Streptosporangium fragile sp. nov.

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A new species of *Streptosporangium* is described, for which we propose the name *Streptosporangium fragile*. This organism produces a new anthracycline antibiotic and is characterized by dark brown to black vegetative mycelium, pink aerial mycelium, brown soluble pigment, and fragile sporangial membrane. In older cultures the fragility of the sporangial membrane results in the coalescence of the sporangiospores into large irregular masses. The type strain of *S. fragile* is SK&F-BC2496 (= ATCC 31519).

The genus *Streptosporangium* was originally described by Couch (4) to include actinomycetes that are characterized by the formation of non-motile sporangiospores which are arranged in a coil within a sporangium. During the course of screening for new antibiotics, we isolated a morphologically and physiologically distinct strain of *Streptosporangium*, strain SK&F-BC2496^T (type strain), which produces a novel anthracycline antibiotic. In this report we provide data to support the recognition of a new species, for which we propose the name *Streptosporangium fragile*. The isolation and characterization of the anthracycline antibiotic have been described (C. H. Nash III, M. C. Shearer, K. M. Snader, J. R. Valenta, and D. Cooper, U.S. patent 4,293,546, October 1981).

MATERIALS AND METHODS

Bacterial strain and culture conditions. Strain SK&F-BC2496^T was isolated by standard soil dilution and plating techniques from a soil sample collected in the Northern Province of Sri Lanka. The soil sample was taken from a cultivated field that was lying fallow after a paddy crop.

Stock cultures were grown on medium 172 (1); temperature relationships were also determined on slants of this medium. The medium used for morphological observations was oatmeal agar (25). Additional media used to characterize strain SK&F-BC2496^T were yeast extract-malt extract agar (25), inorganic salts-starch agar (25), glycerol-asparagine agar (25), peptone-yeast extract-iron agar (25), tyrosine agar (25), potato dextrose agar (Difco Laboratories) with the pH adjusted to 7.0 to 7.2, Czapek-Dox broth (Difco) solidified with agar, Bennett agar (13), Czapek-peptone agar (5), Emerson YpSs agar (7), thin potato-carrot agar (11), defined agar (16), nutrient gelatin (Difco), and litmus milk medium (Difco).

All tests were performed at 28°C. For growth tests under anaerobic conditions, the GasPak system (BBL Microbiology Systems) was used.

Microscopy. For scanning electron microscopy, 14-day-old plate cultures of strain SK&F-BC2496^T on Jensen agar (12) were fixed with osmium tetroxide in situ and gold-shadowed by conventional techniques. The shadowed specimens were viewed with a Jeol 100CX ASID-4D scanning electron microscope.

Physiological tests. The physiological tests used to characterize strain SK&F-BC2496^T were those of Gordon (8, 9) and Gordon and Mihm (10). In the tests for acid production from carbohydrates and utilization of organic acids, all results were confirmed by subculturing onto fresh medium.

Organic growth factor requirements were investigated with a synthetic medium containing the following: dextrose, 15 g; K₂HPO₄, 3 g; KH₂PO₄, 0.5 g; MgCl₂ · 6H₂O, 0.53 g; CaCl₂, 0.01 g; NaCl, 1.0 g; FeCl₃ · 6H₂O, 0.001 g; mineral solution, 10 ml; and distilled water, 1,000 ml. The pH of this medium was 7.1. The mineral solution contained the following: ZnSO₄ · 7H₂O, 2.8 g; CuSO₄ · 5H₂O, 0.125 g; MnSO₄ · H₂O, 1.0 g; CoCl₂ · 6H₂O, 0.1 g; Na₂B₄O₇ · 10H₂O, 0.09 g; Na₂MoO₄ · 2H₂O, 0.05 g; and distilled water, 1,000 ml. The following nitrogen sources were tested: NaNO₃ (3 g/liter), (NH₄)₂SO₄ (3 g/liter), L-asparagine (3 g/liter), and (NH₄)₂SO₄ plus L-asparagine (each 1.5 g/liter). Cultures were grown in 250-ml Erlenmeyer flasks containing 50 ml of medium; the flasks were incubated at 28°C on rotary shakers (250 rpm). Cells were harvested after 7 days, and growth was measured by determining dry weight. Tests were concluded after one subculture on each nitrogen source.

