ACULEXIMYCIN, A NEW ANTIBIOTIC FROM *STREPTOSPORANGIUM ALBIDUM*

I. TAXONOMY OF PRODUCING ORGANISM AND FERMENTATION

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A soil isolate of actinomycete, strain TI-1, was found to produce a new antibiotic aculeximycin which killed insects as well as inhibited the growth of bacteria, yeasts and molds *in vitro*. Yellowish gray colonies on agar media, formation of spherical to oval sporangia at the tip of aerial mycelium and the presence of *meso*-diaminopimelic acid and madurose in the cell wall ascribed this strain to genus Streptosporangium. From its morphological, cultural and physiological characteristics, the strain was determined to be *S. albidum*. Production of aculeximycin was carried out by conventional submerged culture: the highest antibiotic titer obtained was 1,250 μg/ml.

During the course of a screening program for new insecticidal antibiotics using mosquito larvae as the test organism, strain TI-1 was found to produce a new antibiotic in its culture broth. The antibiotic is active against insects, bacteria, yeasts and molds *in vitro* at low concentration and resembles sporaviridin[1], which is produced by *Streptosporangium viridogriseum* but differs from it in some physicochemical properties.

This paper deals with the taxonomy of producing organism and fermentation of aculeximycin. Isolation, physicochemical characterization as well as biological properties of the antibiotic are described in the subsequent paper.

Taxonomic Studies of Strain TI-1

The producing organism, strain TI-1, was isolated from a soil sample collected at Hachijo-island, Tokyo, Japan. Taxonomy was carried out according to the methods described by SHIRLING and GOTTLIEB[2]; several other tests were also used. Observation of the cultures was made after incubation at 28°C for 2 weeks, unless otherwise stated. Color names were assigned according to "Guide to Color Standard"[3]. The characteristics of strain TI-1 were compared with those of known species of actinomycetes described in "The Actinomycetes" Vol. 2 by WAKSMAN[4], "BERGEY's Manual of Determinative Bacteriology"[5] and other recent references on the taxonomy of the family Actinoplanaceae.

Strain TI-1 formed sporangia at the ends of aerial hyphae on various media. Sporangia were

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Plate 1. Light micrograph of strain TI-1 on sucrose - nitrate agar at 28°C for 12 days (×300).

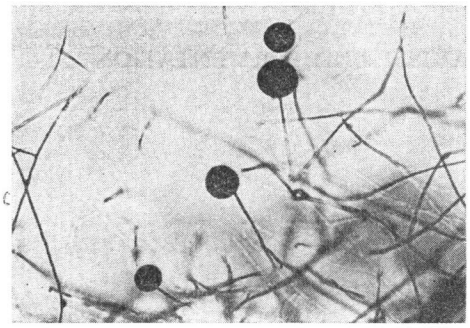


Plate 2. Scanning electron micrograph of strain TI-1 on sucrose - nitrate agar at 28°C for 12 days. Bar represents 50 μm.

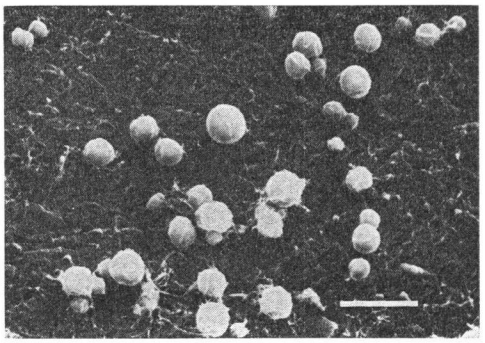


Table 1. Cultural characteristics of strain TI-1.

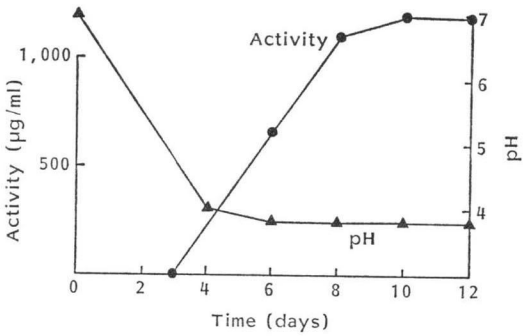
Yeast extract - malt extract agar (ISP 2)	SM: Good, yellowish gray (2–9–10) AM: Good, white to pale yellowish brown (4–8–9) SP: None SG: Abundant
Oatmeal agar (ISP 3)	SM: Abundant, yellowish gray (2–9–10) AM: Abundant, white to pale yellowish brown (4–8–9) SP: None SG: Abundant
Inorganic salts - starch agar (ISP 4)	SM: Poor, colorless AM: Poor, white SP: None SG: Abundant
Glycerol - asparagine agar (ISP 5)	SM: Good, yellowish gray (1–9–10) AM: Poor, white SP: None SG: None
Peptone - yeast extract - iron agar (ISP 6)	SM: Poor, colorless to pale yellow (3–9–10) AM: Moderate, white SP: None SG: None
Tyrosine agar (ISP 7)	SM: Good, pale yellowish brown (4–8–9) AM: Moderate, white SP: None SG: Poor
Nutrient agar (Difco)	SM: Good, yellowish brown (1–9–10) AM: Moderate, white SP: None SG: None
Sucrose - nitrate agar	SM: Good, yellowish gray (1–9–10) AM: Moderate, white to pale yellowish brown (4–8–9) SP: None SG: Abundant
Potato extract - carrot extract agar	SM: Poor, colorless AM: Moderate, white SP: None SG: Abundant

