STUDIES ON A NEW ANTITUMOR ANTIBIOTIC, LARGOMYCIN. I

TAXONOMY OF THE LARGOMYCIN-PRODUCING STRAIN AND PRODUCTION OF THE ANTIBIOTIC

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As a result of taxonomic studies on a streptomycete designated MCRL 0367 which produces the antitumor antibiotic largomycin, it was identified as a strain of *Streptomyces pluricolorescens* OKAMI and UMEZAWA. The production of the largomycin complex is described.

During screening for antitumor antibiotics, a strain (MCRL 0367) in our culture collection was found to show strong antitumor activity. The activity later was found to be due to a mixture of antitumor antibiotics collectively named largomycin.

The present paper is concerned with a taxonomic study of strain MCRL 0367 which was identified as a strain of *Strepomyces pluricolorescens* OKAMI and UMEZAWA[1), and also deals with the production of the largomycin complex. Isolation, purification and properties of largomycin will be reported in the succeeding paper[2).

Taxonomic Study

The largomycin-producing strain MCRL 0367 was isolated from a soil sample collected in the vicinity of Kurihashi City, Saitama Prefecture, in 1963.

Fig. 1. Photomicrograph of *Streptomyces pluricolorescens*, strain MCRL-0367 (BENNETT's agar $\times 500 \times 1/1.5$)

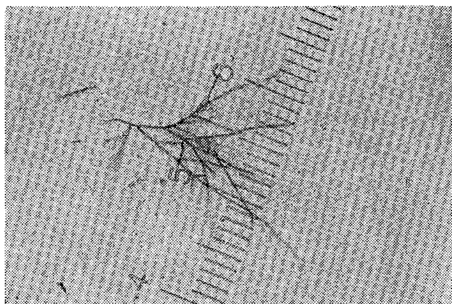


Fig. 2. Electron micrograph of *Streptomyces pluricolorescens*, strain MCRL-0367 (BENNETT's agar $\times 15{,}000 \times 1/1.5$)

Table 1. Cultural characteristics of *Streptomyces pluricolorescens*, strain MCRL-0367

Medium		Cultural characteristics	Medium		Cultural characteristics
Sucrose nitrate agar	G	colorless, transparent	BENNETT's agar	G	colorless
	Rev	colorless		Rev	brown (4-13-5)
	AM	powdery, pale olive (8-18-2), later yellowish gray (7-19-1)		AM	powderly, pale orange (5-19-2)
	SP	pale purple (20-17-3)		SP	reddish brown (3-12-5)
Glycerol nitrate agar	G	grayish red brown (2-14-3)	Nutrient agar	G	colorless to cream (7-19-3)
	Rev	brown purple (24-12-3)		Rev	pale yellow (7-19-4)
	AM	powderly, pale pink (2-18-2)		AM	none
	SP	reddish brown (3-14-4)		SP	none
Glucose nitrate agar	G	light yellow orange (5-18-5)	Glucose nutrieent	G	dull orange (5-17-4)
	Rev	light brown (4-15-4)		Rev	reddish yellow (7-18-6)
	AM	powdery, brownish white (6-19-5)		AM	none
	SP	yellow orange (6-18-6)		SP	none
Glucose asparagine agar	G	pale yellowish brown (7-18-3)	Peptone glucose agar	G	colorless, later dull orange (5-17-4)
	Rev	pale yellowish brown (7-18-3)		Rev	reddish yellow (7-18-6)
	AM	powdery, brownish white (6-19-1)		AM	white, scant
	SP	pale yellow (8-19-2)		SP	none
Calcium malate agar	G	reddish black (2-11-1)	Blood agar	G	cream to yellow (8-15-5)
	Rev	dark brown purple (1-11-4)		AM	none
	AM	powdery, pale yellow (8-19-2) to pale orange (5-19-2)	Egg medium	G	dull yellow (7-18-4)
	SP	brown (4-14-5)		AM	none
				SP	none
Starch agar	G	light reddish yellow (7-19-5)	Gelatin stab	G	cream, becoming brown (4-14-3)
	Rev	reddish yellow (7-18-6)		AM	none
	AM	powdery, brownish white (6-1-1)		SP	later reddish brown (3-14-4)
	SP	yellowish orange (5-17-6)			
Tyrosin agar	G	yellowish brown (6-16-3)	Litmus milk	G	pale yellowish brown (7-17-3), ring
	Rev	pale yellow orange (5-19-2)		AM	none
	SP	pale yellowish brown (7-17-3)		SP	none
Glucose nitrate solution	G	yellowish brown (6-16-3), pellicle	LOEFFLER's coagulated serum	G	colorless cream, wrinkled
	AM	none		AM	none
	SP	pale yellow (7-19-4)		SP	none
Cellulose medium	G	white, scant	Potato plug	G	colorless to cream
	AM	none		AM	none
	SP	none		SP	none

Abbreviation ; G : growth, Rev : reverse, AM : aerial mycelium, SP : soluble pigment.

