NEW ANTITUMOR ANTIBIOTIC, FR-900462

I. TAXONOMY OF THE PRODUCING STRAIN

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(Received for publication September 17, 1988)

A new species of the genus *Streptomyces*, the proposed name of which is *Streptomyces tokashikiensis* sp. nov., is described. Soil isolate, strain No. 7124, produces a new antitumor antibiotic FR-900462. The organism is characterized by the presence of spores on the substrate hyphae. Strain No. 7124 is closely related to *Streptomyces spiralis* in morphological and cultural characteristics, but there are differences in spore surface, growth-permissible temperature, and carbohydrate utilization pattern. Therefore, it was decided to designate strain No. 7124 as a new species within the genus *Streptomyces*.

In the course of a screening for new antitumor substances, a new antitumor substance was discovered in the fermentation broth of actinomycete strain No. 7124 which was isolated from a soil sample which was collected at Tokashiki-village, Okinawa Prefecture. In this report the taxonomy of strain No. 7124 is presented.

Materials and Methods

Bacterial Strains

Strain No. 7124 was isolated from a soil sample obtained from Tokashiki-village, Okinawa Prefecture, Japan. *Streptomyces brasiliensis* ATCC 23727, *Streptomyces carpinensis* ATCC 27116, and *Streptomyces spiralis* ATCC 25664 were obtained from the American Type Culture Collection (ATCC), U.S.A. *Streptomyces cinereus* IFO 12247, was obtained from the Institute for Fermentation, Osaka (IFO), Japan.

Morphological Characterization

The aerial mycelia of the strain grown on oatmeal agar, yeast extract - malt extract agar, and inorganic salts - starch agar were examined directly under an optical microscope. To observe vegetative mycelial spores, the surface of the yeast extract - malt extract agar was taken out with a glass knife. The specimen was observed on a glass slide under a light microscope. Spore surface examinations were made with a transmission electron microscope (model HU-12 Hitachi) and a scanning electron microscope (model S-530 Hitachi).

Cultural and Physiological Characterizations

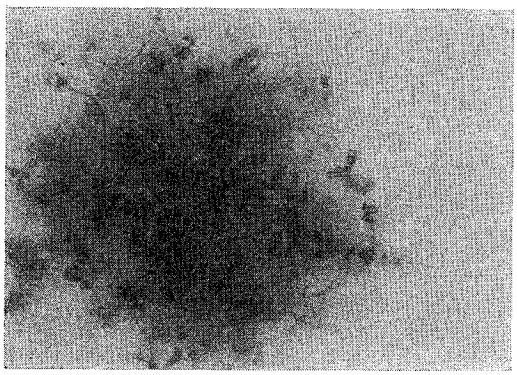
Cultural and physiological characteristics were observed on media as described by WAKSMAN[1] and in the International Streptomyces Project (ISP) report[2]. Color nomenclature in this study was based on the Color Standard (Nihon Shikisai Co., Ltd.). Utilization of carbohydrates was determined by the method of PRIDHAM and GOTTLIEB[3]. The cultures were incubated for 14 days at 30°C.

Growth-permissible temperature range and optimum growth temperature were determined on yeast extract - malt extract agar using a model TN-3 temperature gradient incubator (Toyo Kagaku Sangyo Co., Ltd.).

Cell Wall Analysis

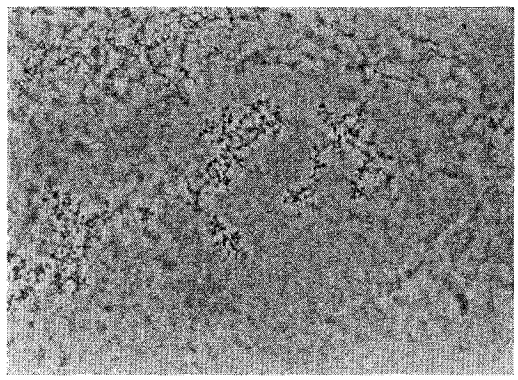
Cell wall analysis was performed according to the method of BECKER *et al.*[4]. Cell wall preparation was obtained by the method of YAMAGUCHI[5].

Fig. 1. Sporophores of strain No. 7124.



Sporophores of strain No. 7124 were observed on aerial mycelium (cultivated on yeast extract - malt extract agar for 10 days at 30°C). Magnification, ×800.

