STUDIES ON A NEW IMMUNOACTIVE PEPTIDE, FK-156

I. TAXONOMY OF THE PRODUCING STRAINS

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Two strains of actinomycetes produce a new biologically active substance, designated FK-156. Examination of the morphological, cultural and physiological characteristics of the producing strains, No. C-353 and No. 6724, identified them as *Streptomyces olivaceogriseus* sp. nov. and *Streptomyces violaceus*, respectively.

In the course of a screening for new biologically active substances, FK-156 was discovered in the culture broth of strains No. C-353 and 6724. These cultures were isolated from soil samples obtained in Kochi Prefecture, and Ishigaki Island, Okinawa Prefecture, respectively. This report contains descriptions of these microorganisms and a discussion of their taxonomic position.

Materials and Methods

The methods described by Shirling and Gottlieb[1] were employed principally for these taxonomic studies. Morphological observations were made on cultures with light and electron microscopes. The cultures were grown at 30°C for 14 days on yeast extract - malt extract agar, inorganic salts - starch agar or oatmeal agar.

Cultural characteristics were observed on ten kinds of media as described by Shirling and Gottlieb[1] and Waksman[2]. The incubation was made at 30°C for 14 days. The color names used in this study were based on Color Standard (Nihon Shikisai Co., Ltd.). The analysis of whole cell hydrolysates was performed by the methods of Becker *et al.*[3] and Yamaguchi[4].

The range of growth temperature and optimum temperature were determined on Bennett agar using a temperature gradient incubator (Toyo Kagaku Sangyo Co., Ltd.). Gelatin liquefaction was examined at 30°C for 14 days on a gelatin medium. Starch hydrolysis was observed by the starch-iodine reaction on an inorganic salts - starch agar plate incubated at 30°C for 14 days. Milk peptonization and coagulation were observed in skim-milk medium at 30°C for 14 days. Melanoid pigment production was observed on tyrosine agar, Tryptone - yeast extract broth and peptone - yeast extract - iron agar. Nitrate reduction was examined on a medium composed of nutrient broth 10 g, KNO_3 10 g, agar 20 g and distilled water 1,000 ml. Formation of nitrite was detected with reagents consisted of 0.8% sulfanilic acid in 5 N acetic acid and 0.5 % α-naphtylamine in the same solvent. H_2S production was examined in a medium composed of nutrient broth 0.3%, glucose 1%, $FeSO_4 \cdot 7H_2O$ 0.02%, NaCl 0.5%, $Na_2S_2O_3$ 0.03%, phenol red 30 μg/ml and agar 2%.

Utilization of carbon sources was examined by the method of Pridham and Gottlieb[5]. The results were determined after 14 days incubation at 30°C.

Results and Discussion

Strain C-353

The chain of mature spores consisted of 5 to 20 spores and formed hooks, loops and occasionally

Plate 1. Aerial mycelium of strain No. C-353.

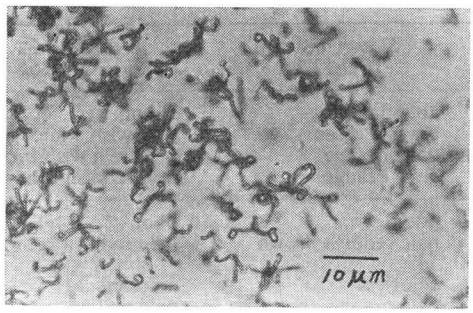
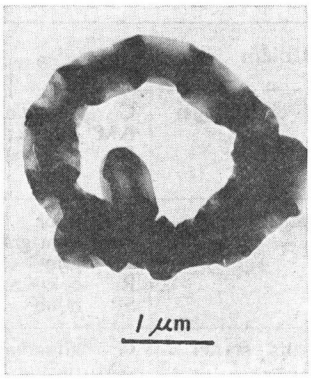


Plate 2. Spores of strain No. C-353.



closed spirals (Retinaculiaperti) (Plate 1). The spores are short cylindrical, phalangiform and 0.5~0.7×1.1~1.3 μm. Spore surfaces are smooth (Plate 2).

