Streptomyces sulfonofaciens sp. nov.

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A new species of bacteria is described, for which we propose the name *Streptomyces sulfonofaciens*. This organism produces a new β -lactam antibiotic, SF-2103A, which is a carbapenem compound. *S. sulfonofaciens* is characterized by its red spore mass, spores with smooth surfaces in straight chains, non-chromogenicity, and carbon utilization pattern. The type strain of *S. sulfonofaciens* is strain SF-2103, which has been deposited in the American Type Culture Collection as strain ATCC 31892.

In the course of screening soil actinomycetes for new antibiotics, we isolated a streptomycete designated strain SF-2103^T (type strain). This organism produces SF-2103A (3), a new type of carbapenem compound (8) which possesses a distinctive sulfonate group in its molecule and shows not only antibacterial activity against gram-positive and gram-negative pathogens but also marked inhibitory activity toward β -lactamase (penicillin amido- β -lactam hydrolase [EC 3.5.2.6]). This carbapenem compound has never been reported before as an actinomycete product.

Strain SF-2103^T belongs to the genus *Streptomyces* Waksman and Henrici because of its morphological features and cell wall composition. In this report we provide a morphological and physiological description of this strain, for which we propose the name *Streptomyces sulfonofaciens* sp. nov.

MATERIALS AND METHODS

Strain SF-2103^T was isolated in our laboratory in May 1979 from a soil sample collected at Nachi-Katsuura, Wakayama Prefecture, Japan. *Streptomyces sindenensis* ISP 5255 (11), *Streptomyces termitum* ISP 5329 (12), and "*Streptomyces alborubidus*" ISP 5465 (13) (not on the Approved Lists of Bacterial Names [14]) were compared with strain SF-2103^T because they appeared to be related.

Morphological characteristics. Morphological observations were made with a light microscope on cultures grown at 28°C for 7 to 14 days on yeast extract-malt extract agar (International *Streptomyces* Project [ISP] medium 2), oatmeal agar (ISP medium 3), and inorganic salts-starch agar (ISP medium 4) (9). The surfaces of the spores and spore chains were studied with an electron microscope (model JEM 100C and scanning electron microscope unit ASID-4D; Japan Electron Optics Laboratory Co., Ltd.).

Cultural and physiological characteristics. The media

and procedures used for cultural and physiological characterization of strain SF-2103^T were those described by Shirling and Gottlieb (9). Each culture was incubated at 28°C for 14 days, except those in milk media (28 and 37°C, 21 days) and gelatin media (25°C, 21 days). The temperature range and optimum temperature for growth were determined on yeast extract-malt extract agar.

Carbohydrate utilization was investigated by using the procedures of Pridham and Gottlieb (6).

Cell wall analysis was performed by the method of Becker et al. (1).

RESULTS AND DISCUSSION

Strain SF-2103^T is considered to be a member of the genus *Streptomyces* because of its morphology and cell wall composition. The characteristics of strain SF-2103^T were compared with the descriptions of known *Streptomyces* species in *Bergey's Manual of Determinative Bacteriology*, 8th ed. (7), ISP reports by Shirling and Gottlieb (10–13), and the classification key of Nomura (5). No previously described species closely matched strain SF-2103^T with regard to the following criteria: red spore mass (the nearest matching tab from the red color series [15] was 5cb, grayish yellowish pink), straight spore chains, smooth spore surface, non-chromogenicity on ISP media, and carbon utilization characteristics. However, *S. sindenensis*, *S. termitum*, and "*S. alborubidus*" were picked as the species most similar to strain SF-2103^T. Recently, Mikami et al. (4) reported that the cell wall of "*S. alborubidus*" ISP 5465 contains meso-diaminopimelic acid, and we confirmed this. The cultural reactions of *S. sindenensis* ISP 5255 and *S. termitum* ISP 5329 were compared with those of strain SF-2103^T (Tables 1 and 2).

Although *S. sindenensis* ISP 5255, *S. termitum* ISP 5329, and strain SF-2103^T had similar

TABLE 1. Cultural characteristics of strain SF-2103^T, *S. sindenensis*, and *S. termitum* on several agar media

Medium	Culture component ^a	Strain SF-2103 ^T	<i>S. sindenensis</i> ISP 5255	<i>S. termitum</i> ISP 5329
Glycerol-asparagine agar	G	Poor, colorless	Good, old rose	Good, none
	AM	None	Abundant, pearl (3cb) ^b	Abundant, pearl pink (3ca-5ba)
Inorganic salts-starch agar	SP	None	None	None
	G	Moderate, bamboo	Good, old rose	Moderate, none
	AM	Moderate, grayish yellowish pink (5ba)	Abundant, shell pink (5ba)	Scant, white(a)
	SP	Light rose beige	None	None
Oatmeal agar	G	Moderate, bisque	Moderate, none	Moderate, none
	AM	Moderate, grayish yellowish pink (5cb)	Scant, white (a)	Moderate, pearl pink (3ca)
	SP	None	None	None
Yeast extract-malt extract agar	G	Good, bisque	Good, butterscotch	Good, butterscotch
	AM	Moderate, grayish yellowish pink (5cb)	Abundant, grayish yellowish pink (5cb)	Moderate, grayish yellowish pink (5cb-5ba)
	SP	None	None	None
Nutrient agar	G	Poor, colorless	Moderate, none	Moderate none
	AM	None	None	Moderate, pearl pink (3ca)
	SP	None	None	None

^a G, Growth; AM, aerial mycelium; SP, soluble pigment.^b The color designations were taken from reference 2.

aerial mass colors on yeast extract-malt extract agar, the two previously described species differed from strain SF-2103^T in carbon utilization and cultural characteristics on glycerol-asparagine agar and nutrient agar.

