CANDIPLANECIN, A NEW ANTIBIOTIC FROM *AMPULLARIELLA REGULARIS* SUBSP. *MANNITOPHILA* SUBSP. NOV.

I. TAXONOMY OF PRODUCING ORGANISM AND FERMENTATION

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A soil isolate of actinomycete, strain No. 43871 produced a new antifungal antibiotic, candiplanecin. Pale brownish to yellow orange color of colonies on agar media, the formation of bottle-shaped, cylindrical sporangia bearing motile spores and the presence of *meso*-DAP and glycine in the cell wall ascribed this strain to genus *Ampullariella*. From its morphological characteristics together with the cultural and physiological features, this strain was determined to be a new subspecies of *Ampullariella regularis* and designated as *Ampullariella regularis* subsp. *mannitophila* subsp. nov. (FERM-P No. 5646). Production of candiplanecin was carried out by conventional submerged culture, in which 2 μg/ml as the highest antibiotic titer was obtained.

In the course of our screening program for new antibiotics produced by organisms of the family *Actinoplanaceae*, a strain of *Ampullariella*, No. 43871 isolated from a soil sample collected at Nogi-gun, Shimane Prefecture, Japan, was found to produce an antifungal antibiotic, candiplanecin.

It is well known that many antibiotics are produced by rare groups of actinomycetes, but it is difficult to isolate these actinomycetes such as genus *Ampullariella, Actinoplanes etc.* because of their poor population in the nature, and slow growth under conventional cultural conditions used for streptomycetes. For the selective isolation of genus *Ampullariella*, PC agar plate containing novobiocin[1] was successfully used and 46 strains of genus *Ampullariella*, including candiplanecin-producing organism, were isolated from 714 soil samples and 81 natural water samples.

The antibiotic is produced by conventional submerged culture in 600-liter fermentor and the maximal potency of the antibiotics, 2 μg/ml, was obtained after 43~93 hours of fermentation. The antibiotic was named candiplanecin because the antibiotic was primarily active against *Candida albicans* and was produced by the strain of family *Actinoplanaceae*.

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

Taxonomic Studies of Strain No. 43871

Morphological and physiological properties of strain No. 43871 were determined by use of conventional media and methods described by SHIRLING and GOTTLIEB[2], along with several supplementary tests. Observations of the culture were made after incubation at 28°C for 2 weeks unless otherwise stated. Color names were assigned according to "Guide to Color Standard" (A manual published by Nippon Shikisai Kenkyusho, Tokyo, Japan). The characteristics of strain No. 43871 were compared with those of the known species of actinomycetes described in "The Actinomycetes, Vol. 2" by WAKSMAN,

Plate 1. Scanning electron micrograph of strain No. 43871 on potato extract - carrot extract agar (PC agar) at 28°C for 10 days. (A mark equals 10 μ).

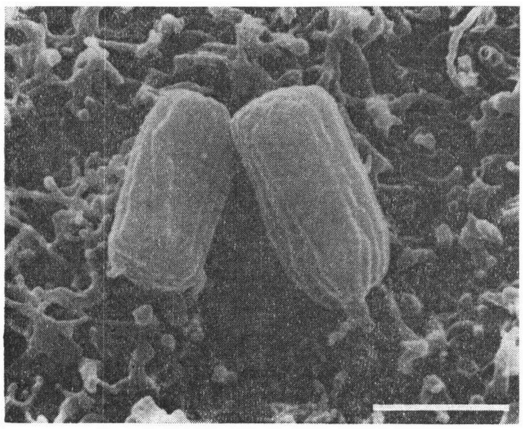
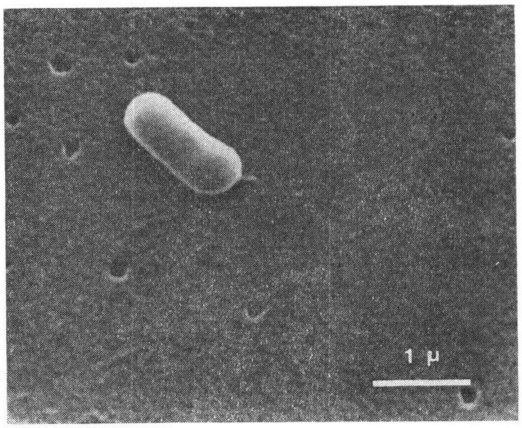


Plate 2. Scanning electron micrograph of zoospore of strain No. 43871. (Potato extract - carrot extract agar, 28°C, 14 days).



