Dactylosporangium roseum sp. nov.

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A new species of *Dactylosporangium* is described, for which the name *Dactylosporangium roseum* is proposed. This organism is characterized by the rose color of its vegetative mycelium and its carbohydrate utilization pattern. The type strain of *D. roseum* is strain SF-2186 (=IFO 14352).

In the course of screening for new antibiotics, we isolated two actinomycetes (strain SF-2107 and strain SF-2186^T [T = type strain]) which produce a new member of the orthosomycin group (8, 16) (antibiotic SF-2107 complex; Japan Kokai Patents 81-156297, 1981; 82-129692, 1982; 83-13392, 1983; assignee, Meiji Seika Kaisha, Ltd.).

These two strains are very similar in their characteristics and were found to belong to the genus *Dactylosporangium* Thiemann, Pagani and Beretta (13) on the basis of their characteristic morphology (i.e., formation of finger-shaped sporangia arising directly from the vegetative mycelium and

new species of the genus *Dactylosporangium*. We designate strain SF-2186 as the type strain, because the characteristic rose color of its vegetative mycelium is more vivid than that of strain SF-2107.

In this report, we compare strain SF-2186^T with previously recognized species of *Dactylosporangium*.

MATERIALS AND METHODS

Bacterial strains. Strain SF-2186^T and strain SF-2107 (= ATCC 31744) were isolated in our laboratory from soil samples collected at Shizuoka, Japan, and Yokohama, Japan,

TABLE 1. Cultural characteristics of strains SF-2186^T and SF-2107^a

Medium	Strain SF-2186 ^T			Strain SF-2107		
	Growth	Reverse color	Sporangia	Growth	Reverse color	Sporangia
Sucrose-nitrate agar	Very poor, thin	Colorless	Abundant	Very poor, thin	Colorless	Abundant
Glucose-asparagine agar	Very poor	Colorless	Abundant	Very poor	Colorless	Abundant
Glycerol-asparagine agar (ISP medium 5)	Very poor	Colorless	Very abundant	Very poor	Colorless	Abundant
Inorganic salts-starch agar (ISP medium 4)	Moderate to good	Rose (8ic)	Very abundant	Poor to moderate	Light rose beige (4ec), sometimes rose (8ic)	Abundant
Calcium malate agar	Very poor	Colorless	Abundant	Very poor	Colorless	Abundant
Oatmeal agar (ISP medium 3)	Moderate	Colorless	None	Poor to moderate	Colorless	None
Yeast extract-malt extract agar (ISP medium 2)	Moderate to good	Rose red (8pc)	Poor	Moderate	Amber (3lc), sometimes rose (8ic)	None or poor
Tyrosine agar (ISP medium 7)	Poor to moderate	Melon yellow (3ga)	Abundant	Poor to moderate	Melon yellow (3ga)	Abundant
Nutrient agar	Poor	Colorless	Poor	Poor	Colorless	None or poor
Bennett agar	Moderate	Pastel orange (4ic) to amber (3lc)	Poor	Moderate	Melon yellow (3ea) to amber (3lc)	None or poor
Sucrose-yeast extract agar	Good	Rose (8ic) to rose wine (8le)	None or poor	Good	Rose (8ic) to rose red (8pc)	Poor

^a True aerial mycelia and diffusible pigments were not formed on all media. For an explanation of color designations, see reference 3.

three or four zoospores arranged in a single straight row inside a sporangium).

Based on a taxonomic comparison with known species, strain SF-2186^T and strain SF-2107 have been identified as a

respectively. For comparative purposes, we studied the following strains: *Dactylosporangium aurantiacum* KCC A-0083^T (= ATCC 23491^T) (14) and *Dactylosporangium thailandense* KCC A-0084^T (= ATCC 23490^T) (13, 14), both of which were obtained from the KCC Culture Collection (Kaken Seiyaku Co. Ltd., Tokyo, Japan); *Dactylosporangium matsuzakiense* SF-2052^T (= ATCC 31570^T) (11) and *Dactylospo-*

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FIG. 1. Finger-shaped sporangia of strain SF-2186^T on glycerol-asparagine agar (ISP medium 5), as observed with a scanning electron microscope. The organism was incubated at 28°C for 14 days. (a) $\times 3,000$. (b) $\times 10,000$.

rangium vinaceum SF-2127^T (= IFO 14181^T) (12), which are strains from our laboratory.

