

A NEW ANTITUMOR ANTIBIOTIC, FR-900482

I. TAXONOMIC STUDIES ON THE PRODUCING STRAIN:
A NEW SPECIES OF THE GENUS *STREPTOMYCES*MORITA IWAMI, SUMIO KIYOTO, HIROSHI TERANO, MASANOBU KOHSAKA,
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A new species in the genus *Streptomyces*, for which the name *Streptomyces sandaensis* sp. nov. is proposed, is described. Soil isolate strain No. 6897 (FERM-P 7654), produces a new antitumor substance FR-900482. The strain has a gray to red spore mass color, *Rectus Flexibilis* to *Retinaculum-Apertum* spore chain morphology, smooth spores, and is nonchromogenic. Strain No. 6897 is closely related to *Streptomyces aburaviensis* and *Streptomyces nitrosporeus*. But, there are many differences in cultural characteristics and in the utilization of carbohydrates.

During the course of a screening for new antitumor substances, FR-900482 was discovered in the fermentation broth of an actinomycete, strain No. 6897 which was isolated from a soil sample. This report deals with the description of the producing organism and its taxonomic position.

Materials and Methods

Bacterial Strain

Strain No. 6897 was isolated from a soil sample obtained from Sanda-shi, Hyogo Prefecture. *Streptomyces aburaviensis* IFO 12830 and *Streptomyces nitrosporeus* IFO 12803 were obtained from the Institute for Fermentation, Osaka (IFO), Japan.

Morphological Characterization

The aerial mycelia of the organism grown on oatmeal agar, yeast extract - malt extract agar and inorganic salts - starch agar were examined directly under a microscope (equipped with long working distance objectives). Spore surface was examined using transmission electron microscope.

Cultural and Physiological Characterizations

Cultural and physiological characterizations were performed using media recommended by WAKSMAN¹⁾ and the International Streptomyces Project (ISP)²⁾. Color names used in this study were based on the Color Standard (Nihon Shikisai Co., Ltd.). Utilization of carbohydrates was determined by the method of PRIDHAM and GOTTLIEB³⁾. 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 YAMAGUCHI⁵⁾.

Results

Morphological Characteristics

The mature spore chains showed *Rectus Flexibilis* to *Retinaculum-Apertum* type morphology (Fig. 1) and consisted of about 10 to 20 spores in each chain. The spores were short-cylindrical, and $0.5 \sim 0.7 \times 0.6 \sim 0.8 \mu\text{m}$ in size. The spore surface was smooth (Fig. 2). Fragmentation of substrate mycelium was not observed (agar media or submerged culture). Sporangia or zoospores were also not observed.

Cultural Characteristics

Cultural characteristics of strain No. 6897 studied on various media are summarized in Table 1. Aerial mycelia formed on oatmeal agar, yeast extract - malt extract agar, or inorganic salts - starch agar were in the gray or red color series. No characteristic reverse side color was observed. No soluble pigment was observed except in the yeast extract - malt extract agar.

Physiological Characteristics

Physiological characteristics of strain No. 6897 are summarized in Table 2. The growth permissible temperature ranged from 10°C to 35°C . The optimum growth temperature was from 30°C to 32°C . No melanoid pigment was produced in tyrosine agar, peptone - yeast extract - iron agar or Tryptone - yeast extract broth. Starch hydrolysis, milk peptonization, and gelatin liquefaction were positive. The pattern of carbohydrate utilization is shown in Table 3.

Cell Wall Analysis

Strain No. 6897 contained LL-diaminopimelic acid and glycine in the cell wall. According to the chemical composition, cell wall type of this strain is type I⁽⁶⁾.

Discussion

The above mentioned morphological characteristics and the cell wall type indicate that strain No. 6897 belongs to the genus *Streptomyces*. Furthermore, from these results, the organism was assigned to the gray (Gy) or red (R), *Rectus Flexibilis* (RF) and *Retinaculum-Apertum* (RA), nonchromogenic (C^{-}), and glabrous (SM) groups as given in BERGEY's manual of determinative bacteriology, 8th Ed.⁽⁷⁾

Fig. 1. Aerial mycelium of strain No. 6897 on yeast extract - malt extract agar (incubated for 14 days at 30°C).

