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Suppression of Collar-rot caused by *Sclerotium rolfsii* Sacc. in chilli by antagonistic bacterial treatments

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Abstract

Chilli (*Capsicum annuum* L.) is an important crop in Sri Lanka. However, chilli cultivation is severely affected in many areas due to several soil-borne pathogens. *Sclerotium rolfsii* Sacc. has been identified as one of the major pathogens that even attack mature plants. Success of control of the pathogen by using merely fungicides is not promising. Rhizobacteria isolated from healthy chilli plants were screened for antagonism against *S. rolfsii* and isolates of a *Pseudomonas* spp. and a *Bacillus* spp. were found to be highly effective on plate assays. Doss-response analysis showed that there is a linear relationship between inoculum dosage and disease incidence. Seed bacterization with individual biocontrol agents significantly reduced the disease incidence in chilli compared to the non-bacterized control and introducing bacterial isolates to root system prior to transplanting of seedlings further enhanced the seedling protection from *S. rolfsii* infection.

Keywords: Biological control, Antagonistic bacteria, *Capsicum annuum, Sclerotium rolfsii*

Introduction

Chilli (*Capsicum annuum* L.) is one of the main spicy crops in Sri Lanka. Several soil-borne pathogens are responsible for the crop losses at different stages of the plant. Collar rot caused by *Sclerotium rolfsii* is one of the most devastating soil-borne pathogens of chilli infecting usually the collar region of the plant. *S. rolfsii* is a ubiquitous soil-borne plant pathogen with a wide range of hosts (Agrios 1997). Most crops are susceptible if grown at a time or place when *S. rolfsii* is present. In the absence of suitable host plants, *S. rolfsii* may live for several years as a saprophyte in the soil or decaying plant debris. Moreover, sclerotia produced by the pathogen provide a survival mechanism under adverse environmental conditions for longer period in soil.

Crop rotation, sanitation, reduced irrigation, resistant varieties and fungicides etc. can help to control the pathogen to some extent, and these methods singly or with combination cannot control the pathogen economically and consistently. It is well demonstrated that many soil and rhizosphere bacterial isolates have antagonistic effects against plant pathogenic fungi *in vitro*. Some of these isolates may also show good

disease suppression under greenhouse conditions and/or in field conditions (Johansson *et al.* 2003). Some of these have also been formulated into commercial biocontrol products (Gerhardson 2002), suggesting that the possibility of use of such bacterial isolates under practical cropping conditions.

Therefore, the main objectives of the present study was screening of bacterial strains isolated from chilli rhizosphere which can antagonize *S. rolfsii in vitro* and evaluate the efficacy of selected isolates for the control of *S. rolfsii* on chilli under greenhouse conditions.

Materials and Methods

A. Isolation of rhizospheric bacteria

Bacterial strains were isolated from the roots of field-grown chilli plants on suppressive soils from Angunukolapalssa. Plants were uprooted and immediately transferred to the laboratory. The root system was gently shaken to remove loosely adhered soil particles. Then the root system was macerated in 10 ml of 0.85% saline with a motor and pestle. Ten fold serial dilutions of the homogenate were plated on Cassamino acid (CAA) medium (Diffco) containing Petri plates. Bacterial representatives of different morphological types present on plates were selected and purified on new CAA plates.

B. Screening of antagonistic bacterial strains in vitro

Screening of antagonistic bacterial strains against *S. rolfsii* was carried out using dual culture plate method. Two streaks of a bacterial isolate (3 cm long) were inoculated at the periphery of agar plate containing 20 ml of CAA. After incubating 24 h at room temperature actively growing *S. rolfsii* agar plug was placed at the center of the agar plate. Plates were incubated at room temperature and checked for any inhibition zone compared to the non-streaked area of the same plate. According to the inhibition zone isolates 5 and 32 were selected for further study. These isolates were identified as a *Pseudomonas* spp. and a *Bacillus* spp. respectively.

