NEW ANTITUMOR ANTIBIOTICS, FR-900405 AND FR-900406

I. TAXONOMY OF THE PRODUCING STRAIN

MORITA IWAMI, SUMIO KIYOTO, MOTOAKI NISHIKAWA, HIROSHI TERANO, MASANOBU KOHSAKA, HATSUO AOKI and HIROSHI IMANAKA†

Exploratory Research Laboratories, †Planning and Development Group, Fujisawa Pharmaceutical Co., Ltd. 2-1-6 Kashima, Yodogawa-ku, Osaka, 532 Japan

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An actinomycete which was isolated from a soil sample produces new antitumor substances. The morphological and cultural characteristics of the strain resemble those of the genera *Streptomyces* Waksman and Henrici 1943 and *Actinomadura* Lechevalier and Lechevalier 1970. Cell wall composition analysis showed that strain No. 6049 contained *meso*-2,6-diaminopimelic acid in its cell wall, and madurose in whole-cell sugars. No sporangia, zoospores or fragmentations of vegetative mycelium are observed. From these results, strain No. 6049 is designated as *Actinomadura pulveracea* sp. nov.

In the course of a screening for new antitumor substance, FR-900405 and FR-900406 were discovered in the culture broth of strain No. 6049. The culture was isolated from a soil sample obtained in Wakayama city, Wakayama Prefecture, Japan. This report contains the description of the microorganism and a discussion of its taxonomic position.

Materials and Methods

Morphological Characterization

The aerial and vegetative mycelium of the organism grown on yeast extract - malt extract agar, oatmeal agar or inorganic salts - starch agar were examined directly under an optical microscope. To observe fragmentation or vegetative mycelium spore, the surface of the medium was taken out with a glass knife. This specimen was observed on a glass slide under an optical microscope.

Cultural and Physiological Characterization

For cultural and physiological characterizations, certain media recommended by WAKSMAN[1] and the ISP media[2] were used. Cultures were incubated for 21 days at 30°C. The color names used in this study were based on Color Standard (Nihon Shikisai, Co., Ltd.). The ability to utilize carbohydrates was determined by the method of PRIDHAM and GOTTLIEB[3].

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 Analysis

The strain was cultured in YM medium (glucose 1%, yeast extract 0.5%, peptone 0.5%, adjusted to pH 7.0 by 0.1 N NaOH) incubated on a rotary shaker (300 rpm) at 30°C. The mycelium grown in YM medium was harvested by centrifugation (6,000×*g*, 20 minutes), and washed twice with distilled water. The mycelium was further washed with ethanol, followed by drying at room temperature. The dried mycelium was used as a whole-cell preparation. Whole cell was analyzed by the procedure of LECHEVALIER[4]. Cell wall preparations were obtained by the method of BECKER *et al.*[5] or YAMAGUCHI[6]. Menaquinones were prepared by the method of COLLINS *et al.*[7], and determined as described

by TAMAOKA *et al.*[8].

Results

Morphology

The mature sporophores were moderately short, and formed mainly hooked or sometimes loose spirals (Fig. 1). The spores were oval, and $0.8\sim1.0\times1.2\sim1.4$ μm in size. The spore surface was warty (Fig. 2). No fragmentation of mycelium was observed on agar medium or in liquid culture. No sporangia, zoospores or vegetative mycelium spores were observed.

Cultural Characteristics

Cultural characteristics of strain No. 6049 studied using various agar media are shown in Table 1.

Table 1. Cultural characteristics of strain No. 6049 and *Actinomadura verrucosospora* ATCC 27299.

	Strain No. 6049		*A. verrucosospora*
Oatmeal agar	G	Poor	Poor
	A	Grayish white	Grayish blue
	R	Colorless	Pink
	S	None	None
Yeast extract - malt extract agar	G	Abundant	Abundant
	A	None	None
	R	Colorless	Pink
	S	None	None
Inorganic salts - starch agar	G	Moderate	Abundant
	A	Pale blue	Pale bluish pink
	R	Pale pink	Pink
	S	None	None
Glucose - asparagine agar	G	Abundant	Abundant
	A	Pinkish gray	Pale bluish pink
	R	Pink	Pink
	S	None	None
Nutrient agar	G	Poor	Moderate
	A	None	None
	R	Colorless	Pink
	S	None	None
Czapek agar	G	Abundant	Poor
	A	White	None
	R	Pink	Pale pink
	S	None	None
Potato - dextrose agar	G	Abundant	Poor
	A	Blue	None
	R	Pink	Pink
	S	None	None
Tyrosine agar	G	Moderate	Moderate
	A	Grayish white	Grayish white
	R	Colorless	Pink
	S	None	None
Peptone - yeast extract - iron agar	G	Abundant	Moderate
	A	None	None
	R	Pale yellow	Pink
	S	None	None

Abbreviation: G=Growth, A=aerial mass color, R=reverse side color, S=soluble pigment.

Fig. 1. Aerial mycelium of strain No. 6049 on inorganic salts - starch agar (incubated for 21 days at 30°C).

The organism was observed with an optical microscope (×500).

