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Effect of Microbial Antagonists on *in vitro* Growth of *Pythium aphanidermatum*

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Abstract: Forty-one isolates of 38 species of fungi and 24 isolates of 8 species of bacteria were tested in dual culture plates. *Penicillium* sp., 2 unidentified bacteria and a sterile fungus were found to inhibit the growth of *Pythium aphanidermatum* producing zones of inhibitions of 7, 11, 4 and 6 mm respectively, around the colony of the pathogen. One strain each of *Rhizobium meliloti*, *Bradyrhizobium* sp., and *Pseudomonas aeruginosa* initially producing zones of inhibition of 15, 20 and 2 mm respectively, but later the pathogen grew and colonies met each other. Similarly, *G. virens* initially produced a zone of inhibition of 20 mm but later on the pathogen over grew the zone of inhibition and colonies intermingled. A strain of *P. aeruginosa* also inhibited the growth of *P. aphanidermatum* without producing zone of inhibition.

Key words: *Pythium aphanidermatum*, microbial antagonists, biological control

Introduction

Of the various soil-borne plant pathogenic fungi, *Pythium* spp., are important pathogens that cause damping-off, root-rot, seed-rot and seedling diseases in economically important crops. Of these, *Pythium aphanidermatum* is reported from a large number of hosts (Plaats-Niterink, 1981) and distributed in the tropical regions of the world. Most efforts on biocontrol of *Pythium* spp., have been made to reduce seed and seedling disease where species of *Trichoderma* , *Penicillium* (Sharif *et al.*, 1988), *Sordaria* (Watanabe, 1991), *Pseudomonads* (Murray *et al.*, 1992), *Bacilli* (Wolk & Sarkar, 1994) and certain actinomycetes (Kusakari & Ueyama, 1975) have proved efficient biocontrol agents against *P. aphanidermatum*. The aim of the present study was to find out new potential antagonists against the pathogen.

Materials and Methods

Cultures of *P. aphanidermatum* and test fungi were prepared on Potato Dextrose Agar medium whereas cultures of bacteria were multiplied on Nutrient Agar and Yeast Extract Mannitol Agar medium. With the help of a 5-mm-diam., sterilized cork borer, a disc was cut from the margin of the antagonist colony and placed 1-cm away from the edge of a Petri plate containing Czapek's Dox Agar. A 5-mm-diam., inoculum disc of *P. aphanidermatum* was placed 1-cm away from the opposite side of the Petri plate. Bacterial isolate was streaked at one end of a Petri plate with the help of a sterilized wire loop, whereas a 5-mm-diam., inoculum disc of *P. aphanidermatum* was placed near the opposite end of the Petri plate. There were 3 replicates of each treatment. The plates were incubated at room temperature (25-30°C) and diameter of fungal colonies was recorded daily.

Results

Of the 41 fungi and 24 bacteria used in the present study, *Penicillium* sp., two unidentified bacteria and an unidentified sterile fungus were found to inhibit the growth of *P. aphanidermatum* producing zones of inhibition of 7, 11, 4 and 6-mm respectively, around the colony of the pathogen. *Gliocladium virens* initially produced a zone of inhibition of 20-mm but later on the pathogen overgrew the zone of inhibition and colonies of both organisms intermingled. During the present study, it was observed that a strain of *R. meliloti* (KUCC-816) and a strain of *Bradyrhizobium* sp., (KUCC-823) initially produced a zone of inhibition of 15 and 20-mm, respectively, around *P. aphanidermatum* colony but later on the pathogen and antagonists colonies met each other and no further growth of either organism was observed. *P. aeruginosa* strain (Pa-5) initially produced a zone of 2-mm but later on *P. aphanidermatum* grew and colonies of both organisms met each other with no further growth was observed. Similarly, *P. aeruginosa* strain (Pa-3) inhibited radial growth of *P. aphanidermatum* without producing zone of inhibition. Other organisms used in this study were failed to check the growth of *P. aphanidermatum* and colonies of the pathogen and the test microorganisms intermingled (Table 1).

Discussion

In the present study one *Penicillium* sp., two unidentified bacteria and a sterile fungus inhibited radial growth of *P. phanidermatum* producing zones of inhibition. Such similar observations have been made by Sharif *et al.* (1988) who reported that a *Penicillium* sp., reduced seed and seedling disease caused by *Pythium* spp. In this study it was also observed that a strain of *P. aeruginosa* inhibited radial growth of *P. aphanidermatum*. Species of *Pseudomonas* especially *P. fluoresecns* have been reported to inhibit the growth of *P. aphanidermatum*. Murray *et al.*, (1992) found 2 strains of *P. fluorescens* antagonistic to *Pythium ultimum* and *P. aphanidermatum in vitro*. Similarly, Zhang *et al.*, (1990) reported that isolates of *P. fluorescens* were especially effective in the control of disease caused by *Pythium* spp., and *R. solani*. In this study radial growth of *P. aphanidermatum* was inhibited by one strain each of *R. meliloti* and *Bradyrhizobium* sp. There are reports where rhizobia releases toxic metabolites (Chakraborty & Purkayashta, 1984) have shown promising results in the control of root infecting fungi viz., *M. phaseolina*, *R. solani* and *Fusarium solani* (Siddiqui *et al.*, 1998). It is interesting to note that in the present study *G.*

