A NEW SPECIES OF *STREPTOMYCES* FROM INDIAN SOILS

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A new species of *Streptomyces* designated as *Streptomyces indicus* CHAKRABARTY which produces abundant aerial mycelium with shrimp-pink spores, antibiotic activity mainly against plant and human pathogenic fungi and melanin pigment, has been described. Its taxonomic relationship with other species of *Streptomyces* as well as the cultural, physiological and biochemical characteristics, are also discussed.

The organism described in this paper was isolated from samples of soil composts from Narit in the district of Howrah, West Bengal, India, in the course of screening for antibiotic-producing actinomycetes. A total of 320 strains were isolated from 42 soil samples collected in this locality. Sixteen cultures showed pigment-producing capacity. The culture was sent to the Institute of Microbiology, The State University, Rutgers, U. S. A., but could not be matched with any known species. The organism studied here is therefore considered to be a new species.

Streptomyces indicus CHAKRABARTY, nov. sp.

This organism possesses a number of identifying characters which distinguishes it from other allied species of *Streptomyces*. The pigment of the mycelium does not diffuse into culture media. The mycelium consists of much branched flexuous hyphae, 1.0～1.6 μ in thickness. Sporophores are flexuous, forming hooks with open loops and primitive spirals; spores in chains, round or oval, 0.6～1.2 $\mu \times 0.5$～0.8 μ (Plates 1 and 2). Spreading growth is formed on glucose-asparagine agar, with abundant aerial mycelium, and shrimp-pink spores. The vegetative mycelium is rose doree and the reverse color scarlet red. Starch is readily hydrolysed; no coagulation of milk with

Plate 1. Aerial mycelium of *Streptomyces indicus* CHAKRABARTY on glucose asparagine agar showing sporophores at young stage (×667)

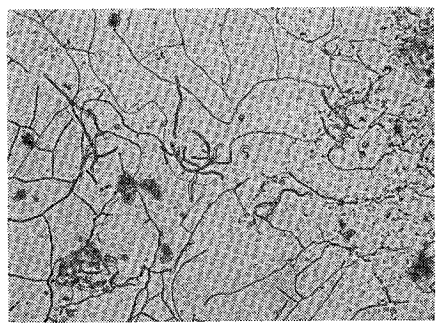
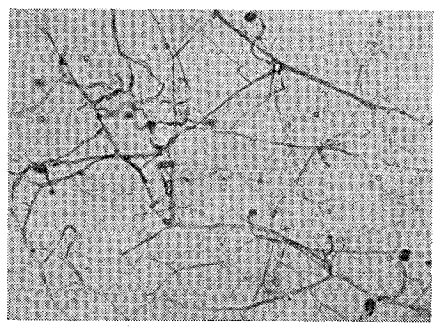


Plate 2. Aerial mycelium of *Streptomyces indicus* CHAKRABARTY showing mature sporophores forming hooks with open loops and primitive spirals (×667)



slow peptonisation and a slightly alkaline reaction. Nitrate is not reduced to nitrite. The proteolytic action on gelatin is weak and liquefaction starts only after the 7th day of incubation. The strain is positive for melanin formation and possesses antibiotic activity against a number of plant and human pathogenic fungi. Optimum temperature for growth and antibiotic production is 28~30°C, at pH 7.0.

The type culture has been deposited in the Institute of Microbiology, The State University, Rutgers, U. S. A., Central Voor Schimell culture and also in the Bose Institute, Calcutta, India as *Streptomyces strain* Ac_{21} 460.

Table 1. Utilisation of carbon sources by *Streptomyces indicus* and *S. albosporeus* A955-Y3

Carbon sources	*S. indicus*	*S. albosporeus* A955-Y3
Glucose	++++	++++
D-Fructose	+	−
Maltose	++++	++++
Lactose	+	+
Sucrose	++	+
Galactose	+++	++
Mannitol	+	+
Raffinose	+++	+
Starch	+++	+++
Xylose	+	+
D-Sorbose	−	−
Na-acetate	−	+
Na-citrate	−	−

Legends : +, ++, +++, ++++ indicate increasing activity.
− indicates absence of activity.

