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Biocontrol of *Pseudomonas fluorescens* and *Bacillus subtilis* Against Fungal Pathogens Isolated from Selected Infected Leaves

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The rhizosphere bacteria are ideal for used as biocontrol agents as they can provide the front line defense for plant against the attack by various plant pathogens. The test fungal pathogens were isolated from the infected leaf samples such as coconut, banana, and rice by using Potato dextrose agar medium. The antagonistic effect of bacteria against test pathogen was determined by dual culture technique. In this experiment *Bacillus subtilis* showed high inhibitory property on the selected infected leaves when compared with *Pseudomonas fluorescens*. The growth of test organism in the Potato dextrose agar medium treated with different concentration of culture filtrate of *Bacillus subtilis* and *Pseudomonas fluorescens* (5%, 10%, and 15%) were studied. The maximum percentage of inhibition of the growth of the test pathogens were at 15% culture filtrate of *Bacillus subtilis* (86.53%) and *Pseudomonas fluorescens* (78.88%). *Bacillus subtilis* culture filtrate contain maximum amount of protein content (50μg) than *Pseudomonas fluorescens* (35μg) was estimated by Lowry's method.

Key words: Biocontrol agents, Fungal pathogens, Dual culture technique, Antagonism, Inhibition.

Biocontrol as the practice or process by which an undesirable organism is controlled by means of another (beneficial) organism. More usually biocontrol is defined as the reduction in attack of a crop species by a pathogen achieved using other living organisms. The suppression in growth of phytopathogenic fungi by bacterial strains indicates the antagonistic effect of bacteria on fungi. Biological control of plant diseases with bacterial antagonists is a potential alternative of chemical control, because chemical control is expensive and results in accumulation of hazardous compounds being toxic to the soil biota. (Gupta *et al.,* 2001).

The rhizosphere bacteria are ideal for use as biocontrol agents as they can provide the front line defense for plant roots against the attack by various plant pathogens. In some cases, the bacterial antagonism of plant pathogens leads to sustainable disease control (Schneider *et al.,* 1982).

Commercial strains of *B. subtilis* have been marketed as biocontrol agents for fungal diseases of crops (Emmert *et al*., 1999; Warrior *et al.*, 2002). *Pseudomonas* strains have been considered to have an attribute to biological control of some soil borne diseases. (Capper *et al.,* 1986).

Biocontrol agent effective in control of soil-borne fungal diseases were identified and used in agriculture. Biocontrol of foliar disease met

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with limited success due to poor survival of introduced biocontrol agents on the phylloplane. Attempts have been made for improved biocontrol of foliar diseases by nutrient supplemented application of the biocontrol agents. Integrated use of biocontrol agents along with the existing disease management technologies is desirable to achieve stable biocontrol of foliar diseases.

MATERIALS AND METHODS

Infected leaf samples (coconut, banana, rice) were collected during the month of December to January from college campus. From that samples, the Fungal pathogens were isolated by using Potato dextrose agar medium. Fungi were identified based on vegetative and spore morphology (Alexopoulos *et al.,* 1996, Gilman, 1957).The test bacterial cultures (*P.fluorescens* MTCC 667, *B.subtilis* MTCC 2057) were procured from Institute of Microbial Type culture collection (MTCC), Chandigarh, India. All the fungal pathogens were subcultured and stored at appropriate temperature. The bacterial inoculum was subcultured in the nutrient broth into sterile test tube. Test pathogens were inoculated and incubated under room temperature. Antagonistic properties of *Pseudomonas fluorescens* and *Bacillus subtilis* were tested against the fungal pathogens such as *Aspergillus flavus, Aspergillus fumigatus, Fusarium* by using dual culture method without addition of antibiotics because it suppressed the growth of bacterium (Skidmore and Dickinson 1976).