Aerial mycelium was in the gray or green color series on oatmeal agar, yeast extract - malt extract agar and inorganic salts - starch agar. Soluble pigment was not produced. Culture characteristics are shown in Table 1.

Whole cell hydrolysates of strain No. C-353 showed that it contained LL-diaminopimelic acid. Accordingly the cell wall of this strain belongs to type I.

Temperature range for growth was from 15 to 40°C and optimum temperature was 28°C. Starch hydrolysis was weakly positive. Gelatin liquefaction, milk peptonization and milk coagulation were negative. Melanin production was positive on tyrosine - yeast extract agar, peptone - yeast extract - iron agar and Tryptone - yeast extract broth.

Most carbon sources examined were utilized except L-arabinose, D-xylose, L-rhamnose, salicin, cellulose, chitin, sodium citrate, raffinose and sodium acetate. These results are shown in Table 3.

After comparing the characteristics of the *Streptomyces* species described in "The Actinomycetes. Vol. II" by WAKSMAN[2], ISP reports by SHIRLING and GOTTLIEB[6,7,8], and other recent literature, *Streptomyces eurythermus* and *Streptomyces galbus* were selected for further detailed comparisons. These two species were differentiated from strain No. C-353 in the following points.

Streptomyces eurythermus IFO-12764

S. eurythermus can assimilate D-xylose, rhamnose, L-arabinose, raffinose, salicin and sodium citrate but not inositol. *S. eurythermus* produces soluble pigment in almost all media tested. Gelatin liquefaction, milk peptonization, urease reaction are positive. *S. eurythermus* can grow in the presence of 7% NaCl but not in the presence of 10%.

Streptomyces galbus IFO-12864

S. galbus can assimilate D-xylose, raffinose and L-arabinose. Assimilation of rhamnose, sodium citrate and sodium acetate are doubtful. In almost all media examined, *S. galbus* produces soluble pigment. Aerial mass color of *S. galbus* is different on yeast - malt extract agar, oatmeal agar, inorganic salts - starch agar and some of the others. Gelatin liquefaction and milk peptonization are positive. *S. galbus* can grow in the presence of 5% NaCl but not in 7%. Optimum temperature is 37°C.

As the results of the above comparisons, it was concluded that strain No. C-353 can be considered a new species and the strain has been designated as *Streptomyces olivaceogriseus* sp. nov. (o.li.va'ce.o.

Table 1. Cultural characteristics of strain No. C-353, *Streptomyces eurythermus* IFO-12764 and *Streptomyces galbus* IFO-12864.

