STUDIES ON THE ANTIBIOTICS FROM *STREPTOMYCES SPINICHROMOGENES* VAR. *KUJIMYCETICUS*. I

TAXONOMIC AND FERMENTATION STUDIES WITH *STREPTOMYCES SPINICHROMOGENES* VAR. *KUJIMYCETICUS*

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Streptomyces sp. TPR-885 which produced the new antibiotics, kujimycins, was isolated from a soil sample from the Kuji District of the Ibaragi Prefecture, Japan. The strain belonged to a chromogenic type of *Streptomyces* similar to *St. spinichromogenes*, *St. griseochromogenes* and *St. cavourensis*. From the taxonomic studies, the strain TPR-885 resembled with *St. spinichromogenes* in the morphological and physiological properties. This strain was accordingly named *Streptomyces spinichromogenes* var. *kujimyceticus*. It produced in excess of 150 mcg/ml of kujimycins in flask and jar fermentation, and the kujimycins showed inhibitory activities against gram-positive bacteria and *Mycobacteria*.

In the course of screening for new antibiotics, a strain, *Streptomyces* sp. TPR-885, was isolated from a sample of soil collected in the Kuji District of Ibaragi Prefecture, Japan.

This strain has produced some antibiotics which showed inhibitory activities against gram-positive bacteria and *Mycobacteria*. Two components were purified and compared with known antibiotics on the basis of biological, chemical and physical properties, and distinguished as new macrolide antibiotics, kujimycins A and B. Then, after taxonomic studies, *Streptomyces* sp. TPR-885 was named *Streptomyces spinichromogenes* var. *kujimyceticus*.

Plate 1. Aerial mycelium of strain No. TPR-885
(On glucose asparagine agar ×150×1/2)

Plate 2. Electron micrograph of spores of strain No. TPR-885
(On yeast extract malt extract agar ×15,000×1/2)

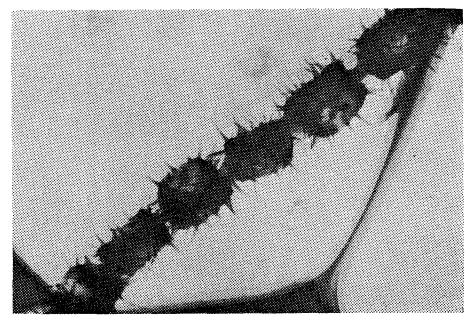


Table 1. Morphological properties of *Streptomyces* sp. TPR-885

Medium	Growth	Aerial mycelium	Soluble pigment	Note
Sucrose CZAPEK's agar (27°C)	good, yellowish brown to blackish brown	good, white to grayish white, partly grayish brown	none	
Glycerol CZAPEK's agar (27°C)	good, yellowish brown to reddish brown	white, later becoming grayish white	none	
Glucose CZAPEK's agar (27°C)	reddish brown, partly blackish brown	white, later becoming grayish white	none	
Starch CZAPEK's agar (27°C)	good, yellow with brownish white	white	none	
Glucose asparagine agar (27°C)	small colony of colorless to pale yellow	white	none	
Glycerol asparagine agar (27°C)	small colony of colorless to pale yellowish white	white	none	
Glycerol calcium malate agar (27°C)	weak, small colony of pale yellowish white	white	none	
Nutrient agar (27°C)	good, moist yellowish brown to grayish brown	scanty, grayish white	dark brown	
Bouillon agar (27°C)	good, moist grayish black to grayish brown	none	dark brown	
Potato plug (27°C)	granular growth yellowish brown to brown	white to grayish white	brown	
Carrot plug (27°C)	weak, whitish yellow to yellowish brown	scanty, white	none or pale brown	
Gelatin stab (27°C)	weak, cream to yellowish brown	scanty, white	dark brown	weak liquefaction
Egg medium (37°C)	weak, small colony of pale yellowish brown to yellowish brown	none	brown	
LOEFFLER's coagulated serum slant (37°C)	cream to pale yellowish brown	none	pale brown	no liquefaction
Cellulose medium (27°C)	none	none	none	
Blood agar (37°C)	moist grayish brown	white to grayish white	none	no hemolysis
Skimmed milk (37°C)	white to pale yellowish brown ring around surface	none	pale yellow	positive peptonization and coagulation

The taxonomic studies with *Streptomyces spinichromogenes* var. *kujimyceticus* and the fermentation procedure of kujimycins are described in this paper.