Cell wall and phospholipid analyses. Cell wall analyses were performed by the method of Becker et al. (3), and whole-cell hydrolysates were analyzed by the methods of Becker et al. (2) and Lechevalier (17). Phospholipid analyses were performed by the methods of Lechevalier et al. (18).

RESULTS

Morphology. Strain SK&F-BC2496^T has the general morphological features that were described by Couch (4) for the genus *Streptospor-*

FIG. 1. Scanning electron micrograph of a sporangium from a 14-day-old culture. Bar = 0.8 μ m.

angium. It is a gram-positive, non-acid-fast organism that forms a substrate mycelium with hyphae about 0.5 to 1.0 μ m in diameter and an aerial mycelium that bears sporangia. The sporangia are usually 6 to 12 μ m in diameter and may be formed apically on main threads or on very short to relatively long lateral branches; a few appear to be sessile. The immature sporangiospores are arranged in coils within the sporangia. Mature sporangiospores are spherical to ovoid and nonmotile. The sporangial membranes are unusually fragile and could be demonstrated clearly only by electron microscopy (Fig. 1 and 2). This fragility of the sporangial membranes results in the coalescence of the sporangiospores into large irregular masses as the cultures age (Fig. 3).

Cell chemistry. Purified cell wall preparations of strain SK&F-BC2496^T contained *meso*-diaminopimelic acid, glutamic acid, alanine, glucosamine, and muramic acid; no characteristic sugars were present. Whole-cell hydrolysates contained madurose. Phospholipid preparations contained phosphatidylinositol, phosphatidylethanolamine, phosphatidylmethylethanolamine, cardiolipin, and unknown glucosamine-containing phospholipids. Therefore, strain SK&F-BC2496^T has a type III cell wall (19) with a type B whole-cell sugar pattern (19) and a type

PIV phospholipid composition (18), which are characteristic of the genus *Streptosporangium* (18, 19).

Soluble pigment. Strain SK&F-BC2496^T produced a brown soluble pigment on nearly all media. The exact nature of this pigment was not determined. However, based on the chromatographic columns used to isolate the antibiotic and perform the phospholipid analyses, we determined that this pigment is a mixture of a number of colored pigments which, when combined, give the brown color.

Appearance on various media. In this study all cultures were observed for 3 weeks. The colors of the cultures were determined by comparison with chips from either the *ISCC-NBS Centroid Color Charts* (15, 21) or the *Munsell Book of Color* (20). The characteristics of the culture on the media tested are given below.

Yeast extract-malt extract agar: growth excellent, black; aerial mycelium scant to moderate, white, turning light pink; sporangia abundant; soluble pigment light brown.

Oatmeal agar: growth good, black (ISCC-NBS 267, black); aerial mycelium moderate, white, turning light pink (ISCC-NBS 8, grayish pink); sporangia abundant; soluble pigment light brown.

FIG. 2. Scanning electron micrograph of a sporangial membrane from a 14-day-old culture. Bar = 0.4 μ m.

FIG. 3. Micrograph of sporangiospores coalescing into irregular masses. The culture was grown on oatmeal agar and examined by bright-field microscopy. Bar = 20 μ m.

Inorganic salts-starch agar: growth good, brownish black; aerial mycelium moderate, white, turning light pink (Munsell 5R 9/2); sporangia abundant; soluble pigment light brown.

Glycerol-asparagine agar: growth fair, flat, brown; aerial mycelium sparse, white; sporangia none to sparse; soluble pigment light brown.

Potato dextrose agar: growth fair to good, black; aerial mycelium abundant, white, turning pink (Munsell 2.5YR 9/2); sporangia abundant; soluble pigment light brown.

Thin potato-carrot agar: growth fair, flat, black; aerial mycelium sparse, white, turning light pink; sporangia abundant; soluble pigment light brown.

Czapek-sucrose agar: growth poor, flat, brown; aerial mycelium scant, white; no sporangia; soluble pigment light brown.

Bennett agar: growth good, black; no aerial mycelium; soluble pigment brown.

Czapek-peptone agar: growth fair, brown; no

aerial mycelium; soluble pigment light brown.

Emerson YpSs agar: growth good, black; aerial mycelium scant, white; no sporangia; soluble pigment brown.

Medium 172: growth excellent, black; aerial mycelium none to sparse, white; sporangia none to moderate; soluble pigment brown.

Tyrosine agar: growth fair, brown; aerial mycelium sparse, white; no sporangia; soluble pigment light brown.

