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A SOIL ACTINOMYCETE ANTAGONISTIC TO *CORTICIUM SALMONICOLOR* CAUSING PINK DISEASE OF RUBBER

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Actinomycetes from the rhizosphere soils of five clones of rubber were tested against *Corticium salmonicolor*, the pathogen causing pink disease on rubber. One of the isolates had an inhibition zone of 40 mm when tested in agar medium and caused lysis of the mycelium upto a distance of 20 mm from the inoculated point, in 72 h. Sterile rubber twigs treated with the actinomycete also prevented the growth of *C. salmonicolor*. The actinomycete was found to survive in the bark of rubber tree for one month under field conditions. Application of actinomycete broth culture on infected trees indicate promise in controlling pink disease.

Key words:- *Hevea brasiliensis*, Pink disease, *Corticium salmonicolor*, Biological control, Actinomycete, India.

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INTRODUCTION

Pink disease of rubber [(*Hevea brasiliensis*) (Willd. ex Adr. de Juss.) Muell. Arg.] caused by the fungus, *Corticium salmonicolor* Bark. & Br., is one of the serious diseases. Application of 10 per cent Bordeaux paste, 0.25 per cent Thiride in petroleum jelly (Edathil and Radhakrishna Pillai, 1976) or 2.5 per cent Calixin in 1 per cent ammoniated rubber latex (Edathil and Kuruvilla, 1983) is the recommended control measure. Though fungicides are indispensable to attain high agricultural productivity, there is a growing concern about their harmful effect to man and wild life. Environmental pollution is another aspect of concern and of late, biological control of plant diseases is considered safe and economic. Among the various biocontrol agencies, actinomycetes are important as they produce antibiotics. A survey conducted by the Rubber Research Institute of India showed that soils of rubber plantations are rich with actinomycetes antagonistic to pathogens of rubber (Kochuthresiamma *et al*, 1988). On testing the actinomycetes isolated from the rhizosphere of rubber and soil for antagonism against *C. salmonicolor*, one isolate recorded very high antagonistic activity. This paper describes the possible use of this actinomycete for controlling the pink disease of rubber.

MATERIALS AND METHODS

Source of actinomycete

Actinomycete isolates from the rhizosphere of five clones of *Hevea* were subjected to cross streak assay against *C. salmonicolor* following the method of Grove

and Randall (1955) in potato dextrose agar (PDA) medium. The actinomycete was streaked as a ribbon and allowed to grow for five days. The inoculum of the pathogen, *C. salmonicolor*, was streaked perpendicular to the antagonist and after three days of incubation the zone of inhibition was measured. One of the isolates, which showed 40 mm inhibition zone was chosen for detailed investigations.

Growth inhibition of the pathogen on rubber twigs

Pieces of rubber twig, 7 cm long and 2 cm in diameter, placed in 250 ml Erlenmeyer flask containing 10 ml of distilled water were sterilised by autoclaving. One set of 12 pieces was inoculated with the actinomycete by dipping the twigs in 10 days old broth culture, followed by inoculation with *C. salmonicolor* mycelial growth in PDA, 5 mm diameter, on each piece. Another set of 12 pieces without any treatment with the actinomycete was inoculated with *C. salmonicolor* culture bits to serve as control. They were incubated at room temperature (24±1°C) visually examined periodically for the mycelial growth of *C. salmonicolor* upto 15 days of inoculation.

In another experiment, the pathogen was grown in PDA medium in 250 ml Erlenmeyer flasks for five days. Then autoclaved pieces of rubber twig smeared with the actinomycete culture were placed on the mycelial mat in the flask. Sterile pieces without the actinomycete inoculation, placed on the mycelial mat served as control.

