AABOMYCIN A, A NEW ANTIBIOTIC. III

TAXONOMIC STUDIES ON THE AABOMYCIN PRODUCING STRAIN, *STREPTOMYCES HYGROSCOPICUS* SUBSP. *AABOMYCETICUS* SEINO SUBSP. NOV.

AKIO SEINO, HIDEO SUGAWARA, SHIROH SHIRATO
and TOMOMASA MISATO*

Kaken Chemical Co., Ltd., Tokyo, Japan
* The Institute of Physical and Chemical Research,
Yamato-machi, Saitama Prefecture, Japan

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A new subspecies of *Streptomyces hygroscopicus* was isolated from a peaty-soil collected in Bibai, Hokkaido, Japan in July, 1965, and numbered as 325-17. The isolate has been named *Streptomyces hygroscopicus* (JENSEN) WAKSMAN *et* HENRICI subsp. *aabomyceticus* SEINO. Freeze-dried cultures of the type strain 325-17 have been deposited in the Fermentation Research Institute, Chiba, Japan and the American Type Culture Collection, Rockvill, Maryland, U. S. A. where they have been assigned accession numbers as FERM-P No. 166 and ATCC 21449, respectively.

A new antifungal antibiotic, aabomycin A, has been isolated from fermented mashes of streptomycete strain 325-17 and its fermentative production, physico-chemical properties and biological activities reported previously[1,2]. The purpose of this paper is to describe the characteristics of *Streptomyces hygroscopicus* subsp. *aabomyceticus* SEINO subsp. nov. for valid publication according to Rules 11 and 12 of the International Code of Nomenclature of Bacteria, 1966[3]. Additionally, the value of using the scanning electron microscope for characterization of fine structure of spore surface showing some irregularities is discussed. Scanning electron microscopy appears to be a useful alternate to preshadowed carbon repligraphy which requires rather complicated techniques and considerable operational time.

Methods

Most of the general procedures suggested by SHIRLING and GOTTLIEB[4] have been followed in this report except that inocula for various media consisted of thrice-washed mycelia. Other media, both synthetic and complex which are described by WAKSMAN[5] also were used. Difco Bacto Agar was employed as a soldifying agent for the various media except that Difco Noble Agar was used for the carbohydrate utilization test. Color comparisons of 21-day old cultures were made under a xenon lamp (Xenolight DL-500, Murakami Shikisai Kenkyusho, Tokyo, Japan) with reference to the Color Harmony Manual (CHM) 4th edition[6] and noted, in succesion, by the CHM code number and CHM name. If the corresponding color name and number of the ISCC-NBS Method of Designating Colors (U. S. Department of Commerce, 1955) was defined, the ISCC-NBS color name and number also noted. Preshadowed carbon replicas of spores were prepared in accordance

with the method reported by DIETZ and MATHEWS[7,8] and carbon repligraphs were taken with a Hitachi HS-8 electronmicroscope. Gold pre-coated spores were prepared by following procedure for scanning electronmicrography:

1 : Touch gently the surface of well-matured aerial growth of test organisms with Scotch Tape No. 465 pre-attached to a copper block.
2 : Place the copper block in a vacuum jar and evacuated to less than 1 μHg.
3 : Incline the copper block at an angle at $\pm 45°$ from horizontal and at the same time rotate slowly during the period of shadowing with Au-metal.
4 : Transfer the block to a JSM-2 scanning electronmicroscope (Japan Electron Optics Co., Ltd. Tokyo, Japan) and photograph.

Results

Micromorphology: Some media such as inorganic salts-starch agar (ISP), yeast extract-malt extract agar, BENNETT's agar, yeast extract-starch agar (yeast extract 2 g, soluble starch 10 g, agar 15 g, distilled water 1,000 ml, pH 7.0) were useful for observation of micromorphology of this strain. Vegetative mycelia does not fragment into

Plate 1. Electronmicrograph of spores of strain 325-17 yeast extract-starch agar, 10 days ($\times 24,000 \times 1/2.5$).