Fig. 2. Vegetative mycelial spores of strain No. 7124.



Within yeast extract - malt extract agar (cultivated for 10 days at 30°C). Magnification, ×800.

Fig. 3. Electron micrographs of spore chains of strain No. 7124.

(A) Transmission electron micrograph of strain No. 7124 spores (10 days culture on yeast extract - malt extract agar).

(B) Scanning electron micrographs of strain No. 7124 spores (10 days culture on yeast extract - malt extract agar).

(A)

(B)

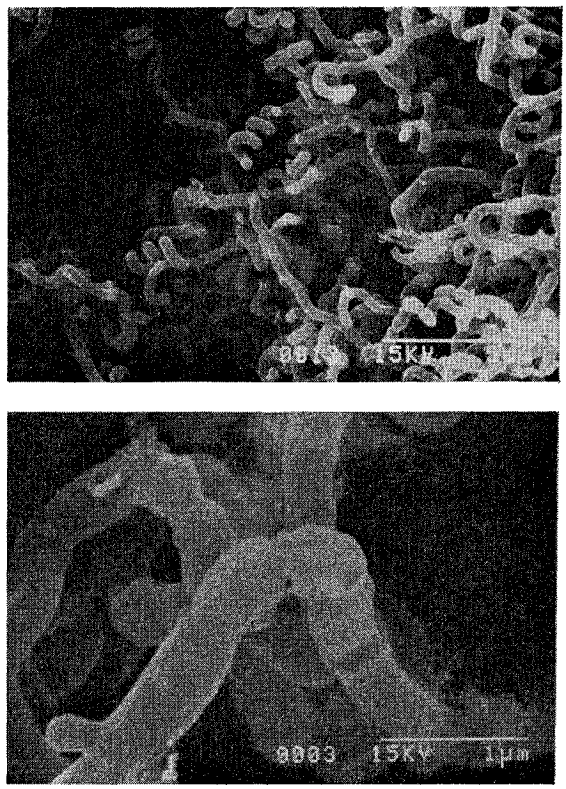


Table 1. Cultural characteristics of strain No. 7124, *Streptomyces brasiliensis* ATCC 23727, *Streptomyces carpinensis* ATCC 27116, and *Streptomyces spiralis* ATCC 25664.

Medium		No. 7124	ATCC 23727	ATCC 27116	ATCC 25664
Oatmeal agar	G:	Moderate	Poor	Moderate	Moderate
	A:	Pale brown	Light gray	Grayish yellow brown	Light gray
	R:	Pale yellowish brown	Colorless	Grayish black	Pale yellow
	S:	None	None	None	None
Yeast extract - malt extract agar	G:	Abundant	Abundant	Abundant	Abundant
	A:	Light gray	None	Grayish pink	Grayish white
	R:	Dark gray	Pale yellow orange	Pale yellow	Light reddish yellow
	S:	Brown	None	None	Brown
Inorganic salts - starch agar	G:	Abundant	Moderate	Moderate	Moderate
	A:	Pale cinnamon pink to gray	Light gray	Grayish yellow brown	Grayish white
	R:	Pale yellowish brown	Yellowish gray	Dark brown	Pale yellowish brown
	S:	None	None	None	None
Glucose - asparagine agar	G:	Abundant	Moderate	Moderate	Moderate
	A:	Pale cinnamon pink	Light gray	Grayish yellow brown	Grayish white
	R:	Brown	Yellowish white	Grayish black	Pale yellow
	S:	Pale yellow orange	None	None	Pale yellow
Glycerol - asparagine agar	G:	Abundant	Moderate	Moderate	Moderate
	A:	Pale yellow orange	Grayish white	Grayish yellow brown	Grayish white
	R:	Brown	Pale yellow	Pale reddish brown	Pale yellow
	S:	Pale yellow orange	None	None	Pale yellow
Sucrose - nitrate agar	G:	Abundant	Poor	Poor	Moderate
	A:	Grayish white	None	Pale reddish brown	Grayish white
	R:	Yellowish brown	Colorless	Pale yellow	Dark orange
	S:	Pale yellow orange	None	None	Light orange
Nutrient agar	G:	Poor	Moderate	Moderate	Moderate
	A:	Grayish white	None	None	None
	R:	Pale yellowish brown	Pale yellow	Colorless	Pale yellow
	S:	Pale yellow orange	None	None	None
Potato - glucose agar	G:	Abundant	Moderate	Moderate	Moderate
	A:	Light gray	Grayish white	Pinkish gray	Grayish white
	R:	Brown	Pale yellow orange	Pale yellow	Grayish brown
	S:	Pale yellow orange	None	None	None
Tyrosine agar	G:	Abundant	Abundant	Abundant	Abundant
	A:	Grayish white	Light gray	Grayish yeollw brown	Yellowish gray
	R:	Brown	Brown	Raw amber	Dark orange
	S:	Pale yellow orange	None	None	None
Peptone - yeast extract - iron agar	G:	Moderate	Moderate	Moderate	Moderate
	A:	Grayish white	None	None	None
	R:	Pale yellowish brown	Pale yellow	Pale yellow	Pale yellow
	S:	Pale yellow orange	None	None	None

