FR-900520 AND FR-900523, NOVEL IMMUNOSUPPRESSANTS ISOLATED FROM A *STREPTOMYCES*

I. TAXONOMY OF THE PRODUCING STRAIN

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A new subspecies of the genus *Streptomyces*, the proposed name of which is *Streptomyces hygroscopicus* subsp. *yakushimaensis* subsp. nov., is described. Soil isolate, strain No. 7238, produces the novel immunosuppressants, FR-900520 and FR-900523. The organism is characterized by its gray aerial mycelium color, hygroscopic spore mass and spiral spore chains with warty or spiny spore surfaces. It is nonchromogenic. Strain No. 7238 shows characteristics most closely related to *Streptomyces antimycoticus* and *S. hygroscopicus*, although there are differences in physiological characteristics and carbohydrate utilization. In terms of morphological characteristics, strain No. 7238 is different from *S. antimycoticus*, but resembles *S. hygroscopicus*. The differences are not sufficient to establish a new species. It would be most suitable to designate strain No. 7238 as a new subspecies within the species of *S. hygroscopicus*.

In the course of a screening program for novel efficient immunosuppressants, FR-900520 and FR-900523 were discovered in a cultured broth of an actinomycete, strain No. 7238 which had been isolated from a soil sample. This paper describes the description of the producing organism and discusses its taxonomy.

Materials and Methods

Bacterial Strain

Strain No. 7238 was isolated from a soil sample collected at Yaku-shima, Kagoshima Prefecture, Japan. *Streptomyces antimycoticus* IFO 12893, and *Streptomyces hygroscopicus* subsp. *glebosus* IFO 13786 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 an optical microscope. Spore surface examinations were observed 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 obtained on media as described by WAKSMAN[1] and in the International Streptomyces Project (ISP) reports[2]. Color nomenclature in this study was based on the Color Standard of 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.).

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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].

Results

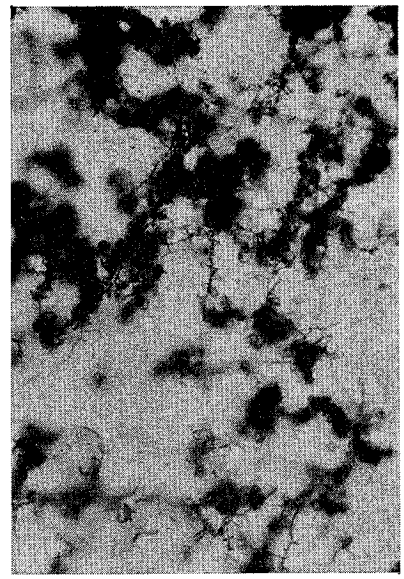
Morphological Characteristics

The mature sporophores were moderately short consisting of about 20 spores in each chain and formed *Retinaculiaperti* and spirales spore chains (Fig. 1). Hygroscopic spore masses were observed in the aerial mycelium on oatmeal agar and inorganic salts - starch agar. Spore surfaces were intermediate between very short, thick spines and watrs (Figs. 2 and 3). No fragmentation of vegetative mycelia, sporangia, zoospores, vegetative mycelium spores, or synnemata was observed.

Cultural Characteristics

Cultural characteristics of strain No. 7238, studied with various media, are shown in Table 1. Aerial mycelia formed on oatmeal agar, yeast extract - malt extract agar, or inorganic salts - starch agar corresponded to the gray color series.

Fig. 1. Micrograph of aerial mycelium of strain No. 7238 on yeast extract - malt extract agar.



The organism was incubated for 14 days at 30°C and observed with an optical microscope (×400).

Fig. 2. Transmission electron micrograph of spore chains of strain No. 7238 on yeast extract - malt extract agar, 10 days culture.

Scale: 1 μm.

Fig. 3. Scanning electron micrograph of spore chains of strain No. 7238 on yeast extract - malt extract agar, 4 days culture.

Scale: 2 μm.

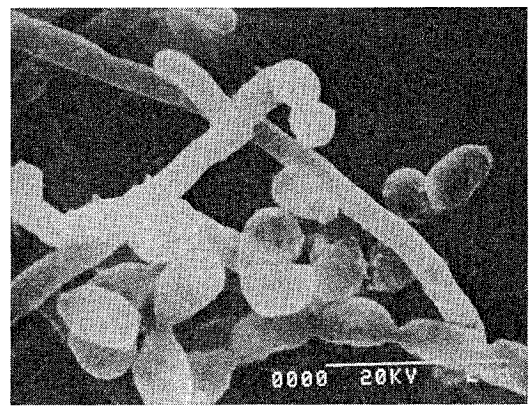


Table 1. Cultural characteristics of strain No. 7238, *Streptomyces antimycoticus* IFO 12839 and *Streptomyces hygroscopicus* subsp. *glebosus* IFO 13786.