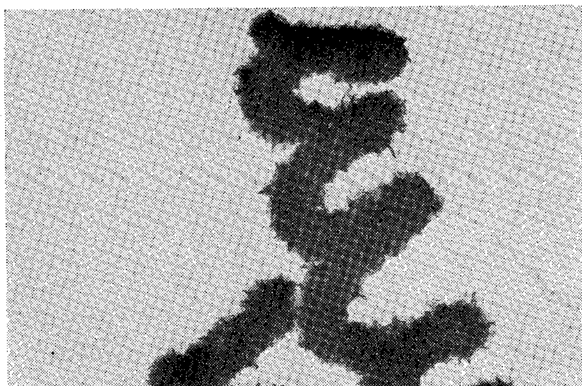


Plate 2. Carbon repligraph of spores of the strain 325-17 (direct magnification $\times 22,000 \times 1/2.5$).

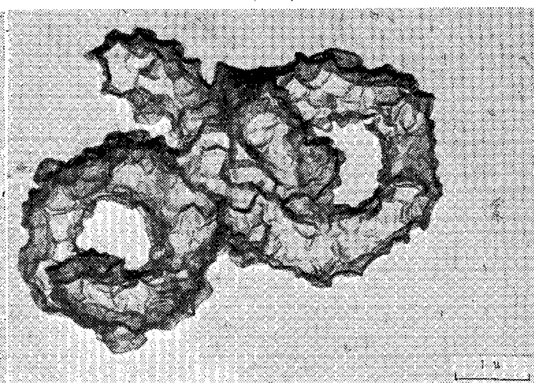


Plate 3. Scanning electron micrograph of spores at lower magnifications (Au-precoated, accelerating voltage 25 KV, magnification $\times 3,000 \times 1/1.5$).

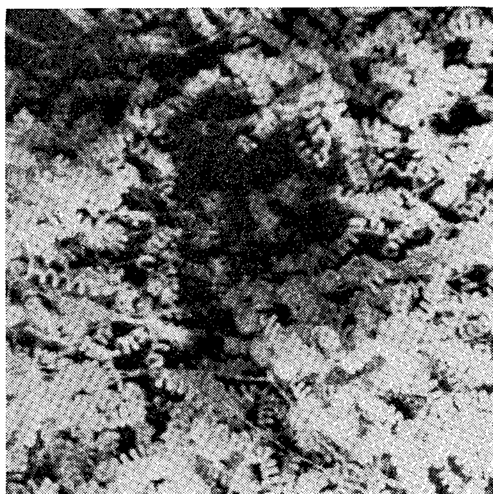
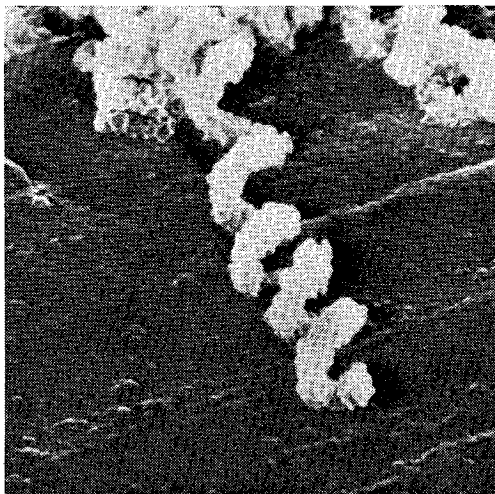
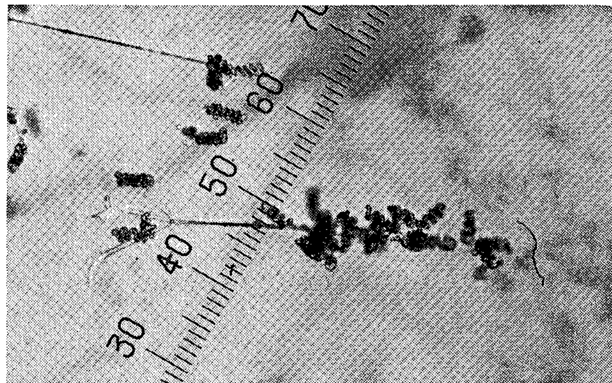


