I-11, A NEW ANTIBIOTIC TAXONOMY OF THE STRAIN I-11, ISOLATION, FERMENTATION, BIOLOGICAL ACTIVITY AND CHEMICAL CHARACTERIZATION OF THE ANTIBIOTIC PRODUCED

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A new antibiotic I-11, was isolated from the mycelial cake of strain I-11, an antibiotic-producing microorganism isolated from soil samples collected in the vicinity of Madras, India. The strain exhibits several modes of growth that differ from those of the Streptomycetes and Actinomycetales, but similar to certain fungi[1].

Strain I-11 grows fairly well on all of the media employed according to the ISP criteria outlined by GOTTLIEB and SHIRLING[2]. Unless otherwise stated, the experiments to determine the cultural characteristics were carried out at 28.5°C for 13~15 days in a pH environment between 7 and 8. The gelatin stab culture was observed after incubation at room temperature for 18~20 days.

Microscopic examination of the culture on an inorganic salts starch agar revealed thick, closely packed and straight, tuft-like aerial mycelium (Fig. 1). The electron micrographs showed spores with a smooth surface (Fig. 2). Scanning electron microscopic examination of the colony grown on tryptic soy agar revealed two different modes of growth. The non-septate substrate mycelium (Fig. 3), which at certain modes grew as small bunches of straight aerial mycelium and then formed small spore-like structures (Fig. 4) as aerial spore chains typical of *Streptomyces* sp. At the same time some of the fragmented aerial mycelium fused and formed large globular structures, from which another aerial mycelium emerged and segmentation was repeated again (Fig. 5).

The cultural characteristics of strain I-11 are shown in Table 1. The ability of the organism to utilize carbon sources was investigated according to the method described by PRIDHAM and

Fig. 1.

Fig. 2.

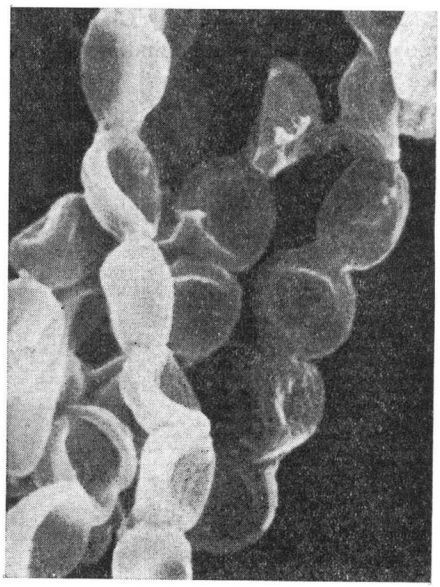

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Fig. 3.

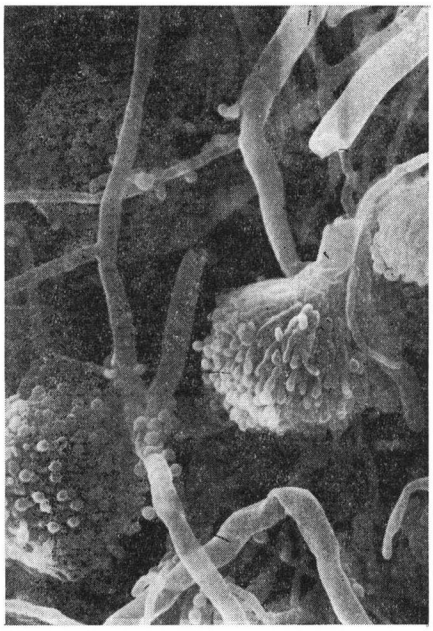


Fig. 4.

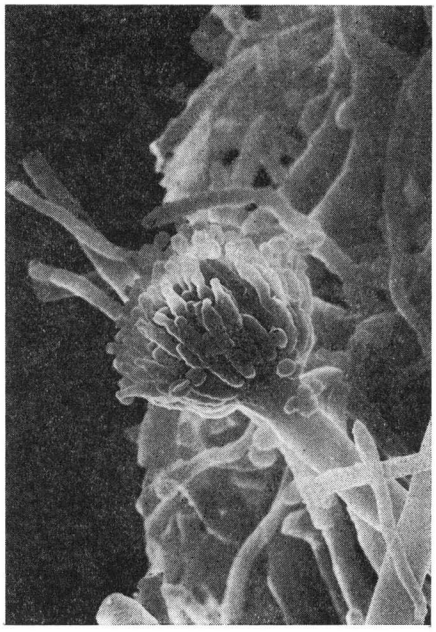
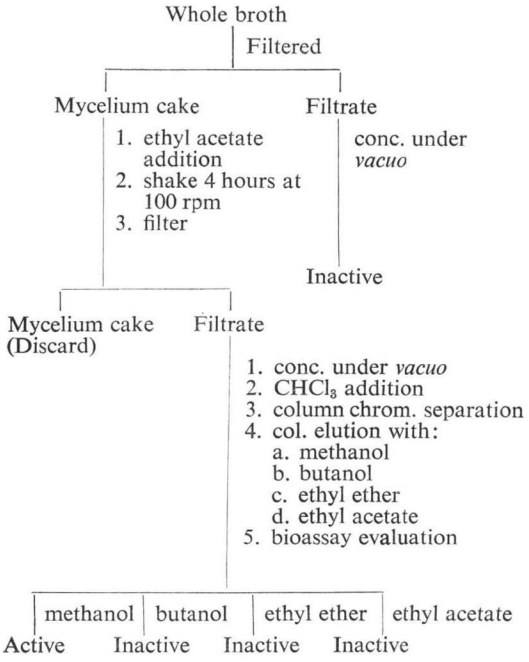


