A NEW SPECIES OF *STREPTOMYCES* PRODUCING VIRGINIAMYCIN FAMILY ANTIBIOTICS

Sir:

In the course of screening for antibiotics, *Streptomyces* sp. No. 16–5 was isolated from a soil sample collected in Kyoto City. Taxonomic studies were conducted in accordance with the methods used in International Streptomyces Project[1]. The morphology of spore chains and spores developed on yeast-malt agar at 28°C for 14 days was observed microscopically. The aerial mycelium of strain No. 16–5 is well branched and the chains of spores are almost straight and flexible (Plate 1). The spores are oval, 0.6~0.8 μ by 1.0~1.5 μ, with smooth surface (Plate 2). The cultural and physiological characteristics can be summarized as follows: aerial mass color is in white color series on oatmeal agar, salts-starch agar and glycerol-asparagine agar; vegetative mycelium is colorless to pale yellow or yellowish gray to yellowish brown on almost all media; no distinctive pigments other than grayish yellow on reverse side of colonies are observed on yeast-malt agar, oatmeal agar, salts-starch agar and glycerol-asparagine agar; melanoid pigments are formed in peptone-yeast-iron agar and tryptone-yeast broth but not in tyrosine agar. The cultural and physiological characteristics of strain No. 16–5 are listed in Tables 1 and 2, respectively. The utilization of carbon sources of strain No. 16–5 was examined on PRIDHAM and GOTTLIEB's agar medium[1]. D-Glucose, D-galactose and D-fructose are utilized for the growth. No or scarce growth is observed on L-arabinose, sucrose, *i*-inositol, D-mannitol, L-rhamnose, raffinose and cellulose. Utilization of D-xylose is doubtful.

When these descriptions were compared with those of known series of *Streptomyces* described in "BERGEY's Manual of Determinative Bacteriology (8th ed.)"[2] and other sources, an agreement was found in the following two species: *Streptomyces albolongus* TSUKIURA *et al.* 1964[3] and *Streptomyces viridaris* PRIDHAM 1970[4]. However, these strains were distinguished from strain No. 16–5 by the following descriptions of their morphological and cultural characteristics. The spore chain of *S. albolongus* usually contains more than 50 spores per chain and this morphology is also seen on glycerol-asparagine agar. The strain forms melanoid pigments on tyrosine agar and utilizes L-arabinose, raffinose and D-xylose but does not utilizes D-fructose. *S. viridaris* forms green vegetative mycelium on some media and utilizes L-rhamnose, raffinose, sucrose and D-xylose.

On the basis of above data, it is reasonable to conclude that *Streptomyces* sp. No. 16–5 is classified as a new species of *Streptomyces*, and the name, *Streptomyces alborectus* OGATA *et* MATSUURA nov. sp. is proposed. The proposed species epithets "*albus*" and "*rectus*" are Latin adjectives meaning "white" and "straight", respectively, after its aerial mass color and spore chain morphology.

The fermentation was carried out in the medium composed of 1.0% glucose, 5.0% C.S.L., 0.05% K_2HPO_4 and 0.01% $MgSO_4 \cdot 7H_2O$ (pH 7.6~8.0) at 28°C for 2 days. From this fermentation broth, antibacterial substances, No. 16–5 A, No. 16–5 B and No. 16–5 D, and a non-antibacterial substance, No. 16–5 C, were

Plate 1. Photomicrograph of strain No. 16–5 (×300)

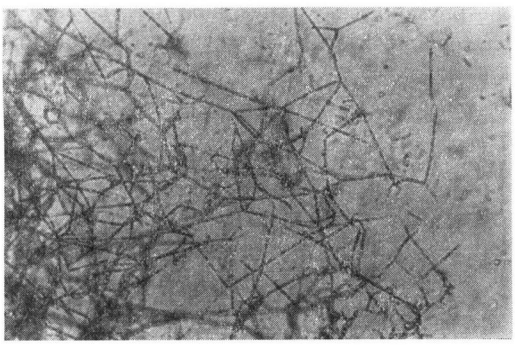
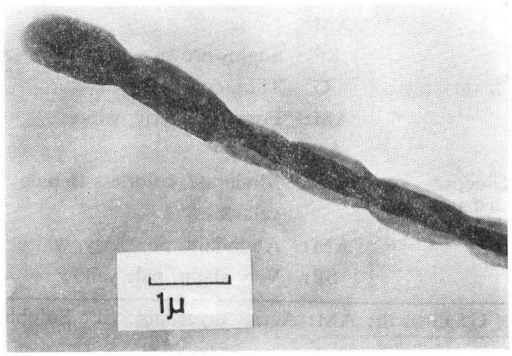


