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In vitro studies on the antagonistic effect of *Streptomyces lydicus* against *Phytophthora meadii.*

Kochuthresiamma Joseph, Jacob Mathew
and R. Kothandaraman
RRI India

ABSTRACT

An actinomycete identified as *Streptomyces lydicus* was tested for inhibition of *Phytophthora meadii* in agar medium and sterile soil. The actinomycete inhibited growth of *P. meadii* in various agar media and reduced the infective propagules in soil. Filter sterilised cultural filtrate and crude extract of antagonistic principle also showed inhibition of *P. meadii* and the test bacteria *Bacillus subtilis.*

Introduction

Abnormal leaf fall disease, caused by *Phytophthora meadii* (Mc. Rae), is a major disease of *Hevea* which accounts for 9 - 16% yield loss[1]. This pathogen is also responsible for diseases such as fruit-rot, shoot-rot[2], patch canker and black stripe in *Hevea*[3]. The primary inoculum of this pathogen is from the soil and dried infected fruits and twigs of the previous season[2]. Many of the soil borne plant pathogens are controlled by antagonistic micro-organisms[4]. Rubber growing soils are rich in actinomycetes showing various degrees of antagonism against *P. meadii*[5]. Actinomycetes are extensively used as biocontrol agents of many diseases[6]. Hence, a study was initiated to test the antagonistic activity of a selected soil actinomycete, *Streptomyces lydicus*, against *P. meadii* under laboratory condition.

Materials and methods

Testing of S. lydicus for inhibition of P. meadii in agar media.

The antagonistic activity of *S. lydicus* against *P. meadii* was studied by the dual culture technique[7]. Actively growing culture of *S. lydicus* was streaked at one side of Petri dishes containing potato dextrose agar, yeast extract glucose agar, soyabean meal agar, czapek's agar and nutrient glucose agar before incubation for 3 days. Seven-day-old 3mm diameter agar culture discs of *P. meadii* were placed in the middle of the Petri dish and allowed to grow. The zone of inhibition was measured and recorded 7 days after inoculation.

Effect of culture filtrate on the growth of P. meadii

50ml of potato dextrose broth medium was prepared in a 250ml Erlin Mayer conical flask, inoculated with actively growing *S. lydicus* and incubated at room temperature in a shaker for 10 days. The broth culture was filtered through Whatman No.1 filter paper and sterilised by passing through a bacteriological filter. The culture filtrate and PDA was mixed in the ratios 10 : 10, 8 : 12, 6 : 14, 4 : 16 and 2 : 18. *P. meadii* was inoculated and growth was recorded.

Extraction of the crude antagonistic active compound and its bioassay

The pH of the filter sterilized culture filtrate was adjusted to 4 and extracted three times with diethyl ether. The pH of the broth was then raised to 10 and again extracted three times with the same solvent. Both extracts were pooled and evaporated to dryness under vacuum using a rotary flask evaporater. The crude antibiotic was tested for activity using *Bacillus subtilis*. Streptomycin assay agar was seeded with 24-hour-old culture of *B. subtilis* and a sterile bioassay disc, soaked with the crude antibiotic extract, was placed over it. The inhibition zone was measured after 24 hours.

Assay of antibiotic by paper chromatography

The crude antibiotic (50ml) was spotted on a Whatman No.1 chromatography paper and a chromatogram was run using a butanol : acetic acid : water mixture (4 : 1 : 1) for 7 hours. This chromatography paper was sliced and placed on an agar plate containing *B. subtilis* in streptomycin assay agar medium. The Rf value of the zone showing inhibition of *B. subtilis* was calculated. The chromatography paper corresponding to the area showing antibiotic activity against *B. subtilis* was cut and placed in PDA plates inoculated with actively growing *P. meadii* and observed for inhibition of growth of the fungi for up to 5 days.