Description

Characteristics on various media

The conventional media for taxonomic studies of *Streptomyces* were employed (WAKSMAN, 1957)[4]. The cultures were incubated at 28°C and the cultural characteristics described according to observations after 14 days of incubation. Nomenclature of color was done according to RIDGWAY's (1912)[3] Color Standards. Detailed description of cultural and physiological character as exhibited on various media are given below :

A. Cultural characteristics

(1) CZAPEK-DOX agar : Good spreading growth, aerial mycelium abundant, spore shrimp pink, vegetative mycelium rose doree, reverse color nopal red, no diffusible pigment.

(2) Nutrient agar : Fair growth, no aerial mycelium, vegetative mycelium pale orange yellow, no diffusible pigment.

(3) Potato dextrose agar : Fair spreading growth, aerial mycelium moderate, spore shrimp pink, vegetative mycelium white, no diffusible pigment.

(4) Oatmeal agar : Good spreading growth, aerial mycelium scanty, vegetative mycelium rose doree, no diffusible pigment.

(5) Egg albumin agar : Good spreading growth, aerial mycelium abundant, spore shrimp pink, vegetative mycelium shrimp pink, reverse color nopal red, no diffusible pigment.

(6) EMERSON's agar : Good spreading growth, aerial mycelium abundant, spore shrimp pink, vegetative mycelium geranium pink, reverse color scarlet, no diffusible pigment.

(7) Calcium malate agar : Fair spreading growth, aerial mycelium moderate, spore shrimp pink, vegetative mycelium colourless, reverse color white, no diffusible pigment, no zone of malate clearing.

(8) Salt nutrient agar : Moderate spreading growth, aerial mycelium abundant, spore shrimp pink, vegetative mycelium rose doree, reverse color nopal red, no diffusible pigment.

(9) Glucose asparagine agar : Good spreading growth, aerial mycelium abundant, spore shrimp pink, vegetative mycelium rose doree, reverse color scarlet red, no diffusible pigment.

(10) Yeast glucose agar : Spreading growth, aerial mycelium abundant, spore shrimp pink, vegetative mycelium Brazil red, reverse color Morocco red, no diffusible pigment.

(11) Starch agar : Spreading growth, aerial mycelium scanty, spore shrimp pink,

Table 2. A comparative study of cultural and physiological characteristics of *Streptomyces indicus*, *S. albosporeus* WAKSMAN *et* CURTIS and *S. albosporeus* A955-Y3

Media		*S. indicus*	*S. albosporeus* WAKSMAN *et* CURTIS	*S. albosporeus* A955-Y3
Glycerol malate agar	G	Moderate, pinkish	Rose to orange red	Pale brownish white
	A	Shrimp pink	White later changing to yellow	White
	SP	None	None	Slight yellowish
Glucose asparagine agar	G	Spreading, pinkish to rose doree	Wrinkled, spreading, red with colorless margin	Pale, yellowish
	A	Abundant, shrimp pink, reverse scarlet red	Appears late, white	White
	SP	None	None	Slight yellowish
Nutrient agar	G	Fair, pale orange yellow	Small, cream colored	Colorless to cream colored
	A	No	No	No
	SP	None	None	None
Starch agar	G	Good spreading, scarlet to Brazil red	Thin, spreading	Spreading
	A	Scanty, shrimp pink	No	White
	SP	None	None	Slight yellowish
	SH	Strong	Strong	Medium to strong
Sucrose nitrate agar	G	Good, spreading, pinkish	Spreading, colorless with pink center	Pale reddish or pinkish
	A	Shrimp pink, sometimes covered with secondary mycelium	White, covering the whole surface, often none	Thin, white with sometimes cottony white mycelium
	SP	None	None	None
Potato plug	G	Spreading, pinkish	Red to brownish grey	Colorless to pale brownish
	A	Abundant, thulite pink	No, sometimes white	No
	SP	None	None	Slight brownish
Gelatin stab	G	Moderate, light brown	Yellow, changing to red	Cream to pale brownish
	A	No	No	No
	SP	None	None	None
	L	Weak	Medium	Medium
Milk	G	Circular ring, pinkish, no coagulation, peptonisation slow	Scanty, pink, no coagulation, no peptonisation	Pale yellowish, no coagulation, peptonisation positive
Cellulose	G	No	No	No
Nitrate reduction	G	None	Fair	None

G : growth. A : aerial mycelium. SP : soluble pigment. SH : starch hydrolysis.
L : liquefaction of gelatin.

vegetative mycelium scarlet, reverse color Brazil red, no diffusible pigment, starch hydrolysed.

