

STUDIES ON THE ANTAGONISTIC ACTINOMYCETES OF THE FOREST SOILS OF PONMUDI

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Soil is a complex heterogenous medium harbouring a varied spectra of microorganisms. Among the microorganisms which have received attention recently are the actinomycetes, which are the producers of the well known antibiotics. They are of particular interest as they possess relatively more antagonistic activity than others. Intensive search for antagonistic organisms among the soil actinomycetes has been in progress in many parts of the world and most of the present day antibiotics in wide use are obtained from soil organisms. Practically little work has been done to isolate and study the antagonistic microorganisms that occur in Kerala soils. Soils from Ponmudi were selected since it is a hilly tract with a cool climate and is possessing a very high nutrient status.

Rangaswamy, Obliswamy and Swaminathan (1967) in their studies on the microbial population in different soil types have found an abundance of antagonistic actinomycetes. Susamma Philip and Sam Raj (1973, 1975) in their studies on different soils of Trivandrum district have found the Ponmudi soils to possess the maximum microbial population at an elevation of 300 m above MSL.

Materials and Methods

The actinomycete population in different soil samples were determined by the soil dilution plate technique as outlined by Timonin (1940). Melted and cooled Kuster's medium (Glycerol or starch - 10.0 g, Casein - 0.3 g, KNO_3 - 2.0 g, NaCl - 2.0 g, $\text{K}_2\text{HPO}_4 \cdot 0.05$ MgSO₄·7 H₂O g, CaCO₃ g, FeSO₄·2 H₂O - 0.01 g, Agar agar - 20.0 g, Distilled water - 1000 ml, pH - ff" 7.0) containing a spore suspension of *Helminthosporium halodes* was immediately added to sterile dishes which contained the soil dilution (Patrick, 1954). The dilution used was 1 in 100000. 1 ml of this diluted suspension was used. The dishes were rotated in a broad swirling motion and incubated at 30°C for 7 days.

Colonies showing clear inhibition zones against *H. halodes* were picked up and cultured in nutrient agar medium. (Peptone 5 g, Beef extract 3 g, Agar agar 15 g, Distilled water 1000 ml, pH 6.8 - 7.0).

The actinomycetes were further tested for antagonism by using different methods (Johnson, Curl, *et. al.* 1959) viz. (1) The antagonist and the test organism were streaked on nutrient agar in petri dishes parallel to each other. (2) The test organism was placed on nutrient agar at 3 equidistant points and the antagonist was seeded in the centre and (3) Cross streak assay method. Here the antagonist was streaked at the periphery of the dish and the test organisms were streaked at right angles to the original streak of the antagonist after 7 days.

The morphological characters of the actinomycetes isolated were studied by growing on slides smeared with inorganic salt starch medium (Gordan and Smith, 1955) and incubated in moist chambers. The antibiotic production was studied by growing the actinomycete in several media like Soyabean peptone broth, Nutrient glucose broth and Yeast extract glucose broth. The medium was dispensed in 250 ml conical flasks, 75 ml/flask and sterilized at 15 lbs pressure per sqinch for 20 minutes. It was then inoculated with spore suspension obtained from 7 day old cultures grown on nutrient slants. The cultures were shaken in a laboratory shaker continuously for 10 days at room temperature. It was then filtered and the residue discarded. *H. halodes* and *Bacillus subtilis* were used as test organisms. They were seeded separately in melted nutrient agar in testubes, mixed thoroughly and poured in to sterile petri dishes. Sterile filter paper discs impregnated with the culture filtrate was kept on the centre. Dilutions from 1/10 to 1/1000 were used and growth observed.

Results and Discussion

Results are presented in Table 1. The actinomycetes, were identified based on their morphological and cultural characters. 40 numbers belonging to different genera viz Streptomyces, Nocardia and Micromonospora were obtained. Majority of them were found to be Streptomyces. One of the Streptomyces spp which showed the maximum inhibition of 25 mm for *H. halodes* and 38 mm for *B. subtilis* was taken up for further study. This was screened against various pathogenic and saprophytic fungi as well as bacteria including phytopathogenic ones.

Of the various media tried nutrient gfcucose broth and soyabean peptone broth were found to give maximum antibiotic and mycelial production. Production of antibiotic was detected from the 4th day onwards. This was assayed by drawing one flask at random every day. From this the culture solution was drawnout, filtered under aseptic conditions and different dilutions were made. Sterile filter paper discs impregnated with the above dilutions were kept in the centre of petri dishes seeded with the test organism. The clear

Table 1
Inhibition of growth by *Streptomyces* sp

Test organism	Particulars	Inhibition in ram.
<i>Helminthosporium oryzae</i>	Rice Pathogen	20
<i>H. halodes</i>	Coconut "	25
<i>Pestalotia palmarum</i>	(5) "	18
<i>Corynespora cassicola</i>	Sesamum "	15
<i>Aspergillus niger</i>	Soil saprophyte	—
<i>Penicillium</i> sp	" "	—
<i>Saccharomyces cerevisiae</i>	Brewer's yeast	22
<i>Staphylococcus</i> sp	Gram negative bacteria	30
<i>Escherichia coli</i>	" "	25
<i>Xanthomonas oryzae</i>	" "	25
<i>Bacillus subtilis</i>	Gram positive bacteria	38

zone of inhibition noticed earlier gradually decreased from the 7th day onwards. Inhibition of growth was noted upto a concentration of 1/500. Further concentrations showed no activity.

The antagonistic actinomycetes isolated from the soils mostly belonged to the genus *Streptomyces*. In Annamalai soils also *Streptomyces* are the predominant naturally occurring actinomycetes (Rangaswamy *et al.*, 1967). That *Streptomyces* spp and also the antibiotics derived from some of them can inhibit the growth of certain plant pathogen is already known (Trinici and Gull, 1970). The *Streptomyces* sp which showed inhibitory property against a variety of fungi and bacteria was however found to be ineffective on soil saprophytes viz. *A. niger* and *Penicillium* sp. Saprophytic fungi in general exhibit considerable resistance to streptomycin is evident from earlier works.

The *Streptomyces* isolate C₆ studied by Rangaswami *et al* (1967) has also shown considerable resistance to soil saprophytes while gram positive and gram negative bacteria including phytopathogenic ones were inhibited.

Forest soils of Ponnudi were screened for antagonistic actinomycetes. One of the *Streptomyces* sp which showed the maximum antifungal and antibacterial activity was tested against a number of plant pathogenic fungi and bacteria. Nutrient glucose broth and soyabean peptone broth were found to be most suitable for the production of antibiotics.

സംഗ്രഹം

പൊൻമുടി വനപ്രദേശത്തുള്ള മണ്ണിലെ സൂക്ഷ്മാണുക്കളെപ്പറ്റി നടത്തിയ ഗവേഷണത്തിൽനിന്നും അതിൽ ധാരാളം വിരോധസ്വഭാവം കാണിക്കുന്ന ആക്ടിനോമൈസറ്റകൾ ഉണ്ടെന്നു കാണാൻ കഴിഞ്ഞു. ഇവയിൽ ഒന്നിനെ പരീക്ഷണശാലയിൽ വളർത്തിയതിൽനിന്നും ഉപദ്രവകാരികളായ സൂക്ഷ്മാണുക്കളുടെയും മറ്റും വളർച്ചയെ സാരമായി തടയുന്നതായി കാണുകയുണ്ടായി.

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