ISOLATION OF BACTERIAL ANTAGONISTS FROM RHIZOSPHERE AND THEIR *IN VITRO* EVALUATION AGAINST PATHOGENS OF COCONUT LEAF ROT DISEASE

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ABSTRACT

Leaf rot disease of coconut is caused by a fungal complex. Among the fungi associated, *Colletotrichum gloeosporioides* and *Exserohilum rostratum* are the main pathogens. Leaf rot is a destructive disease and complete control of it could not be achieved by fungicidal applications. Efforts were made to isolate native bacteria from rhizosphere of coconut and to evaluate their *in vitro* antagonistic potential against leaf rot pathogens so that the potential antagonists could effectively be used in leaf rot disease management. Two fluorescent *Pseudomonas* spp. isolated from rhizosphere were found to be effective antagonists of the main pathogens of leaf rot.

INTRODUCTION

Leaf rot disease (LRD) of coconut is widely prevalent in association with root (wilt) disease (RWD) in Kerala State. An average of 65% of RWD affected coconut palms are infected with LRD. RWD is caused by phytoplasma (Solomon *et al.*, 1983) and LRD is due to a fungal complex. Among the fungi associated with LRD, *Colletotrichum gloeosporioides* and *Exserohilum rostratum* are considered as the main pathogens (Srinivasan and Gunasekaran, 1996a). Control of LRD is very important in view of its destructive potential. Complete control of LRD was not achieved by fungicidal sprayings (Menon and Nair, 1952; Nair and Radha, 1959; Srinivasan and Gunasekaran, 1996b).

Lily *et al.* (1952, 1955) reported that *Bacillus subtilis* inhibited spore germination and growth of *Helminthosporium halodes* (=*Dreschlera halodes*) on solid medium. The culture filtrate of the bacterium inhibited infection of the fungus on leaves. Presently there is a growing interest on the biocontrol of plant diseases (Nallathambi *et al.*, 1997a ; Vidhyasekaran, 1998). As LRD amelioration with chemical control measure is not fully achieved, it becomes necessary to seek alternative methods of disease control like breeding for disease resistance, biological control, etc. Breeding for RWD and LRD resistance is a long term process ; whereas for immediate future, biocontrol can be adopted as such or as part of the measures for integrated disease management. The obvious source of the biocontrol agent is the natural environment itself. Hence, efforts were made to isolate the native bacterial antagonists from coconut rhizosphere and to evaluate their potential as biocontrol agents against the main pathogens of LRD.

MATERIALS AND METHODS

Fungal culture : *Colletotrichum gloeosporioides* and *Exserohilum rostratum* were isolated on potato dextrose agar (PDA) medium from naturally leaf rot affected coconut palms. The cultures were purified and maintained on PDA at 4 ^{0}C until further use.

Isolation of rhizosphere bacteria : The soil from the rhizosphere of 24 coconut palms (from basins at a depth of 0-25 cm) was collected. A composite soil sample was made from rhizosphere soil of three palms after air drying and homogenous mixing. Likewise eight composite soil samples were made and used for isolations.

For the isolation of rhizosphere bacteria, 10 g soil from each composite sample was added to 90 ml of sterile distilled water and stirred for 20 min using magnetic stirrer. Ten-fold dilutions were made and plated onto selective and non-selective media (King *et al.*, 1954; Simon *et al.*, 1973).

All the plates were incubated at 28±1 ^{0}C for 1 to 3 days. For each medium, representative colonies were isolated and repeatedly cultured till pure culture was obtained. The purified cultures were maintained on nutrient agar slants at 4 ^{0}C for further studies.

In vitro antagonism : The bacterial isolates were tested for antagonism against the main pathogens of LRD (C. gloeosporioides and E. rostratum) on PDA by dual culture (Savithiry and Gnanamanickam, 1987).

