



Isolation of *Streptomyces* from tobacco soils that show antimicrobial activity

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ABSTRACT: Twelve isolates of *Streptomyces* spp. from tobacco soils were screened for the production of antibiotics, of which two were found to be efficient. Growth pattern of the two potential isolates was studied and their antimicrobial activity was tested against *Aspergillus flavus*, *A. niger*, *Pythium aphanidermatum*, *Bacillus subtilis* and *Pseudomonas fluorescens*. Isolate 2 was found to be more effective and caused maximum inhibitory zone of 108 mm after 120 hrs against *P. aphanidermatum*. The area of the inhibitory zone observed in both the isolates was directly proportional to the age of the culture for up to 120h.

KEY WORDS: Antibiotics, antimicrobial activity, growth pattern, *Streptomyces*, tobacco soils

INTRODUCTION

Actinomycetes populations form an important component of soil microflora. Waksman and Woodruff (1940) reported soils as the rich source of antagonistic *Actinomycetes* and mentioned that antibiotics liberated by *Actinomycetes* exert inhibitory effects on bacteria and fungi which can be demonstrated by agar streak method (Arai, 1976). The most abundant actinomycetes in the soil are *Streptomyces* spp., which may form > 2/3 of the colonies on dilution plates, with *Nocardia* spp. accounting for up to 1/3 and *Micromonospora* 5% (Alexander, 1961). Lechevalier and Lechevalier (1967) isolated 5000 *Actinomycetes* from 16 soils, of which >95% were *Streptomyces* and only *Nocardia* and *Micromonospora* exceeded 1%. Other genera, although not abundant, were widely distributed in the soils. Formulations of a *Streptomyces* as a biological control agent for *Rhizoctonia* damping off in tobacco seedlings were developed for the first time from vegetative propagules obtained from actively growing, non-sporulating liquid cultures. Alginate beads, durum flour (starch) granules and talcum powder based formulations of this new *Actinomycetes* antagonist (*Streptomyces* Di-944) isolated from the rhizosphere of field-grown tobacco have been developed (Siva Saba Ratnam and Traquair, 2002). This investigation is an attempt to screen *Streptomyces* spp. isolated from tobacco soils and evaluate them for their growth inhibitory effect on bacteria and fungi.

MATERIALS AND METHODS

Soil samples were collected in sterile bags from 6-10 cm depth due to high microbial activity. The samples

were drawn from southern light soils (SLS) of Prakasam district, northern light soils (NLS) of West Godavari district and traditional black soils (TBS) of East Godavari district. The soil samples were pretreated with calcium carbonate and then dried at 45°C for 1h in order to reduce the incidence of bacteria and moulds. This modified procedure was found to be suitable for the isolation and identification of *Streptomyces* spp. (Tsao *et al.*, 1960; Pirous *et al.*, 1999; Alexander, 1961). Soil dilution plate technique was employed for the isolation and enumeration of *Streptomyces* spp. using asparagines-glycerol salts agar (AGS) medium (Pridham and Lyons, 1980). Suppression of growth of bacteria and molds was observed in AGS medium supplemented with streptomycin and amphotericin-B (Williams and Davis 1965). Pure cultures of *Actinomycetes* isolated from the soils were identified up to generic level by comparing the morphology of spore-bearing hyphae spore chain as described in Bergey's manual (Locci, 1989). Slide culture method was employed for morphological studies on *Streptomyces* species (Williams and Cross, 1971).

Streptomyces sp. found to be dominant in tobacco soils was transferred to seed medium containing (gL⁻¹) glucose 4, yeast extract 4, malt extract 10, calcium carbonate 2, pH 7.00 and incubated at 27°C for one week.

After 24h, the seed inoculum was transferred to a fermentation medium comprising (gL⁻¹), glucose 40, proteose 5, peptone 9, calcium carbonate 6, ammonium sulfate 3.5, ammonium chloride 3, manganese sulphate 0.1, and cobalt chloride 0.005 (pH 7.00) and incubated at 27°C for one week to study the growth pattern at 24 hour intervals. Chloroform extracts of culture filtrates

(50 ppm) were concentrated and used for testing their antimicrobial activity against (1) *Aspergillus flavus*, (2) *A. niger*, (3) *Pythium aphanidermatum*, (4) *Bacillus subtilis*, and (5) *Pseudomonas fluorescens*. Antimicrobial activity of these isolates was ascertained by filter paper disc method (Alexander and Strete, 2001; Narayana *et al.*, 2004).

RESULTS AND DISCUSSION

Out of the 12 isolates of *Streptomyces* spp., two were found to be dominant and grew well on AGS medium. Since the AGS medium was supplemented with Streptomycin and amphotericin-B, it prevented the growth of bacteria and moulds and facilitated the development of *Streptomyces*

manure treated plot of permanent manurial trial experiment of TBS of Central Tobacco Research Institute farm. The other two isolates from TBS, four isolates from NLS and other four from SLS are very slow growing and did not show any antagonism against the test organisms. Slide culture studies revealed the presence of substrate mycelium as well as aerial mycelium, as observed by Narayana *et al.* (2004). Isolate 2 was white coloured and powdery in appearance and the other isolate 1 was pale pink. The pink coloured isolate produced dark brown pigment diffusing into the medium. The white colonies did not show any pigmentation.

