Microbispora viridis, a New Species of *Actinomycetales*

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A new species of *Actinomycetales* is described, for which we propose the name *Microbispora viridis*. This organism produces a new peptide antibiotic, SF-2240. *M. viridis* is characterized by its green aerial mass color and longitudinally paired spores, which have rugose surfaces with vertical ridges. The type strain of *M. viridis* is strain SF-2240 (= IFO 14382).

In the course of screening for new antibiotics, we isolated an actinomycete designated strain SF-2240^T (T = type strain). This organism produces antibiotic SF-2240 (8), which is a basic peptide antibiotic having a chromophore in its molecule and showing weak antimicrobial activity against gram-positive and gram-negative bacteria.

Strain SF-2240^T belongs to the genus *Microbispora* Nonomura and Ohara (6), because of its morphological features and cell wall composition. In this paper we describe morphological and physiological properties of this strain, for which we propose the name *Microbispora viridis* sp. nov.

MATERIALS AND METHODS

Bacterial strains. Strain SF-2240^T was isolated in our laboratory by the dry heating method developed by Nonomura and Ohara (7) from a soil sample which was collected in May 1982 at Takayama City, Gifu Prefecture, Japan. For comparative purposes, the type strains of 10 species of the genus *Microbispora* were also studied (see Table 2). These strains were provided by H. Nonomura or were obtained from the Kaken Chemical Co., Ltd. Tokyo, Japan, the American Type Culture Collection, Rockville, Md., or the Institute for Fermentation, Osaka, Japan.

Morphological characteristics. Morphological observations were made with a light microscope on cultures grown at 28°C for 2 to 4 weeks on oatmeal agar (ISP medium 3) and inorganic salts-starch agar (ISP medium 4) (10). Spore morphology was studied with a model JEM 100C electron microscope and a scanning electron microscope (model ASID-4D; Japan Electron Optics Laboratory Co., Ltd. Tokyo).

Cultural and physiological characteristics. The media and procedures used for cultural and physiological characterization of strain SF-2240^T were those described by Shirling and Gottlieb (10) and Waksman (12). Each culture was incubated at 28°C for 2 to 4 weeks. Color determinations were made by comparing the cultures with color chips from the *Color Harmony Manual* (2). The temperature range for growth was determined on yeast extract-malt extract agar (ISP medium 2). Carbohydrate utilization was investigated by using the procedure of Pridham and Gottlieb (9). Cell wall analysis was performed by the method of Becker et al. (1), and whole-cell analysis was performed by the method of Lechevalier (4). Phospholipids were analyzed by the procedure of Lechevalier et al. (5).

RESULTS AND DISCUSSION

Morphological characteristics. The substrate mycelium of strain SF-2240^T was long, irregularly branched, and 0.3 to

0.5 µm in diameter; this mycelium did not fragment into short elements or form spores. The aerial mycelium branched monopodially and was 0.4 to 0.6 µm in diameter. Spores were borne in longitudinal pairs on the aerial mycelium (Fig. 1 and 2). The paired spores were closely arranged along the aerial hyphae, just like a catkin. The spores were oval to ellipsoidal and 0.8 to 1.2 long by 1.5 to 2.5 µm in diameter; the spore surfaces had vertical ridges (Fig. 2). As determined by electron microscopic observation of ultrathin sections, it seemed that the rugose surfaces of the spores were formed by the sheath (Fig. 3). The spores were not motile, and sclerotia or sporangia were not observed.

Cultural characteristics. The cultural characteristics of strain SF-2240^T are summarized in Table 1. The color of the mature aerial masses produced on inorganic salts-starch agar, oatmeal agar, and glycerol-asparagine agar (ISP medium 5) belonged to the green color series (24ge to 24ig; dusty green to sage green [2]). The reverse sides of colonies were pastel yellow to light brown. Neither a soluble pigment nor an insoluble pigment such as iodinin was formed.

Physiological characteristics. The physiological characteristics of strain SF-2240^T are given below. Gelatin liquefaction, hydrolysis of starch, and coagulation and peptonization of milk were positive. Reduction of nitrate and formation of melanoid pigment on peptone-yeast extract-iron agar and

FIG. 1. Light micrograph of sporulating aerial mycelium of strain SF-2240^T on oatmeal agar after incubation at 28°C for 2 weeks. ×700.

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FIG. 2. Scanning electron micrographs of spores of strain SF-2240^T on oatmeal agar after incubation at 28°C for 2 weeks. (a) $\times 2,000$. (b) $\times 8,000$.

tryptone-yeast extract broth were all negative. Strain SF-2240^T grew in media containing NaCl concentrations up to 4%, but 5% NaCl was inhibitory. B vitamins were not essential for growth. The temperature range for growth was 15 to 42°C, with best growth occurring between 28 and 37°C. On ISP medium 9, good growth was obtained with D-glucose, D-mannitol, and L-rhamnose, and moderate growth was obtained with D-fructose, glycerol, L-arabinose, and sucrose. No growth was observed with D-xylose, i-inositol, α -melibiose, or raffinose.

Chemical analysis of cells. The cell wall contained *meso*-diaminopimelic acid as a major constituent. LL-Diaminopimelic acid, glycine, arabinose, and galactose were not de-

tected. An analysis of whole-cell hydrolysates demonstrated the presence of madurose as the principal sugar component. These data indicate that strain SF-2240^T has type III cell walls and a type B whole-cell sugar pattern. A type PIV phospholipid pattern was found.