Taxonomic Studies of *Streptomyces* sp. TPR-885

In order to investigate the growth characteristics of *Streptomyces* sp. TPR-885, the cultures were grown on various media and incubated at 27°C for two weeks. On microscopic observation the aerial hyphae were relatively fine, straight and

Table 2. Utilization of carbohydrates by *Streptomyces* sp. TPR-885

Inositol	+	Galactose	++
Xylose	±	Sucrose	+
Fructose	+	Lactose	+
Rhamnose	−	Raffinose	+
Mannitol	+	Starch	+
Glucose	++	Cellulose	−

++: Strongly positive utilization +: Positive utilization ±: Doubtful utilization −: Negative utilization

Table 3. Physiological properties of *Streptomyces* sp. TPR-885

Tyrosinase reaction	+
Production of hydrogen sulfide	+
Nitrate reduction	−
Hydrolysis of starch	+
Liquefaction of serum	−
Liquefaction of gelatin	+
Peptonization of milk	+
Coagulation of milk	+
Cellulose decomposition	−

closed spirals, especially on glucose asparagine agar (Plate 1). On electron microscopic observation the shape of spores was oval or cylindrical, 0.6~1.1 μ in diameter, and the surfaces were spiny (Plate 2).

The cultural characteristics of the strain grown on various media are shown in Table 1. The aerial mycelium on most of the media was ordinarily white to grayish white. The most remarkable characteristic of this strain was the production of brown pigment in protein-containing media. Accordingly, this strain belonged to the chromogenic type of *Streptomyces*.

Carbon source utilization of the strain TPR-885 was tested by the method of PRIDHAM *et al.*[1] As shown in Table 2, fair growth of the strain TPR-885 was observed on media with the following single carbon source: inositol, fructose, mannitol, glucose, galactose, sucrose, lactose, raffinose and starch. On the contrary, doubtful or no growth was observed with xylose, rhamnose and cellulose. Other physiological properties are shown in Table 3.

Comparison with *Streptomyces* Strains similar to *St.* sp. TPR-885 and the Identification of the Strain

As described above, *Streptomyces* sp. TPR-885 produced closed spirals on sporophores, white to grayish white aerial mycelium on most of the medium and brown pigment on protein-containing media. Compared with known species in literature, the strain was suggested to have close relationship to *Streptomyces spinichromogenes*[2], *Streptomyces griseochromogenes*[3] and *Streptomyces cavourensis*[4]. However, *Streptomyces griseochromogenes* shows orange cinnamon color of growth on sucrose CZAPEK's medium, shows opalescent color of growth on nutrient agar, reduces nitrate and does not coagulate milk. *Streptomyces cavourensis* produces pale brown soluble pigment on glucose asparagine agar, produces grayish aerial mycelium on gelatin and the color of growth is orange brown on nutrient agar.

As a contrast, *Streptomyces* sp. TPR-885 does not produce soluble pigment on glucose asparagine agar, produces aerial mycelium on synthetic agar, shows yellowish brown color of growth on sucrose CZAPEK's agar, changes color of growth from blackish gray to brownish gray on nutrient agar, coagulates milk and does not reduce nitrate. On the basis of the above data, *Stpeptomyces* sp. TPR-885 apparently differs from these organisms.

On the other hand, the strain TPR-885 and *St. spinichromogenes* resemble each

Table 4. A comparative study of culture and physiological characteristics of *Streptomyces* sp. TPR-885, *St. spinichromogenes*, *St. griseochromogenes* and *St. cavourensis*