The two isolates showed similar growth pattern except that isolate 1 was comparatively sluggish. In

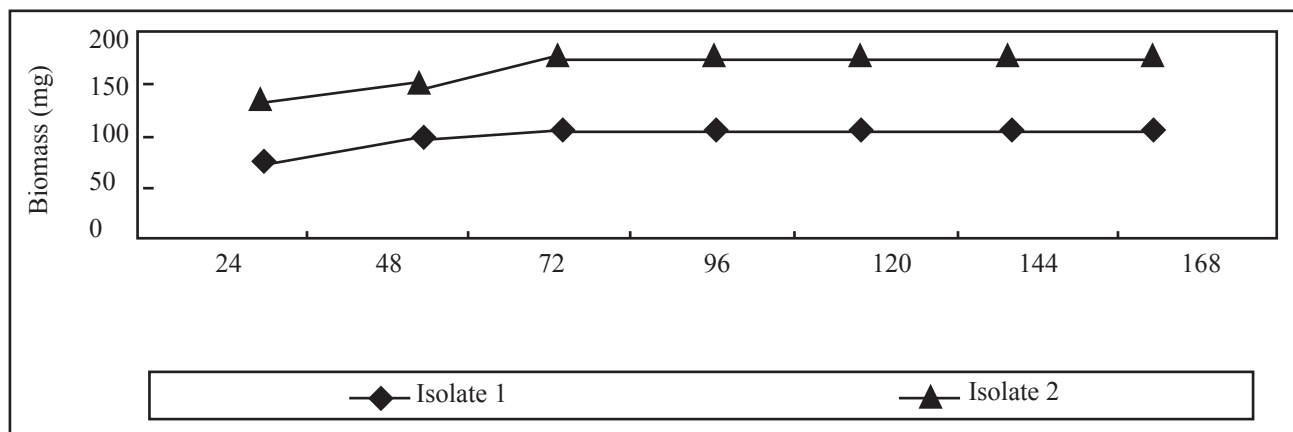


Fig. 1. Growth pattern of *Streptomyces* isolates

alone. Both the isolates (Isolate 1 and Isolate 2) were isolated from the traditional black soils of tobacco. Isolate 2 was isolated from the soil sample collected from farmyard

the isolate1, log phase lasted for 96h and the stationary phase lasted up to 144h (Fig. 1), while in case of isolate 2 the log phase lasted up to 72h and declined later.

Table 1. Antimicrobial activity of chloroform extracts of *Streptomyces* isolates

Age of culture (hrs)	Area of inhibitory zone (mm ²)									
	<i>A. niger</i>		<i>A. flavus</i>		<i>P. aphanidermatum</i>		<i>Bacillus subtilis</i>		<i>P. fluorescens</i>	
	Isolate 1	Isolate 2	Isolate 1	Isolate 2	Isolate 1	Isolate 2	Isolate 1	Isolate 2	Isolate 1	Isolate 2
24	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
48	0.0	0.0	0.0	0.0	0.0	36.0	0.0	0.0	0.0	0.0
72	34.0	48.0	39.0	52.0	42.0	58.0	0.0	5.6	0.0	6.7
96	49.0	63.0	46.0	74.0	58.0	92.0	0.0	18.0	0.0	79.0
120	72.0	98.0	58.0	89.0	62.0	108.0	0.0	25.9	0.0	24.6
144	24.0	83.0	45.0	78.0	51.0	86.0	0.0	29.4	0.0	0.0
168	10.0	52.0	15.0	56.0	21.0	74.0	0.0	12.0	0.0	0.0
SEM ±	0.11	0.05	0.06	0.05	0.07	0.46	NS	0.07	NS	0.02
CD at 5%	0.34	0.16	0.17	0.14	0.20	2.31	NS	0.21	NS	0.07
CV %	4.6	1.5	2.1	1.37	2.38	9.35	NS	4.15	NS	1.7

The data on the antimicrobial activity of the chloroform extract of the two isolates are presented in Table 1. The extract of 1-day-old culture did not show any antifungal activity but the one from 48h old culture inhibited *P. aphanidermatum*. Both the isolates showed inhibitory effect on *Aspergillus flavus*, *A. niger*, and *P. aphanidermatum*. But the isolate 1 showed no inhibitory effect on *B. subtilis* and *P. fluorescens*. The increasing trend of both the isolates on test organisms was maintained for up to 120h. Growth of all the test organisms was inhibited to a great extent by the extract obtained from 120h old culture as shown in Table 1, except *Bacillus*. The subsequent decline in antifungal activity was commensurate with the decrease in the growth of the two isolates. *Aspergillus flavus*, *A. niger*, *P. aphanidermatum*, *B. subtilis*, and *P. fluorescens* were highly sensitive to the extracts of *Streptomyces* isolate 2, as reflected in the form of inhibitory zones. Isolate 1 turned out to be less active as it inhibited the growth of only *P. aphanidermatum*, *A. flavus* and *A. niger* and it was not effective against the bacterial test organisms such as *B. subtilis* and *P. fluorescens*. The maximum inhibitory zone of 108 mm² was observed in the case of *P. aphanidermatum* when treated with the extracts of *Streptomyces* isolate 2. The two isolates of *Streptomyces* obtained from tobacco soils were inhibitory to both bacteria and fungi. Broad-based screening of more and more *Streptomyces* spp. will lead to identification of promising biocontrol agents.

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