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A PIGMENTED, XYLOSE-UTILIZING STRAIN OF *STREPTOMYCES BOBILI*

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IN a preliminary screening of samples collected from special microsites in Agra, viz. mycorrhizosphere of pine; rhizosphere and rhizoplane of potato, gram, guar and chilly; and sewage and earthworm casts, for the isolation of antibiotic-producing actinomycetes, one isolate, J_4, was found to be strongly antagonistic to *Aschochyta rabiei*, which causes blight of gram, and *Alternaria alternata*, which causes leaf spot on chillies, papaya, sunflower, etc. The materials and methods used were similar to those of the previous communication and ISP procedures[1,2].

The screening of these isolates was done by placing the plugs cut from ten-day-old cultures of the actinomycetes in a petri plate previously seeded with test organisms.

The actinomycete isolate J_4 is a melanin-negative, chromogenic type producing soluble pigments of various shades on natural media. It shows strong amylase and tyrosinase reactions, but no cellulolytic, proteolytic or nitrate reduction activity. It is able to utilize glucose, sucrose, maltose, glycerol and xylose as carbon source. Mannitol, lactose and fructose were poorly utilized.

The vegetative mycelium is white, monopodially branched, forming compact growth in agar media. Aerial mycelium is abundant, white (1-A-7)[3] to pinkish-white (2-A-1)[3] turning to rosy pink (table 1). Sporophores are primitive spirals with open hooks (RA). Spores, measuring 3.4–3.3 × 3.3–1.6 μm, are in chains, oval to cylindrical, with smooth surface configuration.

Biological activity

The activity of the isolate J_4 against other micro-organisms was tested by the streak method. The

Table 1 *Cultural characters of X J_4 isolate of Streptomyces bobili on various media*

Medium	Growth	Vegetative mycelium	Aerial mycelium	Reverse of colony	Remarks
Nutrient agar	Moderate	White	Pinkish white (49-B-1)	Mustard yellow to brown (14-K-9)	Dark brown soluble pigment
Tryptone yeast extract (ISP)	Excellent	White	Light pink (1-B-1)	Blackish brown (8-I-11)	Dark brown soluble pigment
Yeast malt extract	Good	White	Light pink (1-B-1)	Pinkish brown (7-J-11)	Maroon soluble pigment
Inorganic salt starch agar (ISP)	Excellent	White	Pinkish white (1-C-7)	Orangeish pink (2-E-8)	Strongly hydrolyses starch
Oat meal (ISP)	Excellent	Off-white	Pinkish white (1-C-7)	Pinkish brown (7-J-11)	Maroon soluble pigment
Glycerol asparagine (ISP)	Good	Dull white	Pink (49-C-1)	Light maroon (S-A-4)	Maroon soluble pigment
Melanin agar	Moderate	White	Pinkish white (10-A-1)	Light yellow (10-B-5)	Negative
Tyrosine agar	Good	White	Light pink (49-B-1)	Blackish brown (8-A-2)	Positive dark brown soluble pigment
Gelatin agar	Good	White	Light pink (9-A-1)	Rust orange (11-A-10)	Orange soluble pigment negative
PDA agar	Good	White	Pinkish white (49-B-1)	Violet (49-K-9)	Maroon soluble pigment

culture was inoculated by spore suspension as a broad streak about the edge of the petri plate on potato dextrose agar medium. After three days of incubation at 28 ± 2°C, the different microorganisms were streaked at right angles to the J_4 streak. The inhibition zone, if formed, was measured as a clear distance between the J_4 streak and the growth of the test organism.

J_4 was found to be active against *Alternaria brassicae*, *A. brassicicola*, *A. solani*, *A. raphni*, *Aspergillus niger*, *A. sulfureus*, *Fusarium solani*, *Curvularia lunata*, *Phoma palmarum*, *Penicillium janthenellum*, *Cladosporium sphaerospermum*, *Humicola fusco-atra*, *Botrydiplodia theobromae*, *Gleosporium candidum*, *Salmonella typhi*, *Pseudomonas pyocanea*, *Escherichia coli*, *Bacillus subtilis*, *Candida albicans*, *Staphylococcus aureus*, *Shigella* and *Proteus*.

Properties of antibiotic substance

The antibiotic produced by J_4 is thermolabile. With rise in temperature from 40 to 80°C the activity decreases gradually, and at temperatures above 80°C the antibiotic substance gets completely inactivated after 30 min.

On storage the activity is lost rapidly at 35–40°C. The antibiotic can be best stored without any appreciable loss in activity for up to 40 days at pH 7.0 at 7°C. The antibiotic is soluble in *n*-butanol, methanol, isopropyl alcohol, ethanol and ethyl acetate and moderately so in benzene, petroleum ether, toluene and chloroform. It was found to give a single spot in two-dimensional paper chromatography as well as by bio-autographic technique. The antibiotic contained only one component with R_f value 0.733 when the chromatogram was run in butanol:oxalic acid:H_2O (50:2.5 g:50), and with R_f value 0.802 when the chromatogram was run in methanol:ethyl acetate (95:5).

The isolate J_4 was identified as a species of *Streptomyces* as its mycelium does not break up in bacilli or coccoid forms and instead forms a tough, textured mycelium.

The *Streptomyces* isolate described above was found to resemble *Streptomyces bobili*[4,5]. It produces active substance very similar to cinerubin produced by a known strain of *S. bobili*. However, it differs from the latter in the production of pigments of various shades in most natural media (table 1) and in the ability to utilize xylose. It has therefore been designated as a pigmented and xylose-utilizing strain of *S. bobili*.

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SCREENING FOR OLEAGINOUS YEASTS USING REPLICA PRINTING TECHNIQUE COUPLED WITH DENSITOMETRIC SCANNING

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MICROORGANISMS have been used as a source of protein and vitamins[1]. Yeast is commercially produced for animal feed[2]. Oleaginous yeasts are capable of accumulating oil within the cells[3]. Microbes can be exploited to produce fat. The advantages of using microbes are their rapid rates of growth and ability to accumulate fat using naturally occurring substrates. Thus single cell oil has recently acquired importance[4]. With this in view, we have screened selected environments for the presence of oleaginous yeast[5]. For screening a large number of isolates, routine methods like pure culture isolation and extraction of fat by chemical methods from every isolated culture are laborious and time-consuming. There is need for a quick screening method for picking up promising oleaginous strains.