Identity of strain SF-2240^T. The formation of longitudinally paired spores on aerial mycelium and a type III cell wall place strain SF-2240^T in the genus *Microbispora*. Strain SF-2240^T is compared with previously described *Microbispora* species in Table 2. All of the previously described species form aerial mycelia having a white to pinkish color; only strain SF-2240^T forms a greenish aerial mass. Moreover, the spores of most of the previously described species

FIG. 3. Transmission electron micrographs of thin sections of strain SF-2240^T spores on oatmeal agar after incubation at 28°C for 2 weeks. (a) Vertical section. $\times 18,000$. (b) Horizontal section. $\times 46,000$.

(except *Microbispora echinospora*) have smooth surfaces, whereas the spores of strain SF-2240^T have rugose surfaces with vertical ridges (Fig. 2 and 4). Enokita et al. (3) described the new subspecies "*Microbispora rosea* subsp. *laenispora*" (not on the Approved List of Bacterial Names [11]), which formed spores having a similar appearance. However, strain

TABLE 1. Cultural characteristics of strain SF-2240^{Ta}

Medium	Growth ^b	Aerial mycelium ^b
Glycerol-asparagine agar (ISP medium 5)	Moderate, colorless	Moderate, putty (1dc) ^c
Inorganic salts-starch agar (ISP medium 4)	Good, buff	Abundant, light almond green (23ie) ^c
Oatmeal agar (ISP medium 3)	Good, pastel yellow	Abundant, dusty green (24ge) ^c
Yeast extract-malt extract agar (ISP medium 2)	Good, yellowish brown	Poor, white (a)
Tyrosine agar (ISP medium 7)	Moderate, light brown	Moderate, bay-berry gray (22fe)
Bennett agar	Moderate, buff	Poor, white (a)
Sucrose-nitrate agar	Poor, colorless	Poor, white (a)
Nutrient agar	Poor, colorless	Poor, white (a)
Calcium malate agar	Poor, colorless	Poor, white (a)

^a Strain SF-2240^T did not produce notable soluble pigment.

^b The color codes and names were taken from reference 2.

^c After 8 weeks of incubation at 28°C, the aerial mass color changed to sage green (24ig) on glycerol-asparagine agar, inorganic salts-starch agar, and oatmeal agar.

SF-2240^T differs clearly from "*M. rosea* subsp. *laenispora*" since the latter produces a white to pink aerial mass.

On the basis of these observations, we believe that strain SF-2240^T represents a new species in the genus *Microbispora*, for which we propose the name *Microbispora viridis* (vi'ri.dis. L. adj. *viridis* green, referring to the green aerial mass). Strain SF-2240 is the type strain of *M. viridis*. A culture of this strain has been deposited in the Institute for Fermentation, Osaka, Japan, as strain IFO 14382^T.

Summary description of *Microbispora viridis* sp. nov. Spores in characteristic longitudinal pairs are formed on the aerial mycelium, but no spores are formed on the substrate mycelium. Spores are oval to ellipsoidal and 0.8 to 1.2 μ m long by 1.5 to 2.5 μ m in diameter and have rugose surfaces with vertical ridges. Sporangia, sclerotia, flagellated spores, and fragmented hyphae are not formed. The color of the aerial mycelium is green on inorganic salts-starch agar, oatmeal agar, and glycerol-asparagine agar.

No distinct soluble or insoluble pigment is formed.

Utilizes D-glucose, D-fructose, L-arabinose, D-mannitol, glycerol, sucrose, and L-rhamnose for growth, but not D-xylose, i-inositol, α -melibiose, or raffinose.

Cell wall type III.

Aerobic.

TABLE 2. Comparison of strain SF-2240^T with type strains of *Microbispora* species

Strain	Medium	Incubation	Aerial mass color ^a	Spore surfaces
<i>Microbispora rosea</i> ATCC 12950 ^T	Oatmeal agar	28°C, 4 weeks	Pink (6ca)	Smooth
<i>Microbispora aerata</i> ATCC 15488 ^T	Tyrosine agar	37°C, 2 weeks	Pink (5ba)	Smooth
<i>Microbispora amethystogenes</i> IFO 14039	Oatmeal agar	28°C, 4 weeks	Pink (5ca)	Smooth
<i>Microbispora bispota</i> ATCC 19993 ^T	Tyrosine agar	55°C, 2 weeks	White (a)	Smooth
<i>Microbispora chromogenes</i> M ₂ -47	Yeast extract-malt extract agar	28°C, 4 weeks	Pink (4ca)	Smooth
<i>Microbispora diastatica</i> KCC A-0023 ^T	Yeast extract-malt extract agar	28°C, 4 weeks	Pink (6ca)	Smooth
<i>Microbispora parva</i> KCC A-0024 ^T	Inorganic salts-starch agar	28°C, 4 weeks	Pink (4ca)	Smooth
<i>Microbispora thermodiastatica</i> ATCC 27098 ^T	Tyrosine agar	45°C, 2 weeks	Pink (5ba)	Smooth
<i>Microbispora thermorosea</i> ATCC 27099 ^T		45°C, 2 weeks	None	
<i>Microbispora echinospora</i> ATCC 27300 ^T	Oatmeal agar	28°C, 4 weeks	Pink (5ca)	Spinose
<i>Microbispora viridis</i> SF-2240 ^T (= IFO 14382 ^T)	Oatmeal agar	28°C, 4 weeks	Green (24ge)	Rugose

^a The color codes were taken from reference 2.

FIG. 4. Scanning electron micrographs of spores of type strains of *Microbispora* species. (a) *Microbispora rosea* ATCC 12950^T. $\times 8,000$. (b) *Microbispora bispora* ATCC 19993^T. $\times 8,000$. (c) *Microbispora chromogenes* M₂-47. $\times 8,000$. (d) *Microbispora echinospora* ATCC 27300^T. $\times 6,000$.

Mesophilic.

Antagonistic properties: produces antibiotic SF-2240.

Habitat: soil.

Type strain: strain SF-2240 (= IFO 14382).

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