Inhibition of P. meadii by S. lydicus in sterile soil

P. meadii and *S. lydicus* were co-inoculated into moist, sterile soil and examined for the survival of the pathogen after 15 days using the baiting technique by the placement of surface sterilised mature fruits of *Hevea*. The degree of infection was recorded. The control was soil inoculated with *P. meadii* only.

Results

Testing of S. lydicus for inhibition of P. meadii in agar media.

The results of the inhibition of *P. meadii* in different media by *S. lydicus* are given in Table 1. An inhibition zone of 17mm was recorded in PDA, next was nutrient glucose agar. Though czapeks agar showed a 9 mm inhibition zone, *P. meadii* had scanty growth in this medium.

Table 1 ***Inhibition of P. meadii by S. lydicus***

Media	Inhibition zone in mm
Potato dextrose agar	17
Yeast extract glucose agar	2
Soybean agar	2
Czapek's agar	9
Nutrient glucose agar	10

Effect of culture filtrate on the growth of P. meadii

Undiluted culture filtrate as well as the filtrate diluted with PDA in a ratio up to 8 : 12 inhibited *P. meadii*. Pathogen growth was almost the same in higher dilutions (Table 2).

Table 2 *Effect of culture filtrate on growth of P. meadii*

Culture filtrate : PDA (ml)	Growth of *P. meadii* in mm
Undiluted	No growth
10 : 10	No growth
8 : 12	No growth
6 : 14	50
4 : 16	50
2 : 18	53

Extraction of crude antagonistic active compound and bioassay

The crude antagonistic active compound extracted with ether was found to inhibit growth of *B. subtilis*. The inhibition zone was 10mm.

Assay of antibiotic by paper chromatography

The antibiotic travelled slowly in the paper chromatographic assay. The slices with an Rf value of 0.4 were found to inhibit *B. subtilis*. The antagonistic active compound of the same Rf value inhibited the growth of *P. meadii* in PDA.

Inhibition of P. meadii by S. lydicus in sterile soil

Dual inoculation of *P. meadii* and *S. lydicus* in the sterile soil showed suppression of the pathogen. 25% of the rubber fruits were infected by the pathogen in the presence of actinomycete while 100% infection was noted in the control.

Discussion

The cross inoculation studies showed inhibition of *P. meadii* by *S. lydicus* in different media, the maximum being in PDA followed by nutrient glucose agar. The variation in the inhibition of the pathogen in different media shows that the antagonistic activity is influenced by the nutrient status of the medium.

The inhibition of *P. meadii* and the test bacteria, *B. subtilis*, by culture filtrate and crude antagonistic active compound indicates extracellular production of antibiotics. However, the involvement of chitinase by the actinomycete in inhibition of the pathogen cannot be ruled out and needs further investigation.

The inhibitory activity of soil actinomycete against *P. meadii* has been well established; rubber growing soils are rich in such actinomycetes[5]. Propagules of *P. meadii* are reduced by *S. lydicus* when inoculated in sterile soil as evidenced by the reduction in infection of rubber fruits.

Garrett[8] suggested that the incidence of the disease caused by the fungal pathogen can be reduced either by the introduction or augmentation of native antagonists, or by changes in the environmental conditions desired for the multiplication and activity of such organisms, or by a combination of both of the processes. Since *S. lydicus* was isolated from rubber growing soils and it is possible to increase the population to the required level then the population of *P. meadii* should be reduced. The growth and antagonistic activity of soil actinomycetes is greatly influenced by soil organic matter[9,10], and hence actinomycete

inoculation needs soil amendment with organic matter. In young rubber plantations, enrichment of soil with organic matter could be achieved by proper establishment of a suitable leguminous cover.

The present study demonstrates the possibility of controlling *P. meadii* using *S. lydicus* by soil inoculation. In addition to reducing the soil inoculum, stem diseases such as patch canker and black stripe may also be controlled by culture filtrate and crude preparation of antibiotics from *S. lydicus*. Further studies on the survival of *S. lydicus* in soil and the properties of antibiotics are essential to arrive at a conclusion on the use of this actinomycete in disease management.

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