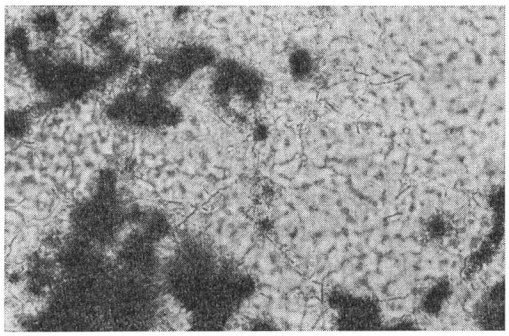

Fig. 2. Electron micrograph illustrating warty spores of strain No. 6049 (incubated on inorganic salts - starch agar for 21 days at 30°C).

Bar indicates 1 μm.

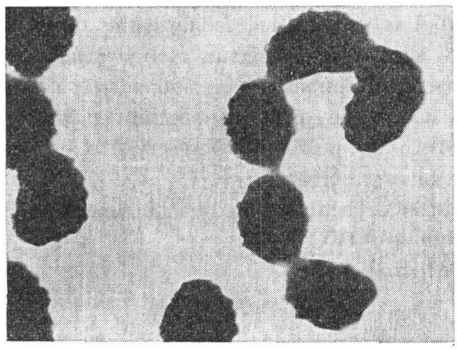

Table 2. Physiological characteristics of strain No. 6049.

Growth-permissible temperature range	20~41°C
Optimum temperature	32°C
Growth-permissible pH range	6~10
Optimum pH	7.5
Nitrate reduction	Negative
Starch hydrolysis	Positive
Milk coagulation	Weakly positive
Milk peptonization	Negative
Gelatin liquefaction	Positive
Melanin production	Negative
H_2S production	Negative
Urease reaction	Negative

Table 3. Carbohydrate utilization by strain No. 6049.

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: ++ Good utilization, + utilization, ± doubtful utilization, − no utilization.

Aerial mycelium formed on oatmeal agar or inorganic salts - starch agar was in the gray or blue color series. The vegetative growth of the organism was pale pink. No soluble pigment was produced in the examined media.

Physiological Characteristics

Physiological characteristics of strain No. 6049 are summarized in Table 2. The optimum growth temperature of the strain was around 32°C. The growth-permissible temperature range was from 20°C to 41°C. Starch hydrolysis and gelatin liquefaction were positive.

Melanoid pigment was not produced in tyrosine agar, peptone - yeast extract - iron agar or Tryptone - yeast extract broth. The strain was able to utilize D-xylose, rhamnose, D-glucose or sucrose as a carbon source. The results are summarized in Table 3.

Cell Analysis

Strain No. 6049 contained *meso*-2,6-diaminopimelic acid in the cell wall (type III). A whole-cell sugar hydrolysate showed the presence of madurose (type B). Nocardomycolic acids were not detected. The predominant menaquinones present in the cells were MK-9 (H_6) and MK-9 (H_8).

Discussion

The above mentioned morphological characteristics and cell wall of strain No. 6049 are similar to those described for members of the genus *Actinomadura*[9]. A comparison of the strain was made with various *Actinomadura* species in the light of the published descriptions[10~15]. Strain No. 6049 was considered to resemble *Actinomadura verrucosospora* Nonomura and Ohara 1971. Therefore, strain No. 6049 was compared with the corresponding *A. verrucosospora* ATCC 27299. As shown in Table 1, cultural characteristics of strain No. 6049 were different from *A. verrucosospora* on oatmeal agar, Czapek agar, potato - dextrose agar and nutrient agar.

Table 4. Differentiation of strain No. 6049 and *A. verrucosospora* ATCC 27299 by carbohydrate utilization and physiological characteristics.

	Strain No. 6049	*A. verrucosospora*
D-Fructose	−	+
L-Arabinose	−	+
Mannitol	−	+
Sucrose	++	±
Glycerol	±	++
Nitrate reduction	Negative	Positive
Milk peptonization	Negative	Positive

Symbols: ++ Good utilization, + utilization, ± doubtful utilization, − no utilization.

Nitrate reduction and peptonization of milk are negative for the strain. They are positive for *A. verrucosospora*.

Strain No. 6049 is unable to utilize D-fructose, L-arabinose and mannitol. *A. verrucosospora* is able to utilize those carbohydrates.

Differentiations of physiological characteristics between strain No. 6049 and *A. verrucosospora* were summarized in Table 4.

Considering the results of comparative studies of strain No. 6049 with the type cultures of the species described above, we have concluded that it is suitable to recognize this taxon as a new species in the genus *Actinomadura*. We propose to designate it *Actinomadura pulveracea* sp. nov. The proposed specific epithet "*pulveracea* (pul. ver. ac'a. L. n. pulvis, pulveris powder; M. L. adj. pulveracea powdery.)" refers to the powdery aerial mycelium on oatmeal agar and inorganic salts - starch agar.

Type strain: Strain No. 6049. A culture of this strain has been deposited in the American Type Culture Collection, Rockville, Md., with the accession number ATCC 39100.

The descriptions of the species are the same as that given above.

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