Medium		C-353	*S. eurythermus* IFO-12764	*S. galbus* IFO-12864
Yeast - malt extract agar	G	abundant	abundant	abundant
	AM	greenish gray	grayish green	yellowish white
	R	yellowish brown	dark orange	pale yellowish brown
	SP	none	dark orange	dull reddish orange
Oatmeal agar	G	poor	poor	poor
	AM	light gray to greenish gray	pale reddish brown	pale reddish brown
	R	colorless	colorless	colorless
	SP	none	pale orange, trace	pale yellow
Inorganic salts - starch agar	G	abundant	abundant	abundant
	AM	olive gray	grayish yellow brown	grayish yellow brown
	R	grayish yellow brown	grayish white	light gray
	SP	none	none	pale yellow
Glucose - asparagine agar	G	abundant	abundant	abundant
	AM	light gray to greenish gray	grayish yellow brown	grayish yellow brown
	R	pale yellow to yellowish brown	light gray	pale yellowish brown
	SP	none	dark orange	pale yellowish brown
Glycerin - asparagine agar	G	moderate	abundant	abundant
	AM	light gray	olive gray to brown	grayish yellow brown
	R	pale yellowish brown	brown	pale yellowish brown
	SP	none	chestnut brown	yellow
Nutrient agar	G	poor	moderate	moderate
	AM	none	grayish white	grayish white
	R	pale yellow	pale yellow	light reddish yellow
	SP	none	none	yellow
Potato-dextrose agar	G	moderate	moderate	moderate
	AM	olive drab	grayish yellow brown	none
	R	grayish black	chestnut brown	yellow
	SP	none	chestnut brown	yellow
Sucrose-nitrate agar	G	moderate	abundant	poor
	AM	none	grayish yellow brown to white	light gray
	R	pale yellow	chestnut brown	yellow
	SP	none	chestnut brown	yellow
Tyrosine agar	G	poor	moderate	moderate
	AM	light olive gray	light gray	light gray
	R	yellowish brown	dark gray	yellow
	SP	light brown	dark brown	dark brown, trace
Peptone - yeast extract - iron agar	G	moderate	moderate	moderate
	AM	none	none	none
	R	colorless to pale yellow	colorless	pale yellow
	SP	light brown	dark brown	dark brown

Abbreviation: G=growth, AM=aerial mass color, R=reverse side color, SP=soluble pigment

gri'se.us; M.L. *adj. olivaceus* olive colored; M.L. *adj. griseus* gray: M.L. *adj. olivaceo-griseus* olive-gray colored), referring to the olive-gray colored aerial mycelium on yeast - malt extract agar, oatmeal agar and inorganic salts - starch agar.

Type Strain: Strain No. C-353. A culture of this strain has been deposited at the American Type Culture Collection, Rockville, Md., under the accession number of ATCC 31427.

The description of species and of the type strain are the same as that given above.

Strain No. 6724

Mature spore chains consisted of 10 to 50 spores and formed spirals (Plate 3). The spores are cylindrical and $0.3\sim0.5\times0.8\sim1.0$ μm. Spore surfaces are spiny (Plate 4).

Cultural characteristics of strain No. 6724 are shown in Table 4. Aerial mycelium was in the red color series on oatmeal agar, yeast extract - malt extract agar and inorganic salts - starch agar. A pink to red soluble pigment was produced. The reverse side pigment and soluble pigment are pH indicators. Whole cell hydrolysates of strain No. 6724 contained LL-diaminopimelic acid. Accordingly, the cell wall of this strain is type I.

Physiological properties of this strain are summarized in Table 5. Temperature range for growth is from 15 to 40°C and optimum temperature is 28°C. Starch hydrolysis and milk coagulation are positive. Gelatin liquefaction and milk peptonization are negative. Melanin production is positive on peptone - yeast extract - iron agar and in Tryptone - yeast extract broth and on tyrosine agar.

Table 2. Physiological properties of strain No. C-353, *Streptomyces eurythermus* IFO-12764 and *Streptomyces galbus* IFO-12864.

	C-353	*S. eurythermus* IFO-12764	*S. galbus* IFO-12864
Temperature range for growth	14~40°C	15~48°C	15~44°C
Optimum temperature	28°C	37°C	37°C
Starch hydrolysis	weakly positive	positive	weakly positive
Gelatin liquefaction	negative	weakly positive	weakly positive
Milk peptonization	negative	positive	positive
Milk coagulation	negative	negative	negative
Melanin production	positive	positive	positive
H_2S production	positive	positive	positive
Urease reaction	negative	positive	negative
Nitrate reduction	negative	negative	negative
NaCl tolerance (%)	<5%	>7%, <10%	>5%, <7%

Table 3. Carbon sources utilization of strain No. C-353, *Streptomyces eurythermus* IFO-12764 and *Streptomyces galbus* IFO-12864.

	C-353	*S. eurythermus* IFO-12764	*S. galbus* IFO-12864		C-353	*S. eurythermus* IFO-12764	*S. galbus* IFO-12864
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

Table 4. Cultural characteristics of strain No. 6724.