Medium		No. 7238	IFO 12839	IFO 13786
Oatmeal agar	G:	Poor	Poor	Poor
	A:	Grayish yellow brown	Grayish yellow brown	Grayish yellow brown
	R:	Pale yellow	Pale yellow	Pale yellow
	S:	None	None	None
Yeast extract - malt extract agar	G:	Moderate	Abundant	Moderate
	A:	Grayish white	Gray	Gray
	R:	Pale yellowish brown	Pale yellowish brown	Dark orange
	S:	None	None	None
Inorganic salts - starch agar	G:	Moderate	Moderate	Moderate
	A:	Gray to black	Gray	Light gray
	R:	Pale yellow orange	Yellowish gray	Pale yellow orange
	S:	None	None	None
Glucose - asparagine agar	G:	Moderate	Moderate	Moderate
	A:	Grayish white	Gray	White
	R:	Pale yellow orange	Pale yellow orange	Pale yellow orange
	S:	None	None	None
Glycerol - asparagine agar	G:	Moderate	Moderate	Moderate
	A:	White	Gray	Light gray
	R:	Yellowish gray	Yellowish gray	Grayish yellow brown
	S:	None	None	None
CZAPEK's agar	G:	Moderate	Moderate	Moderate
	A:	Grayish white	Grayish white	White
	R:	Pale yellowish brown	Pale yellowish brown	Pale yellowish brown
	S:	None	None	None
Nutrient agar	G:	Moderate	Moderate	Moderate
	A:	Grayish white	Grayish white	None
	R:	Pale yellow	Pale yellow	Pale yellow
	S:	None	None	None
Potato - glucose agar	G:	Moderate	Moderate	Moderate
	A:	White, poor	Pale reddish brown	Pale pink to white
	R:	Pale yellow orange	Pale yellow orange	Pale yellowish brown
	S:	None	None	None
Tyrosine agar	G:	Moderate	Moderate	Moderate
	A:	White	Grayish white	Gray to black
	R:	Pale yellowish brown to brown	Brown	Pale yellowish brown
	S:	None	Brown	None
Peptone - yeast extract - iron agar	G:	Moderate	Moderate	Moderate
	A:	None	Grayish white	None
	R:	Pale yellow	Pale yellow	Colorless
	S:	None	None	None

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

No soluble pigment was produced.

Physiological Characteristics

Physiological characteristics of strain No. 7238 are summarised in Table 2. The temperature range for growth was from 18 to 36°C with optimum temperature at 28°C. No melanoid pigment was produced in tyrosine agar, peptone - yeast extract - iron agar, or Tryptone - yeast extract broth. Starch hydrolysis and gelatin liquefaction were positive. The strain did not grow on yeast extract - malt ex-

Table 2. Physiological properties of strain No. 7238, *Streptomyces antimycoticus* IFO 12839 and *Streptomyces hygroscopicus* subsp. *glebosus* IFO 13786.

	No. 7238	IFO 12839	IFO 13786
Temperature range for growth (°C)	18~36	18~38	16~35
Optimum temperature (°C)	28	28	27
Nitrate reduction	Negative	Negative	Negative
Starch hydrolysis	Positive	Positive	Positive
Milk coagulation	Negative	Negative	Negative
Milk peptonization	Negative	Negative	Positive
Melanin production	Negative	Negative	Negative
Gelatin liquefaction	Positive	Positive	Positive
H_2S production	Negative	Negative	Negative
Urease activity	Negative	Negative	Negative
NaCl tolerance (%)	7~10	7~10	5~7

Table 3. Carbohydrate utilization of strain No. 7238, *Streptomyces antimycoticus* IFO 12839 and *Streptomyces hygroscopicus* subsp. *glebosus* IFO 13786.

	No. 7238	IFO 12839	IFO 13786		No. 7238	IFO 12839	IFO 13786
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.

tract agar supplemented with 10% NaCl. Carbohydrate utilization of the strain is summarised in Table 3. D-Glucose, sucrose, lactose, maltose, D-trehalose, inositol, inulin, and salicin were utilized by the strain. Ability to utilize cellulose and chitin was doubtful.