On the basis of these observations, we believe that strain SF-2103^T represents a new species of the genus *Streptomyces*, for which we propose the name *Streptomyces sulfonofaciens* (sul.fon.o.fa'ci.ens. N. L. n. *acidum sulfonicum* sulfonic acid; L. part. adj. *faciens* producing; M.

TABLE 2. Characteristics differentiating strain SF-2103^T, *S. sindenensis* ISP 5255, and *S. termitum* ISP 5329^a

Strain	Nitrate reduction	Carbohydrate utilization			
		L-Arabinose	D-Mannitol	L-Rhamnose	Sucrose
SF-2103 ^T	— ^b	+	+	+	+
<i>S. sindenensis</i> ISP 5255	±	+	+	—	—
<i>S. termitum</i> ISP 5329	±	—	—	—	—

^a All three strains hydrolyze starch, liquefy gelatin, peptonize milk, and utilize D-glucose, D-fructose, and D-xylose. None of the strains coagulates milk, produces melanin (ISP medium 7), or utilizes i-inositol or raffinose.

^b —, Negative response; ±, doubtful response; +, positive response.

FIG. 1. Light micrograph of sporulating aerial mycelium of strain SF-2103^T on inorganic salts-starch agar incubated at 28°C for 14 days. Magnification approximately 700.

FIG. 2. Transmission electron micrograph of smooth spores of strain SF-2103^T on inorganic salts-starch agar incubated at 28°C for 14 days. $\times 10,000$.

L. part. adj. *sulfonofaciens* producing sulfonic acid). A description of this new species is given below.

***Streptomyces sulfonofaciens* sp. nov.: morphology.** The aerial mycelium of strain SF-2103^T branches monopodially and forms spore chains, as shown in Fig. 1. Spores are 0.7 to 0.8 by 1.0 to 1.5 μm and oval to cylindrical and have smooth surfaces (Fig. 2 and 3). The spore chains are nearly straight and moderately short, bearing 10 to 30 spores. Fragmentation of mycelium is not observed on agar or in liquid cultures. Neither sclerotia nor sporangia are formed.

Cultural characteristics. The cultural characteristics of strain SF-2103^T are summarized in Table 3. The colors of the mature aerial masses produced on inorganic salts-starch agar, oatmeal

FIG. 3. Scanning electron micrograph of a spore chain of strain SF-2103^T on inorganic salts-starch agar incubated at 28°C for 14 days. $\times 10,000$.

agar, and yeast extract-malt extract agar belong to the red color series (5cb, grayish yellowish pink [15]). The undersides of colonies are colorless to pale yellowish brown. No diffusible pigments are formed, except for a light rose beige pigment formed on inorganic salts-starch agar. Frequent subculturing of strain SF-2103^T onto fresh agar media results in the loss of the ability to produce aerial mycelium, and small aerial mycelium tends to be formed and then lysed.

TABLE 3. Cultural characteristics of strain SF-2103^{Ta}

Medium	Growth	Aerial mycelium
Sucrose-nitrate agar	Scant, colorless	None
Glucose-asparagine agar	Poor, colorless	None
Glycerol-asparagine agar (ISP medium 5)	Poor, colorless	None
Inorganic salts-starch agar (ISP medium 4)	Moderate, bamboo	Moderate, grayish yellowish pink(5cb)
Oatmeal agar (ISP medium 3)	Moderate, bisque	Moderate, grayish yellowish pink(5cb)
Yeast extract-malt extract agar (ISP medium 2)	Good, bisque	Moderate, grayish yellowish pink(5cb)
Tyrosine agar (ISP medium 7)	Poor, colorless	None
Nutrient agar	Poor, colorless	None
Calcium malate agar	Poor, colorless	None
Bennett agar	Good, yellow maple	None

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

The temperature range for growth of strain SF-2103^T is 15 to 42°C. Optimum growth occurs between 27 and 33°C.

Physiological characteristics. Positive physiological properties for strain SF-2103^T include gelatin liquefaction, starch hydrolysis, and milk peptonization. Negative properties include production of melanoid pigment on peptone-yeast extract-iron agar and tryptone-yeast extract broth, milk coagulation, and nitrate reduction.

Carbohydrate utilization. On ISP medium 9, good or moderate growth is obtained with D-glucose, L-arabinose, D-xylose, L-rhamnose, and D-fructose, and poor growth is obtained with D-mannitol and sucrose. No growth is observed with i-inositol or raffinose.

Cell wall analysis. Analysis of the cell wall hydrolysates by paper chromatography demonstrates the presence of LL-diaminopimelic acid in addition to glycine. Thus, strain SF-2103^T can be considered to have cell wall type I of Becker et al. (1).

Antagonistic properties. Produces a new β -lactam antibiotic, SF-2103A.

Habitat: soil.

Type strain: strain SF-2103 (= ATCC 31892).

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