Testing of actinomycetes for lysis

A loopful of an actively growing culture of the actinomycete was inoculated over fully grown mycelial mat of *C. salmonicolor* in petridishes and was incubated for five days. Mycelia from 0.5 cm, 1 cm., 2 cm and 3 cm away from the actinomycete growth were taken at 24 hr intervals for five days and transferred to petriplates containing PDA medium for growth,. Absence of mycelial growth due to lysis by the actinomycete was recorded as negative.

Production of antibiotics

The actinomycete was grown in potato dextrose broth for 10 days at room temperature with intermittent shaking and the culture was sterilised by filtration through a bacteriological filter. The sterile culture filtrate was incorporated into PDA medium at the rate of 1 ml., 2 ml, 4 ml and 6 ml and the final volume was made to 50 ml. Twenty ml of the above medium was poured into 10 cm petriplates and inoculated with the actively growing culture of the pathogen. The plates were incubated at room temperature (24±1°C) and examined upto 7 days for the mycelial growth.

Selection of stickers

The spores of actinomycete grown on PDA were carefully removed, suspended in sterile distilled water, mixed with different sticking agents in combinations given below and applied on two rubber trees per treatment, all around the trunk in 30 cm band.

a) Starch : 8 g in 35 ml water/100 ml actinomycete culture

b) Carboxy-methyl cellulose : 2 g in 35 ml water/ 100 ml actinomycete culture

c) Refined wheat flour : 8 g in 35 ml water/ 100 ml actinomycete culture

d) Field latex : 1:3 (v/v)

e) Pidivyl(Poly vinyl acetate) : 1:3 (v/v)

Samples of bark scrappings from 2.5 x 2.5 cm area were taken on 1st, 10th and 30th day and the actinomycete population was estimated by dilution plate technique.

Field trials

Field experiments on the control of pink disease of rubber by actinomycete were also conducted. The actinomycete was grown on PD broth for 10 days and mixed with paste made of refined flour (80 g in 350 ml boiled water/l of culture). Rubber trees at various stages of infection by pink disease were selected and infected bark was scrapped and smeared with the actinomycetes preparation. Infected trees with Bordeaux paste application were maintained as control. This experiment was conducted on 10 year old plants of clone RRII 105 at Central Experiment Station, Chethackal, Ranni and on four year old plants of clone PB 217 at Kaliyar estate, Thodupuzha during 1988 and repeated on two year old plants of clone PB 311, at Kaliyar estate during 1989.

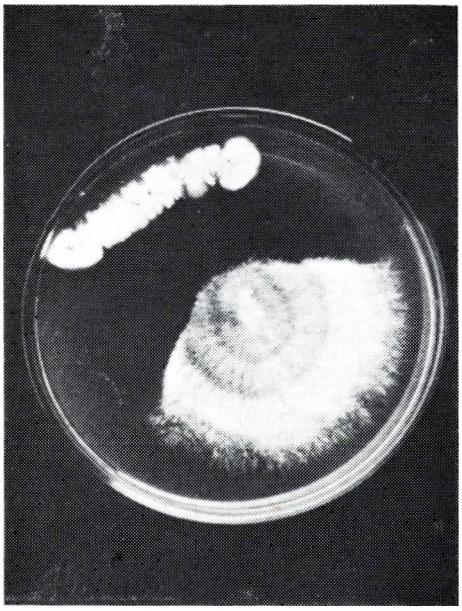
RESULTS AND DISCUSSION

The results on the inhibitory activity of actinomycetes on *C. salmonicolor* are given in Table 1. Out of 107 isolates from different clones of rubber, 51 showed inhibition of varying degrees. Such high percentage of antagonism in the rhizosphere of plants has been reported by Broadbent *et al*, (1971). The maximum zone of 40 mm was exhibited by only one isolate, prolonged incubation of which resulted in browning of mycelia in the edge of the fungus facing the actinomycete ribbon (Fig. 1). The results indicated that rhizosphere of rubber harboured actinomycetes antagonistic to the pathogen, *C. salmonicolor*. Increased population of antagonistic actinomycetes in the rhizosphere is a common phenomenon (Venkatesan and Rangaswami, 1964) as observed in the present study.