Table 1: Interaction of *Pythium aphanidermatum* with microorganisms

Test organisms	Days of incubation	Colony diameter (mm)		Type of reaction* / zone of inhibition (mm)
		T est Pathogen	Organism	
Fungi				
Alternaria alterna (Isolated from soil)	3	26	90	E
Aspergillus candidus (Isolated from air)	3	27	69	E
A. flavus (Isolated from air)	3	22	63	E
A. niger (Isolated from air)	2	24	90	E
A. niger (Isolated from soil)	2	23	90	E
A. niger (Isolated from air)	2	24	90	E
A. nidulans (Isolated from soil)	4	27	70	E
A. oryzae (mungbean isolate)	2	23	75	E
A. sulphureus (KUCC-299)	2	42	72	E
A. terreus (KUCC-478)	2	29	75	E
A. wentii (mungbean isolate)	3	16	80	E
Chaetomium globosum (isolated from soil)	3	29	90	E
Curvularia lunata (Isolate from soil)	4	40	70	E
Drechslera halodes (KUCC-616)	2	31	70	E
D. State of *Cochliobolus spicifer* (KUCC-489)	4	33	70	E
Epicoccum sp. (KUCC-707)	3	24	66	E
Fusarium anthophilum (mango isolate)	4	22	70	E
F. chlamydosporium (*Capsicum* isolate)	4	26	90	E
F. moniliforme (*Zea mays* isolate)	4	31	75	E
F. oxysporum (*Pennisetum typhoides* isolate)	3	34	75	E
F. proliferatum (Tomato isolate)	3	21	64	E
F. semitectum (*Pennisetum typhoides* isolate)	3	31	90	E
F. solani (*Brassica juncea* isolate)	4	34	90	E
F. sporotrichoides (*Capsicum* isolate)	4	25	90	E
F. subglutinans (*Capsicum* isolate)	3	28	71	E
F. scripi (Wheat isolate)	4	23	75	E
Gliocladium virens (KUCC-646)	4	53	69	B
Myrothecium sp. (KUCC-703)	3	26	70	E
Macrophomina phaseolina (KUCC-684)	3	43	90	E
M. phaseolina white strain (KUCC-663)	4	38	75	E
Paecilomyces lilacinus (KUCC-244)	4	13	70	E
P. variotii (Isolated from air)	3	17	60	E
Penicillium sp. (Isolated from soil)	3	9	70	E
Penicillium sp. (Isolated from air)	4	8	65	7
Rhizoctonia solani (KUCC- 581)	3	65	90	E
Stachybotrys atra (mungbean isolate)	4	14	71	E
Thielaviopsis basicola (KUCC-828)	3	29	90	E
Trichoderma harzianum (Isolated from soil)	5	41	57	E
T. koningii (KUCC-427)	4	47	73	E
T. viride (KUCC-656)	3	14	64	E
Sterile mycelium (Isolated from soil)	5	13	56	6
Bacteria				
Bacillus subtilis (KUCC-829)	4	streaked	70	E
Bradyrhizobium japonicum (BJ 3I 1P 110)	4	streaked	75	E
B. japonicum (KUCC-818)	3	streaked	75	E
B. japonicum (KUCC-797)	3	streaked	75	E
B. japonicum (KUCC-569)	4	streaked	69	E
Bradyrhizobium sp. (KUCC-823)	4	streaked	67	C
Bradyrhizobium sp. (KUCC-820)	4	streaked	75	E
Bradyrhizobium sp. (KUCC-825)	4	streaked	75	E
Bradyrhizobium sp. (KUCC-811)	4	streaked	75	E
Bradyrhizobium sp. (KUCC-819)	3	streaked	75	E
Escherechia coli (KUCC-828)	4	streaked	90	E
Pseudomonas aeruginosa (Pa-6)	4	streaked	73	E
P. aeruginosa (Pa-3)	4	streaked	75	D
P. aeruginosa (Pa-5)	4	streaked	68	C
P. aeruginosa (Pa-7)	4	streaked	63	E
P. aeruginosa (Pa-17)	4	streaked	68	D
Rhizobium meliloti (3DOA 29a)	4	streaked	75	E
R. meliloti (KUCC-816)	4	streaked	69	C
R. meliloti (3 DOA 1 139)	4	streaked	78	E
R. phaseoli (KUCC-831)	3	streaked	77	E
Rhizobium sp. (KUCC-809)	4	streaked	75	E
Unidentified bacterium (Isolated from air)	4	streaked	78	E
Unidentified bacterium (Isolated from air)	4	streaked	67	4
Unidentified bacterium (Isolated from air)	4	streaked	66	11

*Types of reaction:

A. A zone of inhibition was produced. No further growth was observed.
B. A zone of inhibition was produced. *P. aphanidermatum* later over grew the zone of inhibition, and colonies of both organisms intermingled.
C. Growth of *P. aphanidermatum* inhibited, *P. aphanidermatum* later over grew and met the colony of test organism. No further growth was observed.
D. Colonies of *P. aphanidermatum* and test organism met each other. No further growth was observed.
E. Colonies of *P. aphanidermatum* and test organism intermingled.

virens, a *P. aeruginosa* isolate and one strain each of *R. meliloti* and *Bradyrhizobium* sp., initially inhibited radial growth of *P. aohanidermatum* producing zones of inhibition but later pathogen over grew the zone of inhibition. This could presumably be due to the production of metabolites by biocontrol agents, which were effective in the initial stages of the growth of *P. aphanidermatum* but development of resistance allowed the pathogen to grow further after temporary inhibition.

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