"The ISP Reports" by SHIRLING and GOTTLIEB, "BERGEY's Manual of Determinative Bacteriology (8th *ed.*) and other recent literatures concerning taxonomy of the family *Actinoplanaceae*.

Strain No. 43871 formed sporangia abundantly on oatmeal, potato extract - carrot extract, inorganic salts - starch and tomato paste - oatmeal agars. The sporangia are cylindrical in shape and $3.5 \sim 6.2 \times 7 \sim 16$ μ in size as shown in Plate 1. When a few drops of sterile water were placed on the surface of the culture on which formation of sporangia was observed and the culture was covered with cover slip, migration of spores was observed at the earliest after 30 minutes. The zoospores with polar flagella were spherical to rod in shape, $0.7 \sim 2.0 \times 1.1 \sim 2.5$ μ in size as shown in Plate 2.

The cultural characterization of strain No. 43871 on various media is shown in Table 1. Development of the aerial mycelium was not observed on almost all of the media tested. The color of the substrate mycelium was usually yellowish orange to yellowish brown and soluble pigment, pale brown in color was produced in several media. Physiological properties and utilization of carbon sources are summarized in Tables 2 and 3, respectively. D-Glucose, L-arabinose, D-xylose, D-fructose, L-rhamnose, D-galactose, D-mannose, sucrose, soluble starch and D-mannitol were utilized, while *i*-inositol, raffinose and cellulose were not. Cell wall analysis of strain No. 43871 was performed by the method described by BECKER *et al.*[3] and *meso*-diaminopimelic acid and glycine were detected as major constituents. This is in accordance with cell wall type II. The results of the taxonomic studies mentioned above show that strain No. 43871 belongs to genus *Ampullariella*. Among known species of genus *Ampullariella*[4~6], the characteristics of strain No. 43871 are closely related to those of *A. regularis* except for the only difference in utilization of carbon sources. Strain No. 43871 utilized D-mannitol but *A. regularis* did not. This difference was not sufficient to consider strain No. 43871 as a new species. From the above, strain No. 43871 was named *Ampullariella regularis* subsp. *mannitophila* subsp. nov. Progeny of the type strain of *A. regularis* subsp. *mannitophila* No. 43871 have been deposited in the Fermentation Research Institute, Agency of Industrial Science and Technology, Ibaragi, Japan, with as accession number of FERM-P No. 5646.

Fermentation

One loopful growth of strain No. 43871 was inoculated into a 500-ml Erlenmeyer flask containing 80 ml of the medium composed of glucose 2.0%, glycerol 1.0%, oatmeal 0.5%, soy bean meal 2.0%,

Table 1. Cultural characteristics of strain No. 43871.

Yeast extract - malt extract agar (ISP 2)	SM: Abundant, raised, yellowish orange AM: None SP: Pale brown SG: None
Oatmeal agar (ISP 3)	SM: Abundant, flat, yellowish orange AM: None SP: None SG: Abundant
Inorganic salts - starch agar (ISP 4)	SM: Abundant, flat, dull yellowish orange AM: None SP: None SG: Abundant
Glycerol - asparagine agar (ISP 5)	SM: Good, flat, pale yellowish orange AM: None SP: None SG: Poor
Tyrosine agar (ISP 7)	SM: Good, flat, grayish yellow brown AM: None SP: Pale brown SG: None
Nutrient agar (Difco)	SM: Moderate, flat, pale yellowish brown AM: Poor, rudimentary SP: Pale brown SG: None
EMERSON'S agar	SM: Moderate, flat, light brown AM: None SP: None SG: None
Water agar	SM: Moderate, flat, brownish white AM: None SP: None SG: Abundant
HICKEY-TRESNER'S agar	SM: Abundant, raised, yellowish brown AM: None SP: Pale brown SG: Abundant
BENNETT'S agar	SM: Abundant, raised, yellowish brown AM: None SP: None SG: Abundant
Sucrose - nitrate agar	SM: Moderate, flat, pale brown AM: Poor, rudimentary, pale brown SP: None SG: Poor
Glucose - asparagine agar	SM: Good, flat, pale orange AM: Poor, rudimentary SP: None SG: Poor
Tomato paste - oatmeal agar	SM: Moderate, raised, pale orange AM: None SP: None SG: Abundant
Glycerol - glycine agar	SM: Moderate, flat, dull yellowish orange AM: None SP: None SG: None
Glucose - nitrate agar	SM: Good, flat, pale yellowish brown AM: Poor, rudimentary SP: None SG: None
Potato extract - carrot extract agar	SM: Good, flat, pale yellowish orange AM: None SP: None SG: Abundant