Defined agar: no growth.

Physiological and biochemical characteristics. Strain SK&F-BC2496^T did not grow under anaerobic conditions. The temperature range for growth was 15 to 45°C; no growth occurred at 10 or 50°C. Hydrogen sulfide was produced. Milk was peptonized. Gelatin was hydrolyzed but not liquefied. Nitrate was reduced to nitrite. Starch, casein, L-tyrosine, and hypoxanthine were hydrolyzed, but urea, adenine, and xanthine were not. Esculin was decomposed, but allantoin and hippurate were not. No growth was produced in lysozyme broth. Catalase was produced. No violet crystals of iodine were produced in any medium.

Acid was produced from L-arabinose, D-cellobiose, dextrin, dextrose, D-erythritol, D-fructose, D-galactose, glycogen, lactose, maltose, D-mannitol, D-mannose, α -methyl-D-glucoside, rhamnose, D-ribose, salicin, starch, trehalose, and D-xylose. No acid was produced from adonitol, dulcitol, inulin, melibiose, raffinose, or α -methyl-D-mannoside. Acid production during an initial culture in medium containing glycerol, D-inositol, D-melezitose, D-sorbitol, L-sorbose, or sucrose was variable, but when the organism was subcultured in media containing these carbohydrates the results were consistently negative. Citrate, malate, succinate, lactate, and pyruvate were utilized; utilization of mucate was weakly positive. During an initial culture in media containing acetate and propionate, these organic acids were utilized, but when the organism was subcultured, the results were variable. Benzoate and tartrate were not utilized. During an initial culture in media containing oxalate and formate, utilization was variable; when the organism was subcultured, the results were consistently negative.

Strain SK&F-BC2496^T did not require vitamins for growth in the synthetic medium used. Either ammonium sulfate or the combination of ammonium sulfate and L-asparagine was a satisfactory nitrogen source; L-asparagine or sodium nitrate permitted little, if any, growth.

DISCUSSION

Strain SK&F-BC2496^T was compared with all of the *Streptosporangium* species listed in *Bergey's Manual of Determinative Bacteriology* (6)

TABLE 1. Comparison of strain SK&F-BC2496^T with *Streptosporangium* species having pink aerial masses^a

Organism	Color of substrate mycelium	Color of soluble pigment	Shape of spores	Growth at 42°C	Nitrate reduction	Starch hydrolysis	Iodinin production	Reference
SK&F-BC2496 ^T	Dark brown to black	Brown	Oval	+	+	+	—	
<i>Streptosporangium amethystogenes</i>	Yellow-brown	Yellow-brown	Oval	—	+	+	+	22
<i>Streptosporangium longisporum</i>	Red to brown-red	None	Cylindrical	—	±	+	—	24
<i>Streptosporangium nondiastaticum</i>	Orange	Yellow-brown	Oval	+	+	—	—	23
<i>Streptosporangium pseudovulgare</i>	Orange to yellow-brown	Yellow-brown	Oval	+	+	+	—	23
<i>Streptosporangium roseum</i>	Red-brown to yellow-brown	Red-brown to purple-brown	Spherical	—	+	+	—	4
<i>Streptosporangium violaceochromogenes</i>	Yellow to orange	Violet	Oval	—	+	+	—	14
<i>Streptosporangium vulgare</i>	Yellow to pale orange	Yellow to pale orange	Oval	—	—	+	—	22

^a +, Positive; —, negative; ±, none or a little.

and on the Approved Lists of Bacterial Names (26); particular emphasis was placed on those *Streptosporangium* species having pink aerial masses (Table 1). Strain SK&F-BC2496^T was easily distinguished from all other species on the basis of its dark brown to black vegetative mycelium. Another distinguishing characteristic was the fragility of its sporangial membranes and the consequent tendency of the sporangiospores to coalesce into large irregular masses. This tendency was observed in only one other species, *Streptosporangium violaceochromogenes*, which was quite different from strain SK&F-BC2496^T in other morphological and physiological characteristics (Table 1). Therefore, we regard strain SK&F-BC2496^T as a new species for which we propose the name *Streptosporangium fragile* (fra'gi.le. L. adj. *fragile* easily broken, fragile, an allusion to the fragility of the sporangial membrane). Strain SK&F-BC2496, the type strain of *S. fragile*, has been deposited in the American Type Culture Collection, Rockville, Md., as strain ATCC 31519.

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