The organism was observed with an optical microscope ($\times 400$).

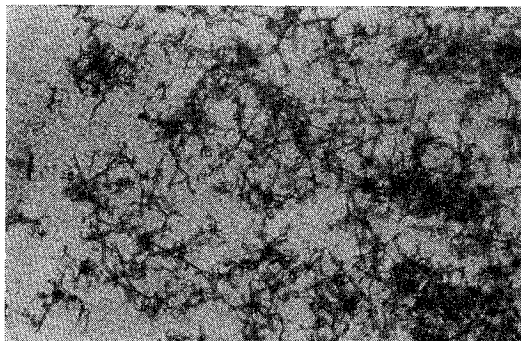


Fig. 2. Electron micrograph of spore chain of strain No. 6897 on yeast extract - malt extract agar, 10 days culture.

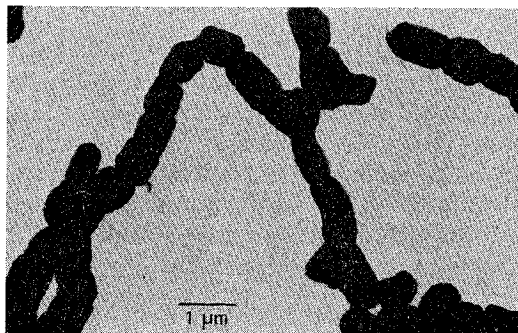


Table 1. Cultural characteristics of strain No. 6897, *Streptomyces aburaviensis* IFO 12830 and *Streptomyces nitrosporeus* IFO 12803.

Medium	No. 6897	IFO 12830	IFO 12803
Oatmeal agar	G: Poor	Moderate	Poor
	A: Grayish yellow brown	Pale reddish brown	Grayish white, poor
	R: Pale yellow	Colorless	Colorless
	S: None	None	None
Yeast extract - malt extract agar	G: Moderate	Abundant	Moderate
	A: Light gray	Grayish yellow brown	Grayish yellow brown
	R: Dark yellow orange	Yellowish brown	Brown
	S: Pale brown	Pale brown	Brown
Inorganic salts - starch agar	G: Moderate	Moderate	Moderate
	A: Grayish yellow brown	Grayish yellow brown	Olive gray
	R: Pale yellow orange	Pale reddish brown	Pale yellow orange
	S: None	None	None
Glucose - asparagine agar	G: Poor	Moderate	Moderate
	A: Pale yellow orange	Gray	Light gray
	R: Pale yellow orange	Dark gray	Pale yellowish brown
	S: None	None	Pale yellow
Glycerol - asparagine agar	G: Abundant	Moderate	Moderate
	A: Grayish yellow brown	Light gray	White
	R: Dark yellowish brown	Yellow orange	Pale yellow orange
	S: None	Pale brown	None
Sucrose - nitrate agar	G: Poor	Poor	Poor
	A: Light gray	None	Grayish white
	R: Colorless	Colorless	Colorless
	S: None	None	None
Nutrient agar	G: Moderate	Poor	Poor
	A: Grayish white	Light gray	Grayish white
	R: Pale yellow	Pale yellow	Colorless
	S: None	None	None
Potato - glucose agar	G: Moderate	Moderate	Moderate
	A: Olive gray	Pale reddish brown	Olive gray
	R: Pale yellow orange	Pale yellow orange	Pale yellow orange
	S: None	None	None
Tyrosine agar	G: Abundant	Abundant	Moderate
	A: Grayish yellow brown	Grayish yellow brown	Gray
	R: Pale yellowish brown to dark brown	Yellowish brown	Grayish yellow brown
	S: None	None	None
Peptone - yeast extract - iron agar	G: Poor	Poor	Moderate
	A: None	None	None
	R: Colorless	Colorless	Colorless
	S: None	None	Pale brown