Plate 4. Scanning electron micrograph of spores at higher magnification ($\times 8,000 \times 1/1.5$) (BENNETT's agar, 14 days, Au-precoated, accelerating voltage 25 KV).



cocoid or bacillary elements in liquid media such as glucose-nutrient broth *etc*. Spores (Electron micrograph, Plate 1); non-segmented, covered with capsule-like membrane, ornamentation of surface is fairly irregular, possibly warty to coarsely spined. Carbon repligraph (Plate 2) and scanning electronmicrograph (Plates 3 and 4) show the surface of spores of this strain to be very rugose and indicate morphological similarity to spores of the Type I group of *Streptomyces hygroscopicus* reported by DIETZ and MATHEWS[7,8]. Sporophores; short side branches located along straight or flexuous main hyphae, terminating in closed coils (*Spirales*) of two or more volutions (Plate 5). Sporophores arranged singly, in pairs, or occasionally in tufts along axial mycelia, but no evidence of true verticillate branching. Average diameter of coils is 2.5 μ. There was no evidence of sporangia, zoospores, ball-like bodies[9], coremia or sclerotia. Hygroscopic patches were observed with cultures on some media such as glucose-asparagine agar and asparagine-dextrose agar.

Plate 5. Sprophores of strain 325-17 by light microscope (1 scale 2.5 μ, yeast extract-malt extract agar, 28°C, 10 days).



Cultural and physiological characteristics: Cultural and physiological studies were carried out at 28°C and the results read at 21 days unless otherwise stated.

Tables 1, 2 and 3 present results obtained in a series of various cultural and physiological tests of strain 325-17. These results place strain 325-17 within the *Streptomyces hygroscopicus* Type I group suggested by DIETZ and MATHEWS[7,8]. However, strain 325-17 differs from the description of *S. hygroscopicus* (WAKSMAN, 1961) as follows: (1) strain 325-17 does not produce yellowish soluble pigment in sucrose-nitrate agar, whereas *S. hygroscopicus* produces such pigment; (2) strain 325-17 gives a weakly positive reaction in melanin formation agar and tyrosine agar ISP; (3) strain 325-17 produces the antibiotic, aabomycin A, whereas any members of microorganisms belonging to the group of S. *hygroscopicus* do not produce aabomycin A, so far reported.

Two antibiotics, flavucidin and venturicidin which are similar to aabomycin A have been reported by SHIBATA *et at.*[10], and RHODES *et al.*[11] separately. However, no taxonomic details have been reported upon flavucidin-producer *Streptomyces* sp. No. 14402. On the other hand, RHODES *et al.*[11] briefly reported that venturicidin is produced by three distinct strains of streptomycete having a general resemblance to *Streptomyces griseolus* and *S. halstedii* and some affinity with *S. xanthophaeus*. As described above, aabomycin A producing strain 325-17 can be identified as one of the species belonging to S. *hygroscopicus*, thus strain 325-17 is easily differentiated from venturicidin producers. Therefore, the strain 325-17 represents a new subspecies of *S. hygroscopicus* whose name is proposed as *Streptomyces hygroscopicus* (JENSEN)