Abbreviations: G, Growth; A, aerial mass color; R, reverse side color; S, soluble pigment.

Results

Morphological Characteristics

The mature sporophores were moderately short spirals consisting of about 10 to 20 spores (Fig. 1). The substrate hyphae bore single or short chain of spores within agar media (Fig. 2). Aerial mycelial spores were cylindrical, oval or reniform, $0.4\sim0.5\times0.7\sim0.9$ μm in size with warty or spiny surfaces (Fig. 3). No fragmentation of vegetative mycelia, zoospores, or synnemata was observed.

Cultural Characteristics

Cultural characteristics of strain No. 7124, observed on various media, are given in Table 1.

Table 2. Physiological properties of strain No. 7124, *Streptomyces brasiliensis* ATCC 23727, *Streptomyces carpinensis* ATCC 27116, and *Streptomyces spiralis* ATCC 25664.

	No. 7124	ATCC 23727	ATCC 27116	ATCC 25664
Temperature range for growth (°C)	18~34	15~38	18.5~42	14~45
Optimum temperature (°C)	23~29	29~31	28~32	26~31
Nitrate reduction	−	+	−	−
Starch hydrolysis	+	+	+	+
Milk coagulation	−	+	−	−
Milk peptonization	+	+	+	+
Melanin production	−	−	−	−
Gelatin liquefaction	+	+	+	+
H_2S production	−	−	−	−
Urease activity	−	−	−	−
NaCl tolerance (%)	≦7	≦5	≦7	≦7

Symbols: +, Positive; −, negative.

Table 3. Carbon sources utilization of strain No. 7124, *Streptomyces brasiliensis* ATCC 23727, *Streptomyces carpinensis* ATCC 27116, and *Streptomyces spiralis* ATCC 25664.

	No. 7124	ATCC 23727	ATCC 27116	ATCC 25664
D-Glucose	+	+	+	+
Sucrose	+	−	−	+
Glycerol	+	+	+	+
D-Xylose	−	+	+	+
D-Fructose	±	+	+	+
Lactose	−	+	+	+
Maltose	+	+	+	+
Rhamnose	−	+	+	+
Raffinose	±	+	+	+
D-Galactose	±	+	+	+
L-Arabinose	−	+	+	+
D-Mannose	+	+	+	+
D-Trehalose	+	+	+	+
Inositol	+	+	+	+
Mannitol	+	+	+	+
Inulin	+	−	+	+
Cellulose	−	−	−	−
Salicin	−	−	+	−
Chitin	−	−	−	−
Sodium citrate	+	+	+	+
Sodium succinate	−	+	+	+
Sodium acetate	−	−	−	−

Symbols: +, Utilization; ±, doubtful utilization, −, no utilization.

Aerial mycelia formed on oatmeal agar, yeast extract - malt extract agar, or inorganic salts - starch agar corresponded to the gray or red color series. Soluble pigment was produced in yeast extract - malt extract agar and other media.

Physiological Characteristics

Physiological characteristics of strain No. 7124 are summarized in Table 2. The temperature range for growth was from 18 to 34°C with an optimum at 26°C. Starch hydrolysis, milk peptonization and gelatin liquefaction were positive.

Carbohydrate utilization of the strain is summarized in Table 3. D-Xylose, lactose, rhamnose, L-arabinose, cellulose, salicin, chitin, sodium succinate, and sodium acetate were not utilized by the strain. The other carbohydrates examined were either utilized or poorly utilized.

Cell Wall Analysis

Analysis of cell wall hydrolysates showed the presence of LL-diaminopimelic acid. Accordingly, it was concluded that the cell wall type of this strain was Type I.