(12) Carbon nutrition agar : Good colonial growth, aerial mycelium abundant, spore eosine pink, vegetative mycelium nopal red, reverse color Brazil red, no diffusible pigment.

(13) Cellulose medium : No growth.

(14) Potato plug : Good spreading growth, aerial mycelium abundant, spore thulite pink, no diffusible pigment.

(15) Carrot plug : Good spreading growth, aerial mycelium abundant, spore shrimp pink, no diffusible pigment.

B. Physiological characteristics

(1) Hydrolysis of starch : Hydrolysis of starch started from the 3rd day of incubation and produced a zone of hydrolysis, 38.0 mm in width on the 14th day.

(2) Coagulation and peptonisation of milk : Growth in a circular ring, no coagulation, peptonisation slow, reaction slightly alkaline.

(3) Liquefaction of gelatin: Growth moderate, weak proteolytic action, liquefaction began on the 7th day of incubation.

(4) Decomposition of tyrosine: Slight decomposition.

(5) Production of nitrite: Nitrate not reduced to nitrite.

(6) Production of acid: Acid produced from xylose only.

C. Specific characteristics

(1) Utilization of carbon sources: Glucose, maltose, sucrose, galactose, raffinose and starch were readily utilized and all supported abundant growth. Fructose, lactose, xylose and mannitol were utilized to a lesser extent and gave reduced growth. Sorbose, Na-acetate and Na-citrate encouraged little growth. Only maltose caused some diffusion of the pigment into the culture medium. The results are given in Table 1.

(2) Antibiotic activity: The strain produced antifungal antibiotic substances which were active against *Curvularia lunata*, *Alternaria solani*, *Fusarium oxysporum*, *Fusarium vasinfectum*, *Helminthosporium oryzae*, *Aspergillus niger*, *Candida albicans*, *Epidermophyton floccosum*, *Trichophyton sulphureum*, *Trichophyton rubrum*, *Microsporum gypseum* and *Trichosporon cutaneum*. The strain exhibited no antibiotic activity against either gram-positive or gram-negative bacteria.

The species described above is close to *Streptomyces albosporeus* WAKSMAN *et* CURTIS[5] with regard to the color of the vegetative mycelium, the production of white secondary mycelium, the insolubility of pigment, mode of growth in some media and the property of hydrolysis of starch; but it showed marked differences in the properties of nitrate reduction, liquefaction of gelatin, peptonisation of milk, antagonistic activity against fungi and in the formation of melanin pigment. The antibiotic vinacetin produced by a *Streptomyces* sp., closely related to *S. albosporeus* (OMACHI, 1953)[2] was chiefly active against *Staphylococcus aureus*, *Corynebacterium diphtheriae* and species of *Mycobacterium* but inactive against gram-negative bacteria and fungi. The strain described in the present communication possesses activity only against fungi and not against gram-positive or gram-negative bacteria. Furthermore nitrate is not reduced to nitrite, thus distinguishing it from *S. albosporeus* WAKSMAN *et* CURTIS. Table 2 represents a comparative account of the characteristics of *Streptomyces indicus*, *S. albosporeus* WAKSMAN *et* CURTIS and *S. albosporeus* A 955-Y 3.

A comparison was also made with a stock culture of *S. albosporeus* labelled as A 955-Y 3 obtained from the National Institute of Health, Tokyo, Japan. This culture did not produce the pink color characteristic of the described isolated strain. The stock culture A 955-Y 3 differed from the strain under study in the solubility of the yellowish pigment, in spore morphology, in antagonistic activity as well as in the pattern of utilisation of different carbohydrates. The tip of the aerial mycelium of the strain described here sometimes formed a spiral, while in strain A 955-Y 3 no such spiral was found. Labilomycin, the antibiotic produced by A 955-Y 3 (AKITA *et al.*, 1963)[1], has activity against *S. aureus* and *Mycobacterium phlei* but the strain under investigation showed no such activity against either of the two test organisms. Moreover, it was melanin positive whereas *S. albosporeus* WAKSMAN *et* CURTIS and *S. albosporeus* strain A 955-Y 3 were melanin negative. Therefore, the strain is considered to be a new species and it has been named after the country of its origin.

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