Strain MCRL 0367 grew well on both synthetic and organic agar media and developed colonies characteristic of the genus *Streptomyces* WAKSMAN and HENRICI, 1943. On most agar media, the formation of abundant aerial mycelia was observed. On synthetic media, sporophores were sympodially branched, forming tufts and some flexuous chains, without spirals or whorls. Mature spore chains were long and bore more than 10 spores per chain (Fig. 1). The spores were cylindrical and measured $0.4 \sim 0.6 \times 0.9 \sim 0.4\ \mu$. Spore surfaces were smooth as determined from electron-micro-

Table 2. Physiological properties of *Streptomyces pluricolorescens* strain MCRL-0367

Solubilization of calcium malate	positive
Nitrate reduction	positive
Hydrolysis of starch	positive
Cellulose decomposition	negative
Tyrosinase reaction	negative
Milk coagulation	positive
Milk peptonization	positive
Hemolysis	strong
Liquefaction of gelatin	positive
Liquefaction of serum	positive
Melanin formatton	negative

Table 3. Utilization of carbon sources by *Streptomyces pluricolorescens*, strain MCRL-0367

Growth	Carbon sources
++	Arabinose, Glucose Glycerol, Maltose, Mannitol, Mannose, Rhamnose, Starch, Xylose
+	Fructose, Lactose, Sucrose
±	Salicin
−	Inositol, Raffinose

++ : Good growth   + : Moderate growth
± : Doutful growth   − : No growth

scope observation (Fig. 2).

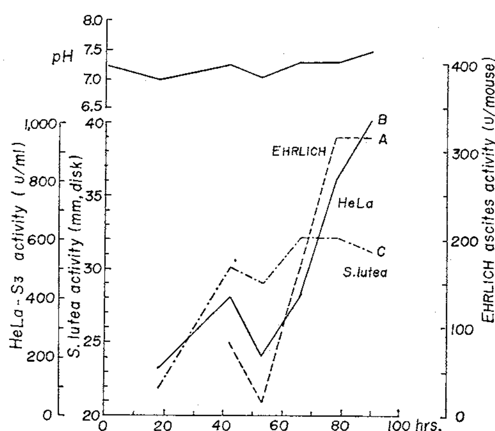
Strain MCRL 0367 was cultivated at either 27°C or 37°C for 3 to 4 weeks on various media used conventionally for the characterization of *Streptomyces*[3]. The data are summarized in Table 1. Color names and designations are those given in "Iro no Hyojun"[4] (The guide to the color standard). Cultures on synthetic media generally were characterized by colorless to yellowish brown vegetative growth with reddish tones, pale pink aerial mycelium and reddish brown soluble pigment. Cultures on organic media were colorless to cream color, and produced no soluble pigment.

The physiological properties of strain MCRL 0367 are shown in Table 2. Strain MCRL 0367 grew well at 27°C as well as 37°C in the pH ranges of 6~9, but showed no growth at 10°C and 50°C at any pH studied.

The method of PRIDHAM and GOTTLIEB[5] was employed to determine the utilization of carbon sources. As shown in Table 3, most of the carbon sources tested were utilized except for inositol and raffinose. Utilization of salicin was doubtful.

From the above-mentioned properties, strain MCRL 0367 was classified as a non-chromogenic *Streptomyces* in the red series and section *Rectus-flexibilis* of PRIDHAM *et al.*[6] According to the classification system proposed by WAKSMAN[7], strain MCRL 0367 was classified as a member of the group I.A. 6 (p. 158). (*Streptomyces* forming no whorls, no melanoid pigment and producing yellowish-brown to reddish-brown soluble pigment). Among the known species listed in this group, *S. pluricolorescens* OKAMI and UMEZAWA[7,8] appeared most similar to strain MCRL 0367 in cultural and morphological characteristics. However, strain MCRL 0367 differs from *S. pluricolorescens* in several properties: with gelatin stabs, *S. pluricolorescens* forms colorless, vegetative

Fig. 3. Production of largomycins.
A. Anti-EHRLICH ascites activity.
B. Anti-HeLa cells (S-3) activity.
C. Anti-*Sarcina lutea* activity.



growth with beige-colored aerial mycelium and produces a slightly yellowish brown soluble pigment. In spite of these minor differences, strain MCRL 0367 and *S. pluricolorescens* showed numerous similarities in other properties, including morphology, physiology and carbon utilization pattern. Thus, the largomycin-producing strain (MCRL 0367) was identified as *S. pluricolorescens* OKAMI and UMEZAWA.

Production of Largomycin

Fermentation studies were carried out in a 2,000-liter fermentor. Production of the largomycin complex was determined by a cup-plate method using *Sarcina lutea* PCI 1001 as the test organism, cytotoxicity by a tissue culture method using HeLa S-3 cells, and antitumor activity by a tumor-inhibition test with EHRLICH ascites carcinoma in mice. The fermentation medium was composed of glycerol, starch and peptone (each at 2 % w/v), meat extract and sodium chloride (each at 0.5 % w/v), and calcium carbonate (at 0.2 % w/v). The pH of the medium was adjusted to 7.2 before sterilizing. One thousand and two hundred liters of the medium were prepared in a 2,000-liter tank fermentor and sterilized at 120°C for 20 minutes. After cooling, 150 liters of seed culture was used to inoculate the production medium. The fermentation was carried out under the following conditions: temperature 27±1.0°C, inner pressure 0.5 kg/cm^2, aeration 600 liters/min., agitation 140～180 r.p.m.

As shown in Fig. 3, the maximum production of largomycin by anti- *S. lutea* activity was achieved after 60 hours. However, antitumor activity reached maximum at 80～90 hours. Culture filtrates obtained at that time remarkably inhibited the increase of ascites of EHRLICH ascites carcinoma even at 300-fold dilution, and also showed cytotoxicity against HeLa S-3 cells at 1,000-fold dilution. A comparable fermentation broth thus obtained was used for the isolation of largomycin as will be detailed in the succeeding paper[2].

Acknowledgement

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