Discussion

The substrate hyphae of strain No. 7124 bore mainly single or sometimes short chains of spores within the agar media. The mature sporophores of aerial mycelia formed spirals consisting of 10 to 20 spores. The cell wall of the strain was of Type I. No fragmentation of vegetative mycelia, zoospores, or synnemata was observed. The morphological characteristics and cell wall type of strain No. 7124 were similar to those of the genus *Streptomyces*. The morphology of strain No. 7124 was compared with those of *S. brasiliensis*[6], *S. carpinensis*[6], *S. spiralis*[6], and *S. cinereus*[7].

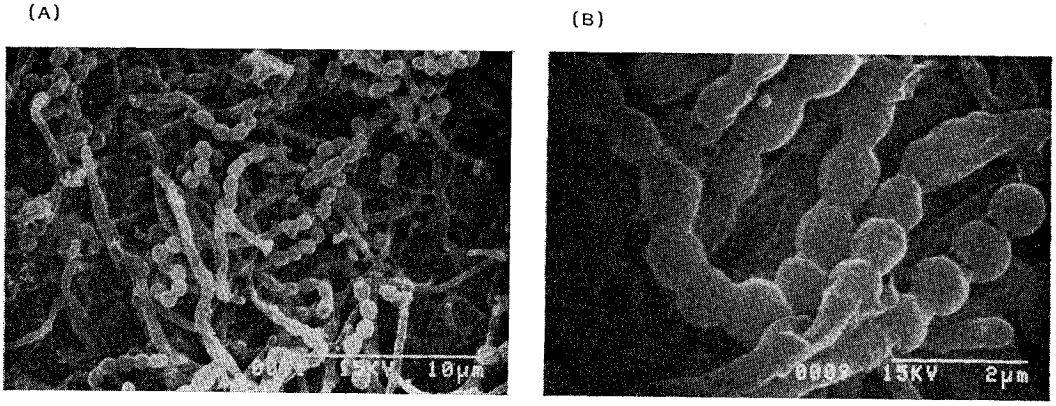
The substrate mycelial spores of strain No. 7124 were morphologically different from those of *S. cinereus*, but in good agreement with those of *S. brasiliensis*, *S. carpinensis*, and *S. spiralis*.

Morphological, cultural, physiological, and carbohydrates utilization patterns of strain No. 7124 were directly compared with those of *S. brasiliensis*, *S. carpinensis*, and *S. spiralis* (see Tables 1~3). From the results of the above comparison, strain No. 7124 was closely related to *S. spiralis*. However, strain No. 7124 was different from *S. spiralis* in the following points.

Morphological Characteristics

Spore surfaces of spores in sporophores arising from the aerial mycelia of strain No. 7124 were rugose (Fig. 3), while those of *S. spiralis* were smooth (Fig. 4). The sporophores of strain No. 7124

Fig. 4. Scanning electron micrographs of *Streptomyces spiralis* spores (10 days culture on yeast extract - malt extract agar).



formed compact spirals, whereas the sporophores of *S. spiralis* formed loose spirals.

Cultural Characteristics

The reverse color of strain No. 7124 was brown on various media, while that of *S. spiralis* was pale yellow. Strain No. 7124 produced soluble pigment in nutrient agar, potato - glucose agar, tyrosine agar, and peptone - yeast extract - iron agar, but *S. spiralis* did not produce a soluble pigment in these media.

Physiological Characteristics

The growth-permissible temperature range of strain No. 7124 was from 18 to 34°C, while that of *S. spiralis* was from 14 to 45°C. Differences were also observed in the utilization of D-xylose, lactose, rhamnose, L-arabinose, and sodium succinate.

The results of the above comparison indicated that strain No. 7124 was a different species from *S. spiralis*. Therefore, it seemed appropriate to consider that strain No. 7124 was a new species in the genus *Streptomyces* and to designate it as *Streptomyces tokashikiensis* sp. nov., referring to the fact that the strain was isolated from a soil sample collected at Tokashiki-village, Okinawa Prefecture.

A culture of this strain has been deposited at the Fermentation Research Institute, Agency of Industrial Science and Technology, Japan, assigned as *Streptomyces tokashikiensis* No. 7124 with an accession No. FERM-P 7393. The description given above for the type strain also serves for the present as the species description.

References

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