SM: Substrate mycelium. AM: Aerial mycelium. SP: Soluble pigment. SG: Sporangium.

Table 2. Physiological properties of strain TI-1.

Nitrate reduction	Positive
Starch hydrolysis	Negative
Gelatin liquefaction	Positive (slow)
Milk coagulation (26°C)	Positive
Milk peptonization (26°C)	Negative
Melanoid pigment formation	
Tryptone - yeast extract broth (ISP 1)	Negative
Peptone - yeast extract - iron agar (ISP 6)	Negative
Tyrosine agar (ISP 7)	Negative
Casein decomposition	Negative
Tyrosine decomposition	Negative
Xanthine decomposition	Negative
Growth temperature (optimum)	18~35°C (24~30°C)
NaCl tolerance	≦2%

Fig. 1. Time course of aculeximycin production in a 500-ml Erlenmeyer flask.



spherical to oval in shape and 10~30 μm in size, as shown in Plates 1 and 2. Sporangiospores were arranged in a single coil in the sporangia. They were oval to elliptical in shape and nonmotile. The cultural characteristics of strain TI-1 on various media are shown in Table 1. The strain produced white to pale yellow aerial mycelium. Substrate mycelium was colorless to yellowish gray and soluble pigment was not produced. Physiological properties of the strain are summarized in Table 2. Glucose, arabinose, xylose, fructose, rhamnose, inositol, galactose, mannose, sucrose, cellobiose, lactose, trehalose, raffinose, dextrin, sodium succinate, glycerol and melibiose were utilized, but dulcitol, inulin and sodium acetate were not. Utilization of maltose, soluble starch and salicin was doubtful. Cell wall analysis of strain TI-1 was performed by the methods described by BECKER *et al.*[6] and LECHEVALIER *et al.*[7]: *meso*-diaminopimelic acid and a trace of madurose were found to be the constituents. These results showed that the cell wall type of this strain is type III.

The results of the taxonomic studies indicated that strain TI-1 belongs to genus Streptosporangium. Among known species of genus Streptosporangium, *Streptosporangium albidum* Furumai, Ogawa and Okuda[8] resembles the strain TI-1 in morphological, cultural and physiological characteristics. Therefore, it is concluded that the present strain TI-1 is a strain of *S. albidum* and designated *S. albidum* TI-1. The progeny has been deposited in the KCC Culture Collection and assigned the accession number KCC A-0240.

Fermentation

One loopful of strain TI-1 growth was inoculated into a 500-ml Erlenmeyer flask containing 80 ml of a seed medium composed of 1.0% glucose, 1.0% glycerol, 1.0% sucrose, 0.5% oatmeal, 2.0% soybean meal, 0.5% Casamino Acids (Difco), 1.0% pressed yeast and 0.1% $CaCO_3$ (pH 7.0, before sterilization). The flask was incubated on a rotary shaker at 28°C for 5 days. A 2-ml aliquot of the culture from the Erlenmeyer flask was inoculated into a 500-ml Erlenmeyer flask containing 80 ml of a production medium composed of 3.0% glucose, 0.05% L-asparagine, 0.05% K_2HPO_4 and 0.2% beef extract (Difco) in distilled water (pH 7.0, before sterilization) and fermentation was carried out at 28°C for 12 days with shaking on a rotary shaker. The maximal potency, approximately 1,250 μg/ml, was obtained after 10 days of fermentation. Antibiotic production during fermentation was monitored by the paper-disc agar-diffusion method using the culture filtrate and *Staphylococcus aureus* FDA 209P and by insecticidal

assay using the first instar of mosquito larvae according to the method described by IKEMOTO[9].

A typical time course of the fermentation in a 500-ml Erlenmeyer flask is shown in Fig. 1.

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