Cell Wall Analysis

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

Discussion

Morphological characteristics and cell wall composition analysis of strain No. 7238 indicated that the strain could be classified as a member of the genus *Streptomyces*. Furthermore, from the above-mentioned results, the strain was assigned to the gray (Gy), spiral (S), nonchromogenic (C^-) and Verrucose (WTY) or Echinulate (SPY) groups as given in BERGEY's Manual of Determinative Bacteriology. 8th Ed.[6], and to the gray (Gy), melanoid pigment (not produced, O), reverse side pigment (not distinctive, O), *Retinaculiaperti* (RA) or *Spira* (S) and warty (wa) or spiny (sp) spore surface groups in NONOMURA's key[7].

Cultural and physiological characteristics and carbohydrate utilization patterns of strain No. 7238 were compared with those of various species described in literature[6,8~10]. Strain No. 7238

was similar to *S. antimycoticus* WAKSMAN 1957 and *S. hygroscopicus* WAKSMAN and HENRICI. Furthermore, morphological characteristics of strain No. 7238 were compared with *S. hygroscopicus* subspecies (*S. hygroscopicus* subsp. *angustmyceticus* IFO 3934, subsp. *decoyicus* IFO 13977, subsp. *glebosus* IFO 13786, subsp. *hygroscopicus* IFO 13472 and subsp. *ossamyceticus* IFO 13983) on yeast extract - malt extract agar, oatmeal agar, inorganic salts - starch agar, glucose - asparagine agar, glycerol - asparagine agar and CZAPEK's agar. Morphological characteristics of strain No. 7238 showed most similarity to those of *S. hygroscopicus* subsp. *glebosus* among the five subspecies grown on the six media. Accordingly, cultural and physiological characteristics, and carbohydrate utilization of strain No. 7238 were compared with type strains of *S. antimycoticus* IFO 12839 and *S. hygroscopicus* subsp. *glebosus* IFO 13786. As shown in Tables 1 to 3, there were many differences between strain No. 7238 and the two strains as indicated in the following.

Streptomyces antimycoticus IFO 12839

Cultural characteristics of strain No. 7238 were different from those of *S. antimycoticus* on yeast extract - malt extract agar, glucose - asparagiol agar, glycerol - asparagine agar, potato - glucose agar and tyrosine agar. Differences were also observed in the carbon utilization of maltose, glycerol, D-fructose, rhamnose, raffinose, D-galactose, D-mannose, mannitol and sodium succinate.

Streptomyces hygroscopicus subsp. *glebosus* IFO 13786

Cultural characteristics of strain No. 7238 were different from those of *S. hygroscopicus* subsp. *glebosus* on yeast extract - malt extract agar, potato - glucose agar and tyrosine agar. Milk peptonization of strain No. 7238 was negative as opposed to that of *S. hygroscopicus* subsp. *glebosus*. Strain No. 7238 grew in the presence of 7% NaCl, but *S. hygroscopicus* subsp. *glebosus* would not. In carbon utilization, strain No. 7238 utilized lactose, inulin and salicin, but *S. hygroscopicus* subsp. *glebosus* did not. Strain No. 7238 did not utilize glycerol, D-xylose, D-fructose, raffinose, D-galactose, D-mannose, mannitol and sodium succinate, but *S. hygroscopicus* subsp. *glebosus* did.

The results of the above comparison indicated that strain No. 7238 was different from the two related strains. However, the formation of hygroscopic spore mass in the aerial mycelia on oatmeal agar and inorganic salts - starch agar, and further morphological and cultural characteristics of strain No. 7238 showed similarity to those of *S. hygroscopicus*. Therefore, strain No. 7238 was regarded as belonging to *S. hygroscopicus*, although it was different from *S. hygroscopicus* subsp. *glebosus* which was most similar to strain No. 7238 in *S. hygroscopicus* subspecies. From the above-mentioned characteristics, it seemed appropriate to consider that strain No. 7238 was a new subspecies of *S. hygroscopicus* and to designate it as *Streptomyces hygroscopicus* subsp. *yakushimaensis* subsp. nov., referring to the fact that the organism was isolated from a soil sample collected at Yakushima. A culture of this strain has been deposited at the Fermentation Research Institute, Agency of Industrial Science and Technology, Japan, assigned as *Streptomyces hygroscopicus* subsp. *yakushimaensis* No. 7238 with an accession No. FERM BP-928. The description given above for the type strain also serves for the present as the subspecies description.

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