Fig. 5.

Fig. 6. Isolation and purification sequence for I-II.



GOTTLIEB[8]. Strain I-11 utilized arabinose, dextrose, fructose, mannitol, raffinose, sucrose and xylose. On most media, yellow centered substrate mycelium developed moderately and the aerial mass was white. A yellowish soluble pigment was produced on paste oatmeal agar, dextrose asparagine agar, and CZAPEK-DOX agar. Strain I-11 did not liquefy gelatin, and the melanin reaction was negative.

Streptosporangium by WAKSMAN[4], the 8th edition of "BERGEY's Manual of Determinative Bacteriology"[5], and according to the key of NONO-

Table 1. Cultural characteristics of strain I-11.

Medium	Amount of growth and degree of sporulation	Aerial mycelium		Substrate mycelium	Soluble pigment
		Formation	Color		
Inorganic salt starch agar	Excellent, sporulation abundant	Abundant	Brown frequently white	Not distinguished	None
BENNETT'S agar	Moderate, sporulation moderate	Good	Brown	Brown	None
Tyrosine agar	Excellent, sporulation abundant	Excellent	Brown	Not distinguished	None
Oatmeal agar	Fair, sporulation fair	Fair	Brown	Brown	Yellow
Tomato paste oatmeal agar	Excellent, sporulation excellent	Good	Deep brown	Not distinguished	Yellow
Glycerol arginine agar	Good growth, sporulation	Good	Brown	Yellow	None
Dextrose asparagine agar	Good sporulation good	Good	Brown	Brick red	Yellow
Potato plug	Poor, sporulation poor	Poor	Gray	Not distinguished	None
Nutrient agar	Poor, sporulation poor	Poor	White	Tan	None
CZAPEK-DOX agar	Good, sporulation and growth	Good	Brown	Dark black	Yellow

Table 2. Antibacterial activity of the antibiotic substance isolated from isolate No. 11 (by paper disc agar diffusion).

Test organisms	Inhibition zones (diameter in mm)
Bacillus subtilis	35
Serratia marcescens	50
Eschrichia coli	45
Proteus vulgaris	40
Pseudomonas fluorescens	35
Salmonella typhi	29
Mycobacterium tuberculosis	32

MURA[6], strain I-11 is considered to be a member of a new genus.

For the production of I-11 (antibiotic), strain I-11 was cultured at 30°C with agitation in fermentation medium composed of 12.5% Pharmamedia (Trader Oil Mill Co., Fort Worth, Texas, U. S. A.) and 12.5% dextrose[7]. I-11 was produced mainly in the mycelium and extracted with ethyl acetate.

The extraction and purification procedure is summarized in Fig. 6. I-11 has a brownish tinge coloration, and melts at 110°C. Molecular formula of $C_{20}H_{27}NOS$ was established by elemental analysis and by mass spectrometric determination of the molecular weight (M.W. 339). I-11 is soluble in most organic solvents and insoluble in water. The UV spectrum in chloroform has absorption maxima at 240 and 265 nm. The IR spectrum (KBr) shows characteristic bands indicating extensive aliphaticity, aromaticity and carbonyl absorption. A comparison of antibiotic I-11 with closely related pigment antibiotics reflects a distinctively different properties and characteristics[8].

The antibacterial activity is shown in Table 2. Antibiotic I-11 was found to be active against T2 DNA phage specific for *Escherichia coli* strain according to the method of KOENUMA[9]. Two zones of inhibition were observed; an inhibitory zone appears in the inner side and a stimulatory zone at the outside.

Comparison with known red, pigmented antibiotics ruled out any similarities in biological activity, and chemical properties.

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