C. Effect of inoculum density of S. rolfsii on percentage of disease incidence

Tests were carried out in order to establish the relationship between numbers of sclerotia/Kg soil and disease incidence measured as number of seeds that failed to emerge and/or post- emergence damping-off of seedlings after 15 days of sowing. From this LD_{50} value was estimated.

To prepare fungal inoculum, *S. rolfsii* was grown on PDA plates for fifteen days and sclerotia were harvested by gently scraping on to SDW. Different amounts of sclerotia

(500,1000, 1500, 2000, 2500, 3000, 4000, and 5000 sclerotia/ kg soil) were mixed pots filled with 2 kg of non-sterile soil (clay:sand:compost at 1:1:1).

D. Preparation of bacterial inoculum

Two bacterial strains were selected namely, CA5 and CA32, from the pool of bacteria isolated from chilli rhizosphere for further investigation on the basis of inhibition zone exhibited in Petri plate assay. A single colony of these cultures was separately inoculated into CAA broth of 100 ml in 250 ml flasks. Cultures were incubated at room temperature for 48 h in a shaker at 100 rpm. Medium containing bacterial cells was centrifuged at 5000 rpm for 10 min. and the pellet was re-suspended in sterilized 0.85% saline. The bacterial suspension was centrifuged again and re-suspended in sterilized distilled water (SDW) to reach 10^8 colony forming units (CFU)/ml approximately.

E. Seed bacterization and root inoculation

Chilli seeds var. MI2 were inoculated with both bacterial strains separately in 50 ml bacterial suspension. Seeds were imbibed for one hour in the respective bacterial suspensions. Bacterized seeds were placed on a filter paper in order to drain out excess suspension prior to sowing. Root system of the seedlings was dipped in a bacterial suspension (6 plants in a 100 ml of 10^8 CFU/ml) for 30 min prior to transplanting in pots.

F. Test for antagonistic effects of selected bacteria on chilli in pot experiments

Chilli variety MI2 known to be susceptible to the pathogen was used as the host plant. The pathogen *S. rolfsii* was isolated from infected stem of chilli plants. Bacterized chilli seeds were sown in pots (50 seeds/pot) containing 2 kg of non-sterile soil (clay;sand;compost 1;1;1). Seeds imbibed in SDW considered as controls. Twenty-one days old seedlings were transplanted in sclerotia amended soil (2.5 sclerotia/g soil) with or without introducing rhizobacteria to the root system (6 seedlings/pot). Sclerotia were introduced to the soil six days prior to the replanting and pots were covered by dried-rice straw in order to maintain high humidity in soil. Four replicates were incorporated per treatment and the experiment was repeated twice.

Number of seedlings infected with *S. rolfsii* at collar region was counted for a period of 30 days from transplanting. A healthy plant scored 2, a plant with minor symptom scored 1, and a dead plant scored 0. From these scores, a disease suppression index (DSI) was established ranging from 12 for a pot with all six plants healthy to 0 for a pot with all six plants dead.

G. Statistical analysis

SAS (version 6.0 USA) was used for analysis of variance (ANOVA) and the means separated using Duncan's multiple range-test ($P< 0.05$).

Results

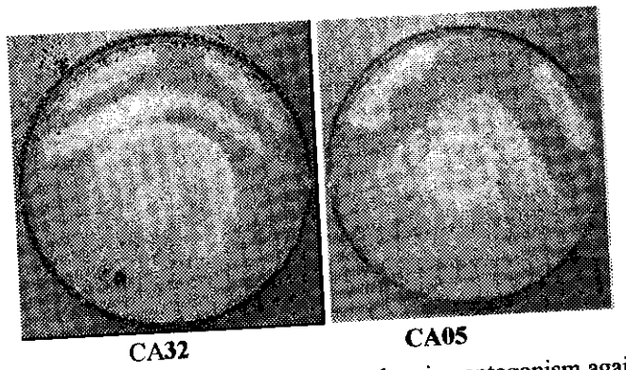
A. In vitro screening of rhizospheric bacteria

Among the total 40 bacterial isolates only five isolates showed antagonism against *S. rolfsii* in plate assay (Table 1). From these five isolates only two isolates were significantly inhibit the mycelial growth (Figure 1) and reduced the formation of sclerotia. Isolate CA05 produced diffusible yellow pigment and fluorescent under UV and Gram-negative identified as a *Pseudomonas* spp. Isolate CA32 white in color, and Gram-positive identified as a *Bacillus* spp.