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

Casamino acids 0.5%, pressed yeast 1.0%, $CaCO_3$ 0.1% and Nissan Disfoam CB-442 (Nissan Chemical Co., Japan) 0.01%. The pH of the medium was adjusted to pH 7.0 before sterilization. The flasks were incubated on a rotary shaker at 28°C for 120 hours. A 35-ml aliquot of the culture from the Erlenmeyer flask was inoculated into a 2-liter Erlenmeyer flask each containing 700 ml of the medium composed of glucose 0.5%, glycerol 2.5%, pressed yeast 1.0%, soy bean meal 1.0%, corn steep liquor 1.0%, KH_2PO_4 1.0%, $CaCO_3$ 0.5% and trace salts solution ($FeSO_4 \cdot 7H_2O$ 100 mg, $MnCl_2 \cdot 4H_2O$ 100 mg and

Table 2. Physiological properties of strain No. 43871.

Nitrate reduction	positive
Starch hydrolysis	negative
Gelatin liquefaction	positive (slow)
Milk poptonization 26°C	negative
37°C	positive (pH 6.9)
Milk coagulation 26°C	negative
37°C	positive
Melanin formation	
Tyrosinase reaction	positive (++)
Tryptone - yeast extract broth (ISP 1)	positive
Peptone - yeast extract - iron agar (ISP 6)	positive
Growth temperature range*	15~40°C
Casein decomposition	positive
Tyrosine decomposition	negative

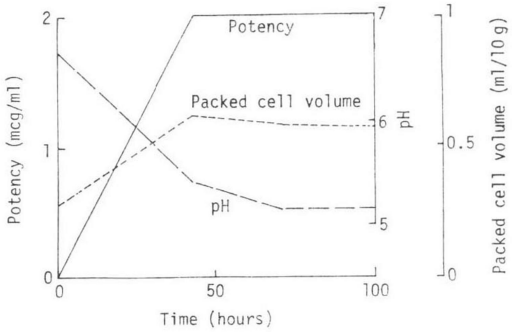
* Yeast extract - malt extract agar (ISP 2)

Table 3. Carbon utilization pattern of strain No. 43871.

D-Glucose	+	D-Mannose	++
L-Arabinose	+	Sucrose	+
D-Xylose	+	Raffinose	−
D-Fructose	+	D-Mannitol	+
L-Rhamnose	+	Soluble starch	+
i-Inositol	−	Cellulose	−
D-Galactose	+	Control	−

++: Strongly positive utilization. +: Positive utilization. −: Negative utilization.

Fig. 1. Fermentation of candiplanecin in 600-liter fermentor.



$ZnSO_4 \cdot 7H_2O$ 100 mg in 100 ml H_2O) 1 ml/liter. The pH of the medium was adjusted to pH 7.2 before sterilization. A 2.5 liters aliquot of the culture from the 2-liter Erlenmeyer flasks was further inoculated into a 100-liter fermentor containing 50 liters of the medium described above and incubated for 48 hours with agitation (390 rpm) and aeration (50 liters/minute) at 28°C as a seed culture. After inoculation of 15 liters of the seed culture into a 600-liter fermentor containing 300 liters of the medium composed of the same composition as the seed culture, fermentation was carried out for 80~120 hours with agitation (240 rpm) and aeration (300 liters/minute) at 28°C. Mycelial growth was expressed as the packed cell volume (ml) after centrifugation of 10 g of the culture broth at 3000 rpm for 15 minutes. Antibiotic production during fermentation was monitored by the disc-plate method using *Candida albicans* YU 1200 as a test organism. An example of a typical time course of the fermentation in 600-liter fermentor is shown in Fig. 1. The maximal potency of the antibiotic, approximately 2 μg/ml, was obtained after 43~93 hours of fermentation.

References

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