Media. Washed cells were inoculated onto the following media: ISP media 2 to 7 (10); sucrose-nitrate agar; glucose-asparagine agar; nutrient agar; Bennett agar; and calcium malate agar of Waksman (15). Sucrose-yeast extract agar (sucrose, 10 g; yeast extract [Difco Laboratories, Detroit, Mich.], 5 g; CaCO₃, 1 g; agar [Difco], 15 g; tap water added to 1,000 ml; pH not adjusted) was used as an additional medium. Inoculated plates were incubated at 28°C.

Morphological characterization. The morphologies of the sporangia and spores of the strains grown on glycerol-asparagine agar (ISP medium 5) and inorganic salts-starch agar (ISP medium 4) were studied by both light microscopy and electron microscopy. For scanning electron microscopy, an agar block containing numerous sporangia was fixed in 2.5% glutaraldehyde for 16 h and in osmium tetroxide fumes for 4 h. The block was then gradually dehydrated by washing with increasing proportions of acetone and finally dried by the critical-point method (1).

FIG. 2. Electron micrograph of a zoospore of strain SF-2186^T. $\times 13,950$.

Each specimen was sputter-coated with gold-palladium and then examined with an electron microscope (model JEM100C-ASID; Japan Electron Optics Laboratory Co., Ltd.). For observation of zoospores, drops of a suspension containing zoospores were gently placed onto collodion-coated grids and air dried. The specimens were shadowed with platinum-palladium and examined with the electron microscope described above.

Cultural characterization. The cultural characteristics of the strains on various media were observed after 14 to 21 days of incubation at 28°C. Color determinations were made by comparing the cultures with color chips from the *Color Harmony Manual* (3).

Physiological characterization. The media and procedures used for physiological characterization were the same as those outlined by Waksman (15) and Shirling and Gottlieb (10). The temperature range for growth was determined on ISP medium 4. NaCl tolerance was measured on Luedemann agar medium (6) containing different concentrations of NaCl (0, 1.5, 3, 4, 5, and 7%). Carbohydrate utilization was determined by the procedure of Luedemann and Brodsky (7).

Cell wall analysis. Cell walls were analyzed by the procedure of Becker et al. (2), and whole cells were analyzed by the method of Lechevalier (4).

RESULTS AND DISCUSSION

Morphological characteristics. The vegetative mycelium of strain SF-2186^T was fine, 0.5 to 0.7 μ m in diameter, long,

irregularly branched, and twisted and penetrated into the agar. Fragmentation of hyphae usually did not occur either on agar or under submerged conditions. Aerial mycelia were not formed by agar cultures except for traces of rudimentary hyphae developing on oatmeal agar (ISP medium 3). Strain SF-2186^T produced finger-shaped sporangia. The sporangia emerged directly from the vegetative hyphae and were found singly or in clusters on the surface of the solid media. Sporangia were abundantly formed on all chemically defined media used, but were rare on organic media, such as yeast extract-malt extract agar (ISP medium 2), nutrient agar, and Bennett agar (Table 1).

With the scanning electron microscope, the sporangia appeared to be pod-shaped structures enveloping the spore chains (Fig. 1). The sporangia were 0.8 to 1.1 by 2.5 to 5.5 μ m in size. Each sporangium usually contained three to four spores in a single straight row. The spores were elliptical and measured 0.8 to 1.1 by 0.6 to 1.5 μ m. If the surface of a sporangium-containing culture was flooded with sterile water or soil extract solution, motile spores were observed after 30 to 60 min at room temperature. Electron microscopy of the spores revealed the presence of long polar flagella (Fig. 2). The formation of globose bodies, which have been described by Thiemann et al. (14) and Sharples and Williams (9), was not clearly observed in strain SF-2186^T. The morphological characteristics of strain SF-2107 were identical to those of strain SF-2186^T.

Cultural characteristics. Table 1 summarizes the degree of growth, formation of sporangia, and reverse color of the vegetative mycelia of strains SF-2186^T and SF-2107 on various media. Neither strain formed true aerial mycelia or diffusible pigments on the agar media used. The most significant character of strain SF-2186^T was the rose color of its vegetative mycelium on selected media, such as inorganic salts-starch agar (ISP medium 4), yeast extract-malt extract agar, and sucrose-yeast extract agar. Strain SF-2107 showed the same rose color on sucrose-yeast extract agar, but rarely on inorganic salts-starch agar and yeast extract-malt extract agar. The rose color of the vegetative mycelium was not sensitive to pH changes, since no color change was observed when it was exposed to either 0.05 N NaOH or 0.05 N HCl.

