NOTE

PRODUCTION OF SPECTINOMYCIN BY A NEW SUBSPECIES OF *STREPTOMYCES HYGROSCOPICUS*

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In the course of screening for new antibiotics, spectinomycin (abbreviated as SPM)-like activity was discovered. The producing culture, strain No. MK 43 was unlike the known SPM-producers, *Streptomyces spectabilis*[1] and *S. flavopersicus*[2]. Since SPM has recently become more important for the treatment of disease such as gonorrhea[3], identification of the products and the organism was carried out.

Fermentation of MK 43

The composition of seed medium and fermentation medium was the same (glucose 2 %, yeast extract 0.5 %, peptone 0.5 % and $CaCO_3$ 0.1 % in tap water, pH 7.2 before autoclaving). Eighteen liters of fermentation medium in a 30-liter jar fermentor were inoculated with 10 % inoculum size and fermented for 5 days at 30°C with aeration and agitation.

Isolation and Purification of Active Substances

The fermented broth contained two activities, XK 43-1 and XK 43-2. One of them, XK 43-1, was adsorbed on IRC 50 (H^+) resin, and eluted with 0.5 N HCl. The further purification procedure was similar to that of SINCLAIR and WINFIELD[4].

The other active component, XK 43-2 was unadsorbed and recovered in the effluent from the IRC 50 (H^+) resin. The active substance was extracted with 1-butanol and purified according to MANN *et al.*[5].

Identification of XK 43-1 and XK 43-2

The materials from the eluate (XK 43-1) or the effluent (XK 43-2) of the IRC 50 (H^+) resin were examined by paper chromatography and thin-layer chromatography. The Rf values of XK 43-1 and XK 43-2 were identical with those of SPM and of hygromycin A (HM-A), respectively. The physicochemical properties of XK 43-1 and XK 43-2 were also identical with those of SPM and HM-A, when the elemental analysis, melting point, optical rotation, U.V. spectrum, I.R. spectrum and solubility were compared with literature values for SPM[4,6] and HM-A[5,7]. Then XK 43-1 and XK 43-2 were definitely identified as SPM and as HM-A, respectively.

Taxonomy of *Streptomyces* sp. No. MK 43

The taxonomy of *Streptomyces* sp., strain No. MK 43, isolated from a soil sample obtained near Lake Sagami, Kanagawa, Japan, was studied according to the method of SHIRLING and GOTTLIEB[8]. Media for the observation of physiological and cultural characteristics were prepared according to SHIRLING and GOTTLIEB[8] and also WAKSMAN[9]. All media were incubated at 30°C for 14 days unless otherwise stated. The color determination of the cultures were made with reference to the Color Harmony Manual, 4th edition[10]. Morphological observations of mature culture on inorganic-salts-starch agar *etc.* were made with a light microscope and an electron microscope. Samples were observed directly with a conventional electron microscope but when a scanning electron microscope was used, samples were pre-coated with gold and carbon (50 : 50).

Table 1 shows cultural characteristics of MK 43 on various media. The substrate mycelium is generally yellowish brown and aerial mycelium is white or gray. On some media such as glucose-asparagine agar or inorganic-salts-starch agar, the aerial mycelium turns black with formation of hygroscopic area after 2 or 3 weeks incubation. Physiological characteristics are summerized in Table 2. A loopful of the aerial mass of MK 43 was inoculated to ISP Medium 1 in a tests tube and shaken

Table 1. Cultural characteristics of strain MK 43

Medium	Growth	Aerial mycelium	Soluble pigment
Sucrose-nitrate agar	poor; no color	good; white (a)~gray (5 ih)	none
Glucose-asparagine agar	poor; no color	moderate; white (a)~gray (5 fe)	none
Glucose-nutrient agar	moderate; yellowish brown (2 ie)	good; white (a)	none
Oat meal agar	poor; no color	moderate; gray (10 fe), (3 nl)	none
Starch agar	moderate; yellowish brown (2 gc)	good; white (a)~gray (5 ih)	none
Tyrosine agar	poor; no color	poor; gray (b)	none
Yeast ext.-malt ext. agar	moderate; yellowish brown (2 gc)	good; gray (5 ih), (3 ba)	none
Glycerol-asparagine agar	moderate; yellowish brown (2 ec)	good; white (a)~gray (5 fe)	none