Effect of culture filtrate : Bacterial culture filtrates were prepared from 2 day old cultures by filtering through 0.22 µm millipore filters. A central well of 8 mm diameter was made in the PDA plates using sterile cork borer. Agar blocks with mycelial growth of leaf rot fungi were placed on either side of the well at a distance of about 25 mm. The central well was then filled with 250 micro litre of culture filtrate. The plates were incubated at 28±1 ^{0}C for 5 to 7 days and the inhibitory effect of culture filtrate, if any, was recorded qualitatively (- = no reaction ; + = positive reaction) for seven days at regular intervals.

Identification of selected bacterial isolates : The bacterial isolates were identified upto generic level with the help of morphological, cultural and biochemical characteristics as per the "Bergey's Manual of Systematic Bacteriology" (Volume I and II).

RESULTS AND DISCUSSION

Isolation of rhizosphere bacteria and screening for antagonistic activity : Twenty one isolates from rhizosphere were obtained and they were purified. The antagonistic activity of these isolates against *C. gloeosporioides* and *E. rostratum* were graded qualitatively as –ve and +ve based on the presence or absence of antagonism.

The growing cultures of nine out of 21 rhizosphere isolates showed clear antagonism by inhibiting the mycelial growth of both fungi. Four isolates showed weak antagonism whereas eight showed no reaction.

In vitro biocontrol potential of rhizosphere isolates : The reactions of nine rhizosphere isolates selected from the above study were found to be variable in general, all the isolates had more inhibitory effect on *E. rostratum* (Table 1). However, isolate 9 was found to be effectively inhibiting the mycelial

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growth of both *C. gloeosporioides* and *E. rostratum* followed by isolate 8 (Table 2).

Table 1 Antagonistic potential of fluorescent Pseudomonads from rhizosphere

Isolate Number	Inhibition zone (mm) * after 7 days in PDA	
	C. gloeosporioides	*E. rostratum*
1	3.0	9.0
2	3.0	12.0
3	2.5	6.5
4	4.0	9.0
5	2.0	4.0
6	1.5	5.0
7	2.5	9.0
8	5.5	10.0
9	7.0	12.0

* Mean of 6 replications

Table 2 Growth of *C. gloeosporioides* and *E. rostratum* (radius in mm) as affected by fluorescent *Pseudomonas* spp.

Fungus	Per cent inhibition (after days)*				
	1	2	3	5	7
C. gloeosporioides					
Isolate 8	0	8.7	38	45	55
Isolate 9	0	12.5	30	45	61
E. rostratum					
Isolate 8	0	12.0	34	60	60
Isolate 9	0	25.0	40	68	68

* Mean of 6 replications.

The cell-free culture filtrates of all the nine bacterial isolates also inhibited the mycelial growth of *C. gloeosporioides* and *E. rostratum*, thereby indicating the secretion of antifungal substance into the growth medium. The level of antagonism detected was more or less the same as observed with the growing cultures.

Identification of selected bacterial isolates : The bacterial isolate numbers 8 and 9, which showed maximum antagonism against both the main fungal pathogens of LRD, were found to be gram negative rods, nonsporulating, motile, aerobic, catalase positive, oxidase positive, produced both diffusible fluorescent and non-fluorescent pigments, gelatinase positive, amylase negative and lysine decarboxylation positive, acid production from glucose. On the basis of these characters both the isolates were identified as fluorescent *Pseudomonas* spp.

The micro-organisms isolated from environments, such as rhizosphere, may prove to be promising biological control agents for use on leaves, the prime consideration being the ability to grow fast (Campbell, 1989). The surface growth of pathogens like *Colletotrichum* may be more vulnerable to nutrient competition from such biocontrol agents.

As the rhizosphere fluorescent *Pseudomonas* spp. are naturally occurring antagonists, they may establish themselves to an effective population levels in competition with the existing ones. For successful biocontrol, colonization by an antagonist is of prime importance.

Efforts have been made to promote the activity of naturally occurring bacteria to control many plant diseases (Arya and Parashar, 1997; Nallathambi *et al.*, 1997a, 1997b; Vidhyasekaran, 1998). In the present study some attempts have been made to discern the existence of certain native bacteria in coconut rhizosphere and two isolates from rhizosphere have been catalogued as effective antagonists of LRD pathogens. Their further evaluation and field testing are important before introducing them as biocontrol agents in LRD management.

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