain RY35-23^T represents the novel species of the genus *Dactylosporangium*, for which the name *D. sucinum* (type strain RY35-23^T = JCM 19831^T = TISTR 2212^T = PCU 333^T) is proposed.

Table 2 Differential characteristics between strain RY35-23^T and closely related type strains

Characteristics	*RY35-23^T*	*D. fulvum JCM 5631^T*	*D. roseum JCM 3364^T*	*D. darangshiense JCM 17441^T*
Gelatin liquefaction	−	−	+	−
Skimmed milk coagulation	+	−	+	−
Starch hydrolysis	+	+	−	+
Nitrate reduction	−	−	+	−
Growth at pH 4	+	+	−	−
NaCl tolerance (%)	3	2	3	1
Acid production from				
L-Arabinose	+	w	−	+
D-Mannitol	+	+	−	+
D-Melezitose	+	+	w	+
D-Melibiose	+	−	−	+
L-Rhamnose	+	−	−	+
Salicin	+	+	w	−
D-Sorbose	w	+	−	−
D-Xylose	+	+	−	+
Utilization of				
L-Arabinose	w	−	w	+
D-Cellobiose	+	+	w	w
D-Mannitol	+	+	−	+
D-Mannose	+	w	+	+
D-Melezitose	w	w	−	w
D-Melibiose	−	−	−	w
Salicin	w	w	w	+
D-Sorbose	−	−	−	−
Sucrose	+	+	+	+
D-Raffinose	−	−	−	w
D-Xylose	+	+	−	+

Abbreviations: +, positive; w, weakly positive; −, negative.

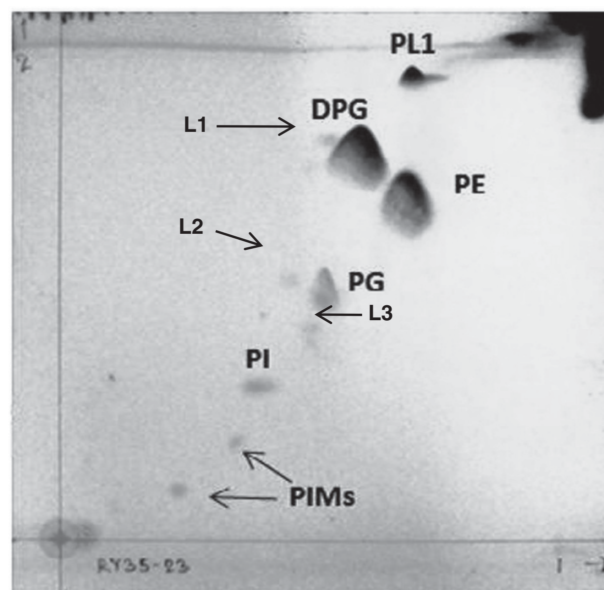

Figure 2 Polar lipid profiles of strain RY35-23^T on a two-dimensional thin-layer chromatogram that were detected with 5% phosphomolybdic acid in ethanol as spraying reagent (for total lipids). DPG, diphosphatidylglycerol; L, unknown lipids; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PIMs, phosphatidylinositol mannosides; PL, unknown phospholipid. A full color version of this figure is available at *The Journal of Antibiotics* journal online.

Table 3 Cellular fatty acid compositions (%) of strain RY35-23^T and closely related type strains

Fatty acid	*RY35-23^T*	*D. fulvum JCM 5631^T*	*D. roseum JCM 3364^T*	*D. darangshiense JCM 17441^T*
Saturated fatty acids				
$C_{14:0}$	—	2.6	1.1	—
$C_{15:0}$	0.5	1.4	1.2	0.3
$C_{16:0}$	1.0	10.1	7.8	1.3
$C_{17:0}$	5.1	0.6	3.0	1.3
$C_{18:0}$	1.9	9.6	4.7	1.8
$C_{19:0}$	0.7	0.4	0.2	—
Unsaturated fatty acids				
$C_{16:1}\omega7c$	—	1.0	1.3	—
$C_{18:1}\omega9c$	1.1	2.7	6.4	0.8
Branched fatty acids				
iso-C14:0	0.7	2.6	2.2	0.8
iso-C15:0	31.5	19.8	29.2	23.2
anteiso-C15:0	6.5	13.3	2.8	6.5
iso-C16:0	19.8	11.9	30.0	35.5
iso-C17:0	9.6	2.4	3.2	7.1
anteiso-C17:0	19.0	0.9	4.3	20.1
iso-C18:0	0.6	0.3	0.4	0.8
Summed in feature 8	1.6	1.2	2.2	0.4

—, not detected. Summed in feature 8 comprises $C_{18:1}\omega8c$.

Description of *Dactylosporangium sucinum* sp. nov.