Physiological characteristics. The physiological characteristics of strain SF-2186^T are described below. Reduction of nitrate and liquefaction of gelatin are positive. Hydrolysis of starch, peptonization and coagulation of milk, and formation of melanoid pigment are all negative. Strain SF-2186^T grows within a temperature range of 20 to 42°C, with an optimum

TABLE 2. Comparison of *D. roseum* SF-2186^T with the type strains of four other *Dactylosporangium* species

Strain	Reverse color on: ^a		Diffusible pigment	Hydrolysis of starch	Utilization of:	
	Oatmeal agar	Sucrose-yeast extract agar			D-Mannitol	L-Rhamnose
<i>D. roseum</i> SF-2186 ^T	Colorless	Rose (8ic)	None	— ^b	—	—
<i>D. aurantiacum</i> KCC A-0083 ^T	Light apricot (4ea)	Shell (3ca)	None	+	+	+
<i>D. thailandense</i> KCC A-0084 ^T	Russet orange (4pc)	Orange rust (4pe)	Occasional, amber or brown	+	+	+
<i>D. matsuzakiense</i> SF-2052 ^T	Burnt orange (5nc)	Luggage-tan (4ne)	Occasional, light brownish pink	+	+	+
<i>D. vinaceum</i> SF-2127 ^T	Wine (7pg)	Ebony brown (8pn)	Wine to deep red	+	+	+

^a For an explanation of color designations, see reference 3.

^b +, Positive; —, negative.

range of 28 to 37°C. Strain SF-2186^T tolerates 1.5% NaCl, but no growth occurs on more than 3% NaCl.

When a Luedemann-Brodsky medium was used as the basal medium, this organism utilized D-glucose, D-fructose, and sucrose, but not D-mannitol, D-inositol, raffinose, or L-rhamnose. Utilization of D-xylose and L-arabinose is doubtful. The physiological characteristics of strain SF-2107 are identical to those of strain SF-2186^T described above.

Cell wall analysis. An analysis of cell wall hydrolysates of both strain SF-2186^T and strain SF-2107 by paper chromatography demonstrated the presence of 3-hydroxydiaminopimelic acid and a trace of *meso*-diaminopimelic acid. No LL isomer was detected. Sugar determination indicated that xylose and a trace of arabinose were present. Thus, both strain SF-2186^T and strain SF-2107 can be considered to have type II cell walls and whole cell sugar pattern D according to the classification scheme of Lechevalier and Lechevalier (5).

Identity of strain SF-2186^T. The formation of finger-shaped sporangia containing motile spores, the absence of true aerial mycelia, and type II cell walls place strain SF-2186^T in the genus *Dactylosporangium* Thiemann et al. (14). Four species, *D. aurantiacum* (14), *D. thailandense* (13, 14), *D. matsuzakiense* (11), and *D. vinaceum* (12), have been described previously as species of the genus *Dactylosporangium*. We compared strain SF-2186^T directly with the type strains of these four previously described species. No outstanding differences in morphology were apparent among these four type strains and strain SF-2186^T, regardless of whether sporangia or spores were compared. However, strain SF-2186^T differs clearly from the other four type strains in its cultural and physiological characteristics, as described below. *D. vinaceum* produces a wine to deep red diffusible pigment on almost all media used, whereas no obvious diffusible pigment was observed in strain SF-2186^T on the same media. As shown in Table 2, the reverse colors of *D. aurantiacum*, *D. thailandense*, and *D. matsuzakiense* are apricot to orange on oatmeal agar, whereas the reverse of strain SF-2186^T is colorless. On the other hand, strain SF-2186^T shows a distinctive rose reverse color on yeast extract-malt extract agar, on inorganic salts-starch agar, and especially on sucrose-yeast extract agar, whereas the other three species do not show such color on these media. In addition, strain SF-2186^T and the other four species differ in some physiological properties, such as starch hydrolysis and utilization of D-mannitol and L-rhamnose (Table 2).

In view of the characteristics described above, strain SF-2186^T can be considered a new species of the genus *Dactylosporangium*, for which we propose the name *Dactylosporangium roseum* sp. nov. (ro'se.um. L. neut. adj.

roseum rose colored, referring to the color of the vegetative mycelium). Strain SF-2186 is the type strain of *D. roseum*; a culture of this strain has been deposited in the Institute for Fermentation, Osaka, Japan, as strain IFO 14352.

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