Plate 2. Electromicrograph of the spores of strain No. 16–5 (×5,000)



isolated and purified by benzene extraction and successive silica gel column chromatographies. Physical and chemical properties, PMR and IR spectra and other analytical data of each component were used to determine the structures of these components. It was concluded that No. 16–5 A is identical with virginiamycin M because the PMR spectrum of No. 16–5 A was completely superimposable upon that of ostreogrycin A[5] (virginiamycin M[6]). Amino acids and other components in No. 16–5 B were analyzed to be identical with those in virginiamycin S, and mass spectrum of No. 16–5 B was virtually identical with that of staphylomycin S[7] (virginiamycin S[6]). Furthermore, PMR spectrum of No. 16–5 B was also superimposable upon that of virginiamycin S[8]. No. 16–5 C was proved to be L-prolyl-L-leucine anhydride by direct comparison with chemically synthesised L-prolyl-L-leucine anhydride on the method described by CHEN[9] in their IR spectra, $[\alpha]_D$ values and some other analytical data. No. 16–5 D was conceivable to be virginiamycin M_2 because PMR spectrum in the double bond region ($\delta = 5 \sim 9$ ppm) of No. 16–5 D was well superimposable upon that of ostreogrycin G[10] (virginiamycin M_2[6]).

Table 1. Cultural characteristics of strain No. 16–5

Medium	Cultural characteristics
Yeast extract-malt extract agar (ISP)	G: Thin, colorless to yellowish brown
	AM: Abundant velvety, light brownish gray
	SP: Pale yellowish brown
Inorganic salts-starch agar (ISP)	G: Moderate, yellowish gray
	AM: Moderate, powderly, white
	SP: Very slight, pale yellow
Oatmeal agar (ISP)	G: Thin, colorless to pale yellow
	AM: Abundant, powderly, white
	SP: None
Glycerol-asparagine agar (ISP)	G: Moderate, pale yellow
	AM: Poor, powderly, white
	SP: Very slight, pale yellow
Tyrosine agar (ISP)	G: Moderate, pale yellow
	AM: Abundant, powderly, white
	SP: Very slight, pale yellow
Nutrient agar	G: Thin, colorless to yellowish gray
	AM: None
	SP: Slight, brown
CZAPEK agar	G: Thin, colorless
	AM: Poor, powderly, white
	SP: None
Glucose-asparagine agar	G: Moderate, colorless to pale yellow
	AM: Abundant, powderly, white
	SP: Very slight, pale yellow

G: Growth; AM: Aerial mycelium; SP: Soluble pigment

Table 2. Physiological properties of *Streptomyces* sp. No. 16–5

Melanin formation	+
Tyrosinase reaction	−
H_2S production	+ (weak)
Nitrate reduction	+
Hydrolysis of starch	+
Liquefaction of gelatin	+
Peptonization of milk	+ (slow)
Coagulation of milk	−
Temp. range for growth	5~37°C

Only one species of *Streptomyces*[11], *Streptomyces virginiae*, has been reported as the strain producing virginiamycin M, S and M_2, simultaneously. However, strain No. 16–5 is clearly different from *Streptomyces virginiae* by its aerial mass color, utilization of carbon source and so on. In addition, the present strain produced No. 16–5 C, L-prolyl-L-leucine anhydride, which was reported as a retardative material for the growth of plant seedlings and roots[9]. Streptomycin-producing strain of *Streptomyces griseus* was reported to produce this compound[12], but strain No. 16–5 is clearly distinguishable from *Streptomyces griseus*. The simultaneous production of virginiamycin family antibiotics and the physiologically active substance, L-prolyl-L-leucine anhydride, by an organism has not been known.

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