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

and to the gray (Gy), melanoid pigment (none, O), reverse side pigment (not characteristics, O), *Rectus Flexibilis* (RF) and *Retinaculum-Apertum* (RA), and smooth spore surface (sm) groups in NONOMURA's key⁸⁾. Cultural and physiological characteristics, and carbohydrates utilization pattern of strain No. 6897 were compared with those of various species^{7,9~11)} selected for comparative studies. Strain No. 6897 was closed to *Streptomyces aburaviensis* NISHIMURA *et al.* 1957 and *Streptomyces nitrosporeus* OKAMI 1952, although there were some differences in cultural and physiological characteristics, and carbohydrates utilization (see Tables 1~3).

Cultural characteristics of strain No. 6897 were different from those of *S. aburaviensis* on glucose - asparagine agar, sucrose - nitrate agar and potato - glucose agar. Gelatin liquefaction and NaCl

Table 2. Physiological properties of strain No. 6897, *Streptomyces aburaviensis* IFO 12830 and *Streptomyces nitrosporeus* IFO 12803.

	No. 6897	IFO 12830	IFO 12803
Temperature range for growth	10~35°C	NT	NT
Optimum temperature	30~32°C	NT	NT
Nitrate reduction	Negative	Negative	Positive
Starch hydrolysis	Positive	Positive	Positive
Milk coagulation	Negative	Negative	Negative
Milk peptonization	Positive	Positive	Positive
Melanin production	Negative	Negative	Negative
Gelatin liquefaction	Positive	Negative	Positive
H ₂ S production	Negative	Negative	Negative
Urease activity	Negative	Negative	Positive
NaCl tolerance (%)	>5~<7	>3~<5	>7~<10

NT: Not tested.

Table 3. Carbon utilizations of strain No. 6897, *Streptomyces aburaviensis* IFO 12830 and *Streptomyces nitrosporeus* IFO 12803.

	No. 6897	IFO 12830	IFO 12803		No. 6897	IFO 12830	IFO 12803
D-Glucose	+	+	+	D-Mannose	+	-	+
Sucrose	-	-	-	D-Trehalose	±	±	+
Glycerol	+	+	+	Inositol	-	-	-
D-Xylose	+	-	+	Mannitol	-	-	-
D-Fructose	-	-	-	Inulin	-	-	-
Lactose	-	-	+	Cellulose	-	-	-
Maltose	+	+	+	Salicin	+	-	+
Rhamnose	+	-	+	Chitin	-	-	-
Raffinose	+	-	-	Sodium citrate	-	-	+
D-Galactose	+	-	+	Sodium succinate	+	+	+
L-Arabinose	+	-	+	Sodium acetate	-	+	-

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

tolerance of strain No. 6897 were different from those of *S. aburaviensis*. Differences were also observed in the utilization of D-xylose, rhamnose, D-galactose, L-arabinose, D-mannose, raffinose, salicin, and sodium acetate.

Cultural characteristics of strain No. 6897 were different from those of *S. nitrosporeus* on oatmeal agar, yeast extract - malt extract agar, glucose - asparagine agar, glycerol - asparagine agar, potato - glucose agar, and tyrosine agar. Nitrate reduction, urease activity, NaCl tolerance properties of strain No. 6897 were different from those of *S. nitrosporeus*. Differences were also observed in the utilization of lactose, sodium citrate, and raffinose.

Considering results of comparative studies of strain No. 6897 with the type cultures of the species described above, it seems appropriate to consider that strain No. 6897 is a new species within the genus *Streptomyces*, for which the name *Streptomyces sandaensis* sp. nov., referring to the soil obtained at Sanda-shi from which the organism was isolated.

Type strain: Strain No. 6897. A culture of the strain has been deposited at the Fermentation Research Institute, Agency of Industrial Science and Technology, Japan and assigned as *Streptomyces sandaensis* No. 6897 with the accession number FERM-P 7654. The description given above for the type strain also serves for the present as the species description.

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