Table 1. Cultural characteristics of strain 325-17

Medium	Growth	Aerial mycelium	Soluble pigment	Remarks
Sucrose-nitrate agar plate	Good, spreading, 2 ea (Light Wheat)	Thin, powdery, almost white with 1 ea (Light Yellow) center	None	
Glycerol-nitrate agar plate	Good, spreading, 2 fb (Buff) (Light Yellow, 86)	Thin, powdery, white with g (Gray) center	Pale Yellow	
Glucose-asparagine agar plate	Good, spreading, 2 ea (Light Wheat)	Abundant, powdery, 3 fe (Silver Gray) with moistened patches	None	Hygroscopic
Asparagine-dextrose agar plate	Same as glucose-asparagine agar	Abundant, powdery, 5 fe (Ashes) (Light Grayish Reddish Brown, 45) with black moistened patches	None	Hygroscopic
Glycerol-asparagine agar plate	Good, spreading, 2 ca (Light Ivory) (Pale Yellow, 89)	Thin, powdery, 1 ba (Yellow Tint) (Pale Yellow, 89) to 3 dc (Natural)	Very faintly pink	
Glycerol-calcium malate agar plate	Restricted, elevated 2 ic (Light Gold)	Abundant, powdery, 1 ic (Citron Yellow) to 3 ig (Beige Brown) (Grayish Yellowish Brown, 80)	None	Calcium malate partially solubilized
Inorganic salts-starch agar plate (ISP)	Abundant, spreading, 1 la (Lemon Yellow)	Abundant, powdery, 4 li (Beaver) (Brownish Gray, 64)	Light Yellow	Hydrolytic activity is positive
Tyrosine agar plate (ISP)	Good, elevated, 3 le (Cinnamon) to 4 nl (Dark Brown)	Abundant, powdery, 4 li (Beaver) (Brownish Gray, 64)	3 le (Cinnamon)	Tyrosinase reaction weakly positive
Yeast extract-malt extract agar plate (ISP)	Abundant, 1 ia (Lemon Yellow) to more dark colored	Abundant, powdery, 4 li (Beaver) (Brownish Gray, 64)	Light yellow	
Oatmeal agar plate (ISP)	Good, restricted, not elevated, 1½ ca (Cream)	Good, powdery, 4 li (Beaver) (Brownish Gray, 64)	None	
BENNETT's agar plate	Elevated, good, 1 ga (Light Yellow)	Abundant, powdery, nearly 5 fe (Ashes) (Light Grayish Reddish Brown, 45)	1 ca (Lemon Yellow)	
Yeast extract-starch agar plate	Abundant, elevated, spreading, 1 ga (Light Yellow) with dark colored center	Abundant, powdery, 3 ig (Beige Brown) (Grayish Yellowish Brown, 80)	Light Yellow	
Nutrient agar (Difco Bacto Nutrient Agar)	Moderate, 1½ ca (Cream)	Good, powdery, white	None	Non-chromogenic

WAKSMAN *et* HENRICI, 1948 subsp. *aabomyceticus* SEINO subsp. nov.

Description of *Streptomyces hygroscopicus* (JENSEN) WAKSMAN *et* HENRICI, 1948 subsp. *aabomyceticus* SEINO subspecies novis

a. a. b. o. my. ce. ti. cus. M. L. adj. of aabomycin

Micromorphology: vegetative mycelia never fragments into bacillary or coccoid elements, branched finely.

Sporophores: occur singly, in pairs or sometimes in whorl-like arrangement but not as true veticils and terminated in compact sinistrose coils with two or more volutions.

Spores: non-segmented, possibly warty or spiny, surface irregular and much folded.

Special characteristics: hygroscopic.

Color of vegetative mycelia: Light Wheat (CHM, 2 ea) with sucrose-nitrate agar.