	Streptomyces sp. TPR-885	*Streptomyces spinichromogenes*	*Streptomyces griseochromogenes*	*Streptomyces cavourensis*
Sporophores	closed spiral	open spiral	closed spiral	spiral
Spore surface at magnification	spiny	spiny	—	—
Glycerol CZAPEK's agar	G : good, yellowish brown to reddish brown A : white, later becoming grayish white S : none	G : pale yellow A : none S : none		
Sucrose CZAPEK's agar	G : good, yellowish brown to blackish brown A : white to grayish white S : none		G : spreading orange cinnamon A : white or neutral light gray S : none	G : yellow A : chalky white to yellow S : none
Glucose asparagine agar	G : pale yellow to colorless A : white S : none	G : pale brown A : pale brownish gray S : brown	G : restricted ivory yellow A : none, later becoming white S : none	
Glycerol calcium malate agar	G : pale yellowish white A : white S : none	G : colorless A : white S : none		G : weak, yellowish brown A : scanty, white S : yellowish brown
Nutrient agar	G : good, moist blackish gray to brownish gray A : none S : dark brown	G : brownish gray A : none S : brown	G : restricted opalescent A : none S : brown	G : orange brown A : scanty, chalky white to yellow S : light brown
Gelatin stab	G : cream to yellowish brown A : scanty, white S : dark brown	G : dark brown A : none S : brownish black	G : wrinkled yellowish in liquefied portion A : scanty, white S : dark brown	G : scanty, brown wrinkled A : scanty, gray S : brown
Skimmed milk	G : pale yellowish brown ring A : none S : pale brown	G : dark brown A : none S : grayish dark brown	G : surface ring brown	G : white to yellow ring around surface
Nitrate reduction	—	+	+	—
Production of hydrogen sulfide	+			
Tyrosinase reaction	+	+	—	
Cellulose decomposition	—	—	—	
Milk peptonization	+	+	+	+
Milk coagulation	+		—	+
Gelatin liquefaction	±	+		+
Hydrolysis of starch	+	+		+
Hemolysis	—	+		
Liquefaction of serum	—	—		
Solubilization of Ca-malate	+	+		
Antibiotic produced	Kujimycin (macrolide group)	Coumermycin (similar to novobiocin)	Blasticidins A, B and C	Flavensomycin

Abbreviation G: Growth A: Aerial mycelium S: Soluble pigment

other except nitrate reduction and hemolysis in physiological properties as shown in Table 4. But, *St. spinichromogenes* produces soluble pigment on glucose asparagine agar and does not produce aerial mycelium on glycerol-CZAPEK's agar. In this view point, the strain TPR-885 differs from *St. spinichromogenes*.

From these considerations, the strain TPR-885 was classified as a novel variety of *St. spinichromogenes* and was designated *St. spinichromogenes* var. *kujimyceticus*.

Fermentation of Kujimycins

The antimicrobial activity was detected by the paper disc plate method using *Sarcina lutea* NIHJ as the test organism and by thin-layer chromatography on Silica Gel G.

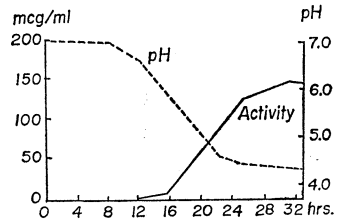
1. Flask fermentation

The seed culture of *Streptomyces spinichromogenes* var. *kujimyceticus* was incubated at 27°C under shaking for 48 hours in a medium containing 1 % glycerin, 0.5 % meat extract, 0.5 % peptone and 0.3 % NaCl. One ml of this culture was inoculated into each shake flask (500 ml, in vol.) containing 100 ml of the production medium of 2 % glucose, 2 % soluble starch, 1 % yeast extract, 0.25 % NaCl, 0.2 % $CaCO_3$, 0.0003 % $MnCl_2 \cdot 4H_2O$, 0.0003 % $ZnSO_4 \cdot 7H_2O$ and 0.0003 % $CuSO_4 \cdot 5H_2O$. The fermentation was carried out at 27°C on a reciprocating shaker for 40~48 hours, and maximum production of kujimycins was found to contain 100~150 mcg/ml in broth.

2. Jar fermentation

In a 30-liter fermentor, 20 liters of medium containing 2 % glucose, 2 % starch, 1 % yeast extract, 0.25 % NaCl, 0.2 % $CaCO_3$, 0.0003 % $MnCl_2 \cdot 4H_2O$, 0.0003 % $ZnSO_4 \cdot 7H_2O$, 0.0003 % $CuSO_4 \cdot 5H_2O$ was prepared, and the pH of the medium was adjusted to 7.0, followed by autoclaving. After inoculating with a 2 % seed culture which was incubated at 27°C under shaking for 48 hours in same medium, fermentation was carried out under the conditions of 20 liters/minute in aeration, 300 r.p.m. in agitation and 27±1°C in temperature. As an antifoam agent, one ml of silicon oil was added.

Fig. 1. Culture process in kujimycin production



As shown in Fig. 1, the maximum production of kujimycins was observed after 28~32 hours, and at the time of harvest, kujimycins in broth reached a 150 mcg/ml line on average. At this time, the pH of cultured broth was 4.2~4.8.

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