Table. Screening for antagonistic activity by different bacterial isolates against *S. rolfsii* based on inhibition zone.

Bacterial isolates	Inhibition zone in (mm)[1]
CA 32	5.9[a]
CA05	3.6[b]
CA12	1.4[c]
CA21	1.8[c]
CA28	1.2[c]

[1] Values are means of three replications. Data followed by the same letter in a column are not significantly different from each other according to Duncan's multiple range test (P=0.05).



CA32 CA05

Figure 1: Dual petry plate assay showing antagonism against *S. rolfsii* in CAA plates.

B. Disease incidence vs. inoculum density relationship

LD_{50} value for untreated control was calculated and this value was 2.5 sclerotia/g of soil. There is a direct relationship between inoculum dosage of *S. rolfsii* and disease incidence

(Table 2). Therefore, for further experiments 2.5sclerotia/g soil used as initial inoculum of the pathogen.

Table 2. Percentage of damping-off seedlings when different amounts of sclerotia added to soil.

No. of Sclerotia/Kg soil	Experiment I	Experiment II	Experiment III	Mean
0	7	6	7	6.7
500	7	8	7	7.3
1000	10	11	10	10.3
1500	11	12	15	12.7
2000	45	46	46	46.3
3000	55	52	49	52
4000	58	58	56	57.3
5000	60	59	62	60.3

C. Performance of antagonists in pot experiments as biological control agents

Table 3. Mean disease suppression index (DSI) values for different treatments against *S. rolfsii* infection in chilli seedlings under greenhouse conditions.

Treatment	DSI values in the greenhouse screening[1]		
	Experiment I	Experiment II	Mean
Healthy control	45	46	45.5a
Infected control	23	20	21.5c
Isolate CA32 seed bacterization only	34	32	33b
Isolate CA05 seed bacterization only	32	34	33b
Isolate CA32 seed bacterization cum root inoculation	42	45	43.5a
Isolate CA05 seed bacterization cum root inoculation	41	43	42a

[1]Figures with the same letter within a column are not significantly different according to Duncan's multiple range test ($P < 0.05$)

Discussion

Isolation of rhizobacteria resulted mainly Gram-negative isolates closely related to pseudomonads and few *Bacillus* spp. These results are similar to other investigations using comparable approaches (Gerhardson 2002). Screening of rhizobacteria against *S. rolfsii* showed that the majority of bacteria that reside in the chilli rhizosphere are not able to antagonize the pathogen *in vitro*. Among the antagonists differences in antagonistic ability were found in the plate assay. This phenomenon was probably correlated with the differences in levels of hydrolytic enzymes, antibiotics or any other toxins produced by each species or isolates (Radjacommare *et al.* 2004).

Crop protections from different soil-borne pathogens have been demonstrated by applying antagonistic bacteria as seed treatments (seed bacterization) or/and applying as a soil drench in different host-pathogen systems (Sarma *et al.* 2002). In the present study, it was attempted to investigate the impact of introducing antagonists not only as a seed treatment but also to the root system prior to transplanting. Due to the enhanced protection observed in this study it can be concluded that the application of antagonists to root system by dipping in the individual bacterial suspension prior to transplanting clearly indicates the important of such application in this respect.

Previous studies often emphasized a lack of performance and consistency under field conditions of *in vitro* and greenhouse screened disease-suppressing microorganisms (Knudsen *et al.* 1997) and this has been seen as a major drawback in the search for potential biological control agents. Therefore, testing of these two antagonists in field conditions is of paramount importance.

Acknowledgments

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