Color of aerial mycelia: Beaver (CHM, 4 li) (Brownish Gray, ISCC-NBS, 64) with

Table 2. Physiological reactions of strain 325-17

Test	Media employed	Response
Melanin formation	Peptone iron agar (Difco) +0.1% yeast extract	Negative (4 days)
	Melanin formation agar stab (WAKSMAN, No. 42)[5]	Doubtful (21 days) Light brown
	Tyrosine agar (ISP)	Doubtful (21 days) Light brown
Tyrosinase reaction	Tyrosine agar (ISP)	Doubtful
Xanthine decomposition	Xanthine agar (Gordon & Mihm)	Negative
Hydrogen sulfide production	Peptone iron agar (Difco) +0.1% yeast extract	Negative (over night)
Nitrate reduction	Glucose-nitrate broth	Negative
	Nitrate broth (Difco)	Negative
Liquefaction of gelatin	20% Difco Bacto Gelatin	Positive
Liquefaction of serum	Difco LÖFFLER serum medium	Positive
Digestion of milk	10% Difco Bacto skim milk	Positive
Cellulolytic activity	Filter paper+CZAPEK broth without carbon source	Negative
Temperature range	BENNETT's agar, pH 7.0	No growth at 5°C & 50°C
Oxygen requirement	Glycerol nutrient broth under CO_2 atmosphere	Aerobic

Table 3. Carbohydrate utilization pattern of strain 325-17 in PRIDHAM and GOTTLIEB's synthetic agar

Carbohydrate	Response*
Arabinose	±
Glucose	++
Galactose	++
Glycerol	++
Lactose	++
Levulose	++
Mannose	++
Maltose	++
Melezitose	−
Melibiose	++
Raffinose	++
Rhamnose	++
Sorbose	−
Sucrose	++
Trehalose	++
Xylose	++
Aesculin	−
Salicin	−
Inulin	+
Adonitol	++
Dulcitol	−
i-Inositol	++
Mannitol	++
Sorbitol	−

* ++ : strongly positive utilization
+ : positive utilization
± : utilization doubtful
− : utilization negative

inorganic salts-starch agar ISP.

Chromogenicity: doubtful positive reactions with tyrosine agar and melanin formation agar and negative reaction with peptone-iron agar.

Soluble pigment other than melanoid: pale yellow with various media.

Physiological properties: starch, gelatin and milk are hydrolyzed; cellulolytic activity, xanthine decomposition, nitrate reduction and hydrogen sulfide production are negative; carbohydrate utilization patterns positive for most compounds tested.

Temperature relationship: mesophilic.

Oxygen relationship: aerobic.

Habitat: soil.

Antagonistic properties: produces aabomycin A.

Type strain: strain 325-17 is designated as the type of this new subspecies and has been deposited in the Fermentation Research Institute, Chiba, Japan and the American Type Culture Collection, Maryland, U. S. A. where they have been assigned accession numbers as FERM-P No. 166 and ATCC 21449, respectively.

Discussion

The advantages of the scanning electron microscope to perform a micromorphological examination upon the fine structure of actinomycetes has been discussed by WILLIAMS and DAVIES[12]. It have been reported that spore surfaces of *S. hygroscopicus* shows apparently wrinkled using carbon repligraphy with the usual transmission type electronmicroscope (DIETZ and MATHEWS[7,8]). Using adhesive Scotch Tape No. 465, specimens of *S. hygroscopicus* subsp. *aabomyceticus* were prepared simply by a direct printing method from petri dish cultures. The selection of a suitable adhesive has an important effect to resultant images. Probably, the adhesive, Scotch Tape No. 465, does not generate some undesirable

refractions of primary- and secondary-electrons which disturb resultant images. Although, the scanning electronmicroscope has a disadvantage in markedly lower resolving power than the transmission type electronmicroscope, the direction of rotation of coils can be clearly observed at lower magnifications. However, even with scanning electron-micrography the segmentation of catenulate spores of strains 325-17 has not yet been observed. In scanning electron-micrography, a metalic coating of spore surfaces under higher vacuum is rather indispensable procedure for clear resultant images. The coating process, however, needing some carefulness is an undoubtedly defect in scanning electron-micrography. Because of the lower resolving power of the scanning electronmicroscope, the condition of the instrument also influences resultant images. In spite of the disadvantages, scanning electron-microscope can be very useful for observation of fine structure of spore surfaces, especially for characterization of strains belonging to the *S. hygroscopicus* Type I group, and for routine examination of sporophore morphology.

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