Plate 1. Sporophores of strain MK 43 by light microscope. (oatmeal agar medium, 30°C, 7 days)

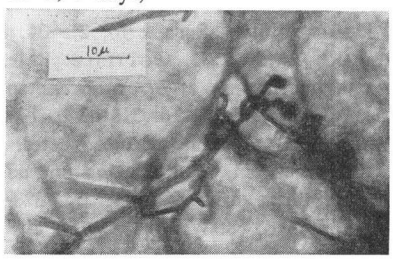
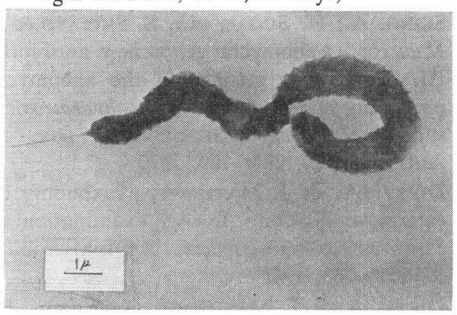


Plate 2. Electron micrograph of spores of strain MK 43. (inorganic salts starch agar medium, 30°C, 14 days)

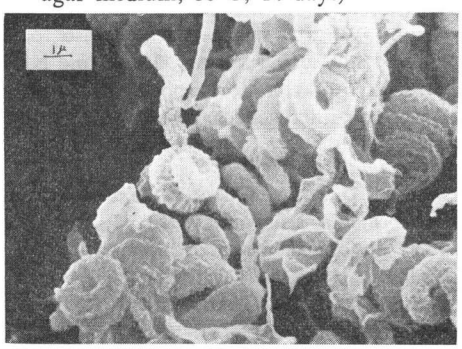


for 2 days for the test of optimum growth temperature. Some media such as inorganic-salts-starch agar and yeast extract-malt extract agar were used for the morphological observation. The aerial mycelium is developed from the substrate mycelium with simple branching. The sporophores occur along the aerial axis occasionally in tufts, the top of which makes a compact spiral (Plate 1).

Table 2. Physiological properties of MK 43

Utilization of carbon sources	± glucose, fructose, mannitol, glycerol, mannose + salicin ± xylose, sucrose, lactose − arabinose, *i*-inositol, rhamnose, raffinose
Growth temperature	25°~45°C
Liquefaction of gelatin	positive
Hydrolysis of starch	positive
Milk peptonization	positive
Chromogenecity	no soluble pigment on tyrosine agar medium and tryptone-yeast extract agar medium; slightly brown pigment on peptone-yeast extract iron agar medium

Plate 3. Scanning electron micrograph of spores of MK 43. (inorganic salts starch agar medium, 30°C, 14 days)



Zoospores, sporangia and screlotia were not observed. Spores are not segmented, and the surface is warty (Plate 2). Scanning electron microscopy shows the surface of spores to be rugose (Plate 3). Scanning electron microscopy was used for the observation of spore surface according to SEINO[11] instead of preshadowed carbon repligraphy of MATHEWS and DIETZ[12]. Judging from the data obtained above, the strain MK 43 belongs to the *S. hygroscopicus* Type I group of MATHEWS and DIETZ[12,13]. However, MK 43 differs from other strains of *S. hygroscopicus* in several points.

1) None of the strains of *S. hygroscopicus* produces SPM, although some strains in this species have been reported to produce HM-A[14]. MK 43 produces SPM as the main product and HM-A as a minor product.

2) *S. hygroscopicus* does not form melanoid pigments in peptone-yeast-iron agar, whereas MK 43 forms slightly brown pigments in this medium.

3) *S. hygroscopicus* utilizes rhamnose well as a carbon source, whereas MK 43 does not utilize it.

Besides strains of *S. hygroscopicus* cited above, *S. noboritoensis*[7] has been also reported as a HM-A producer, and *S. spectabilis*[1] and *S. flavopersicus*[2] have been reported as SPM producers. These three species are, however, definitely different from *S. hygroscopicus*. The strain, MK 43, was therefore determined to be a new subspecies of *S. hygroscopicus* and named *Streptomyces hygroscopicus* (JENSEN) WAKSMAN *et* HENRICI, 1948 subsp. *sagamiensis* YAMAMOTO subsp. nov. A type strain of this new subspecies has been deposited in the American Type Culture Collection, Maryland, U.S.A. with acession number ATCC 21703.

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