Medium	Aerial mass color	Reverse side of colony	Soluble pigment
Sucrose - nitrate agar	purplish white, powdery	colorless, small colonies	purple
Glucose - asparagine agar	purplish white, powdery	yellowish red, small colonies	pink
Glycerin - asparagine agar	pinkish white to pink, powdery	yellowish red, small colonies, slightly wrinkled	pink to red
Inorganic salts - starch agar	whitish red, short cottony	yellowish red, slightly wrinkled	red
Tyrosine agar	none	red, wrinkled colonies	none
Nutrient agar	none or very thin, powdery	colorless to purple, flat	reddish purple
Yeast - malt extract agar	pink to purplish pink, short cottony	reddish brown to purplish brown, wrinkled colonies	reddish purple
Oatmeal agar	pink to purplish pink, powdery	colorless to purplish pink, small colonies	pink to purple
Peptone - yeast extract-iron agar	none	colorless, wrinkled colonies	brown

Plate 3. Aerial mycelium of strain No. 6724.

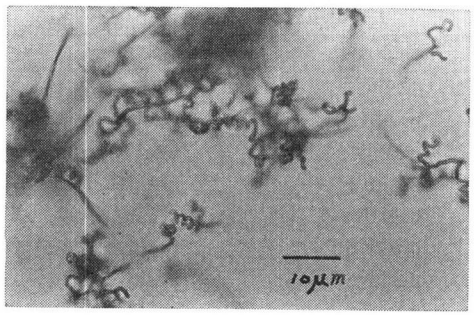


Plate 4. Spores of strain No. 6724.

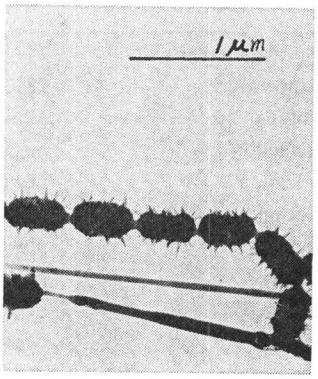


Table 6. Carbon source utilization of strain No. 6724.

L-Arabinose	+	Inulin	±
D-Xylose	+	Cellulose	−
L-Rhamnose	+	Chitin	−
D-Glucose	+	Glycerol	+
D-Fructose	+	D-Mannitol	+
D-Mannose	+	Salicin	+
D-Galactose	+	Inositol	+
Sucrose	+	Sodium acetate	−
Lactose	+	Sodiun citrate	+
Maltose	+	Sodium succinate	+
Raffinose	+		

Symbols: +=utilization, ±=doubtful utilization, −=no utilization

Table 5. Physiological properties of strain No. 6724.

Temperature range for growth	15~40°C
Optimum temperature	28°C
Starch hydrolysis	positive
Gelatin liquefaction	negative
Milk peptonization	negative
Milk coagulation	positive
Melanin production	positive

Cell wall type: Type I (LL-diaminopimelic acid)

The results of carbon source utilization tests are shown in Table 6. All carbon sources tested were utilized except inulin, cellulose, chitin and sodium acetate.

After comparison of the characteristics of the *Streptomyces* species described in "The Actinomycetes. Vol. 2" by WAKSMAN[2], ISP reports by SHIRLING and GOTTLIEB[6,7,8] and other recent literature,

Streptomyces violaceus was recognized to be quite similar to strain No. 6724. Thus taxonomic comparison was carried out using the standard culture of *S. violaceus* IFO-13103. No significant difference was observed between the two cultures and the properties of strain No. 6724 showed good agreement with those of *S. violaceus* IFO-13103. Strain No. 6724 has, therefore, been identified as *Streptomyces violaceus* No. 6724.

Strain No. 6724 has been deposited in the American Type Culture Collection under the accession number of ATCC 31481.

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