Dactylosporangium sucinum (su.ci' num. L. neut. adj. *sucinum* amber gold color). Gram-positive, mesophilic, aerobic actinomycete. Amber vegetative mycelia are formed on ISP 2, ISP 3 and ISP 6 media. Finger-shaped sporangia can be observed on ISP 4 and ISP 5 media. Globose bodies are formed on ISP 2, 3, 4, 5, 6 and 7 media. Light wheat pigment is produced on ISP 2, ISP 4 and ISP 7 media. The strain shows good growth on ISP 2 medium, moderate growth on ISP 3, 4, 6 media and nutrient agar, and poor growth on ISP 5 medium. Optimal temperature for growth is 25–37 °C; no growth observed at 45 °C. Growth pH is 4–9 (optimum pH 6–7). The maximum NaCl concentration for growth is 3%. Hydrolysis of esculin and starch is positive. Liquefication of gelatin is negative. Nitrate is not reduced to nitrite. Peptonization of milk is positive but coagulation of milk is negative. Produces acid from salicin, D-melezitose, D-xylose, D-mannitol, D-mannose, L-rhamnose, L-arabinose, D-cellobiose and D-glucose but not *myo*-inositol and D-sorbitol. Utilizes D-glucose, L-arabinose, D-mannose, D-xylose, D-mannitol, sucrose, D-melezitose, D-cellobiose but not D-melibiose, D-arabitol, D-sorbose and D-raffinose. Cell wall contains 3-hydroxy-diaminopimelic acid as a major diaminopimelic acid and small amount of *meso*-diaminopimelic acid. The major phospholipids were diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylinositol and phosphatidylinositol mannosides. The major isoprenoid quinones were MK-9(H_8) and MK-9(H_6). The major cellular fatty acids were $C_{17:0}$, $C_{18:0}$, $C_{18:1}\omega9c$, anteiso-$C_{15:0}$, iso-$C_{15:0}$, iso-$C_{16:0}$, iso-$C_{17:0}$ and anteiso-$C_{17:0}$. The G+C content of type strain is 72.5 mol%.

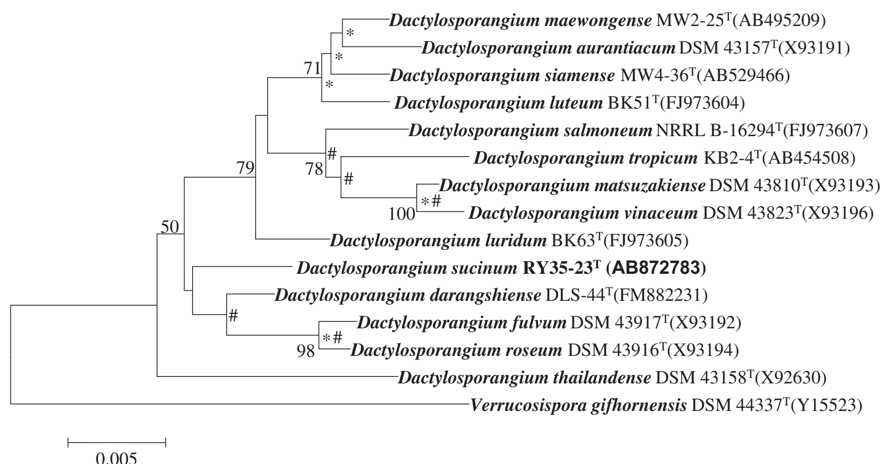

Figure 3 Phylogenetic relationships based on neighbor-joining analysis (Saitou and Nei, 1987) of 16S ribosomal RNA gene sequences of strain RY35-23^T and all members in the genus *Dactylosporangium*. *Verrucosispora gifhornensis* DSM 44337^T was used as an out group. Asterisk (*, #) indicated that the branches were recovered in the maximum-likelihood tree and maximum-parsimony tree, respectively. The number at branch nodes indicates bootstrap percentages derived from 1000 replications (only value >50% are shown). Bar=0.01 substitutions per nucleotide position.

Table 4 DNA–DNA relatedness of strain RY35-23^T and closely related type strains

	DNA–DNA relatedness (%) with labeled strains[a]			
Strain	*RY35-23^T*	*JCM 5631^T*	*JCM 3364^T*	*JCM 17441^T*
RY35-23 ^{T}	100±0.0	36.1±2.9	37.1±3.7	32.9±3.5
D. fulvum JCM 5631^T	27.4±3.1	100±0.0	60.9±2.2	30.1±3.4
D. roseum JCM 3364^T	39.5±1.4	67.1±10.7	100±0.00	23.0±6.0
D. darangshiense JCM17441^T	25.0±2.9	28.9±1.0	31.0±1.5	100±0.0

[a]Average from six independent determinations.

The type strain RY35-23^T (=JCM 19831^T= TISTR 2212^T= PCU 333^T) was isolated from peat swamp forest soil collected from Nong Jum Rung area, Rayong province, Thailand.

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