Table 1. **Occurrence of actinomycetes antagonistic to *C. salmonicolor* in different clones of rubber.**

Source	Population of actinomycetes $\times 10^8$ $^{-}g^1$	Number tested	% actinomycete showing inhibitory zones			
			1–5 mm	6–10 mm	11–20 mm	>20 mm
Fx 516	125	22	27.27	9.00	0.00	4.55
RRIM 701	251	20	25.00	15.00	5.00	
F 4542	180	22	36.36	18.18	4.22	
PR 107	148	20	10.00	25.00	10.00	
RRIM 600	147	15	33.33	20.00	6.67	
Control (non rhizosphere soil)	71	8	12.50	12.50	12.50	

It is of interest to note that the pathogen failed to grow on sterile rubber twig pieces pre-inoculated with the actinomycete. But in the absence of the actinomycete, it exhibited profuse growth. The actinomycete when inoculated on fully grown mycelia of *C. salmonicolor* caused lysis upto a distance of 20 mm from the inoculated site in three days (Table 2) and mycelia in this area turned to white from pink.



Eig. 1. Actinomycete inhibiting *C. salmonicolor* in agar medium

Table 2. **Lytic action of the antagonist on** *C. salmonicolor*

Time after inoculation hr	Distance (mm) of mycelial sample from the actinomycete		
	10	20	30
24	—	+	+
48	—	—	+
72	—	—	+

— mycelium lysed + mycelium viable

The results of the study on the production of antagonistic principle in liquid media indicated that the antagonistic principle was extra cellular and the culture filtrate even after ten fold dilutions was effective.

The population of actinomycetes applied on bark with stickers were found to be reduced on 10th day of application. But the population with refined flour paste was comparatively more (Table 3). The actinomycete culture, when applied to pink disease affected 10 year old RRII 105 and 4 year old PB 217 trees, controlled the disease (Table 4). However the effect was less than that of Bordeaux paste application in both the clones in 1988 season. But in 1989 season the actinomycete treatment was on par with Bordeaux paste treatment when tested on two year old trees of clone PB 311. The wounds of the bark applied with actinomycete culture healed faster than those treated with Bordeaux paste. Both *in vitro* and *in vivo* studies on the inhibition of the pathogen *C. salmonicolor* thus showed that the actinomycete could be used for the control of pink disease of rubber.

Though the actinomycete grew and inhibited *C. salmonicolor* on sterile rubber wood pieces, it failed to multiply under field conditions. Hence the recovery from disease may be mainly due to the antibiotic principle capable of causing inhibition of

Table 3. **Survival of actinomycetes on tree bark as influenced by stickers (number/sq cm)**

Day	Stickers used				
	Starch	CMC	Refined wheat flour	Latex	Pidivyl
1	560	920	800	160	512
10	320	392	400	0	88
30	24	41	48	0	14

Table 4. **Pink disease recovery**

Year	Clone	Treatment	Number of trees		Recovery (%)
			treated	recovered	
1988	RRII 105	Actinomycete	31	25	80.65
	RRII 105	Bordeaux paste	32	28	87.50
	PB 217	Actinomycete	50	40	80.00
	PB 217	Bordeaux paste	45	41	91.00
1989	PB 311	Actinomycete	60	57	95.00
	PB 311	Bordeaux paste	60	57	95.00

growth and lysis of mycelia. While controlling the pink disease of *Hevea* the actinomycete treatment also hastened the process of healing which is an advantage over Brodeaux paste treatment.

The success of biocontrol depends on the survival and multiplication of the antagonists at the site of application without altering the biological equilibrium (Baker and Cook, 1974). Therefore investigations to find out optimum conditions that favour growth of the actinomycete and production of antagonistic principles *in situ* as well as their effect on nontarget microorganisms are necessary.

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