Antibiotic interaction (Narasimha rao *et al.,* 2001) was carried out the culture filtrate of *Pseudomonas fluorescens* and *Bacilius subtilis* were collected from the nutrient broth and added separately to the cooled PDA medium to give the concentration of 5, 10, and 15 %. The PDA medium was dispersed in Petri plates and allowed to solidify. After solidification agar blocks (5mm) cut from the actively growing margin of the test fungi like *A.flavus, A.fumigatus, Fusarium* were inoculated at the centre of the plates. The plates were incubated at 37º C for five days. The radial growth was measured periodically. The percent inhibition of growth was calculated as follows

$$\% \text{ of inhibition of growth} = \frac{\text{Growth in control - Growth in treatment}}{\text{Growth in control}} \times 100$$

Protein estimation was carried out from *Pseudomonas fluorescens* and *Bacillus subtilis* in nutrient broth by Lowry *et al.,* 1951.

Statistical analysis

All the experiments were conducted at least thrice. The results were statistically analyzed by using arithmetic mean or average, standard error (Gupta, 1977).

RESULTS AND DISCUSSION

The antagonistic ability of the bacterium such as *Pseudomonas fluorescens* and *Bacillus subtilis* against test fungal pathogens were evaluated invitro condition. In the present study *Bacillus subtilis* was found very effective in the suppression of *Aspergillus flavus, Aspergillus fumigatus* and *Fusarium*, grown on Potato dextrose agar medium and also *Pseudomonas fluorescens* was proved effective in controlling the infection (Table 1). The maximum percentage of inhibition of the growth of the test pathogens were at 15% culture filtrate of *Bacillus subtilis* (86.53%) and *Pseudomonas fluorescens* (78.88%) (Table 2). *Bacillus subtilis* culture filtrate contain

Table 1. Antagonistic effect of *P.fluorescens* and *B.subtilis* against different fungal pathogens

Antagonistic bacteria	Growth inhibition zone (mm)		
	A.flavus	*A.fumigatus*	*Fusarium*
P.fluorescens	32.3±0.05	24.3±0.05	20.6±0.80
B.subtilis	52.6±0.11	63.6±0.2	71.3±0.05

Values are represented as Mean ± standard error in mm

Table 2. Effect of culture filtrate of *B.subtilis* and *P.fluorescens* on the growth of foliar pathogen

S. No	Name of the culture	Concentration (%)	*A.flavus*		*A.fumigatus*		*Fusarium*	
			Growth rate (mm/day)	% of growth inhibition	Growth rate (mm/day)	% of growth inhibition	Growth rate (mm/day)	% of growth inhibition
1		5	12	60	10	66.66	14	53.33
	P.fluorescens	10	9	70	7	76.66	12	60
		15	3	90	6	80	10	66.66
2		5	10	66.66	8	73.33	15	50
	B. subtilis	10	4	86.66	5	83.33	10	66.66
		15	2	93.33	1	96.66	9	70

maximum amount of protein content 50µg than *Pseudomonas fluorescens* 35µg was estimated by Lowry's method(Table 2). Results of present research were supported by the work done by various workers. Tested the indigenous *Pseudomonas* strains isolated from rhizosphere of rice cultivated in coastal agricultural ecosystem against rice sheath blight disease and reported that they are effective under saline soil conditions.(Sunitha Rangarajan *et al.,* 2003).

In our results were correlated with Molly *et al.,* 2009 observed the antagonistic effect of *Pseudomonas* strains which can control not only root pathogens but also some leafy pathogens like *Curvularia. Oidium, Pestalotia* isolated from infected crop plants. *Phanerochaete chrysosporium* degrade both cellulose and lignin and to cause separation of cells into fibers. The antagonistic effect of Fungi such as *T.viridae* and *A.niger* against *P.chysosporium* were analysed. In that study *T.viride* highest control pathogen, while *A,niger* moderately control the pathogen. The present research was correlated with other findings in related to the bacterial antagonist such as *B.subtilis, P.fluorescens, E.aerogenes* and mixed culture of bacteria were effectively control the pathogen (Jothilakshmi *et al.,* 2009).Similarly Ramachandaran 2009 reported the organic solvent extracts from the leaf of *Euphorbia fusiformis* were tested against four important aflatoxin producing fungi namely *Aspergillus flavus, Aspergillus niger, Aspergillus fumigatus, Aspergilus erythrocephalus* and *Fusarium* species.

The findings of the present study justify the claimed use of the rhizosphere bacteria such as *P.fluorescens* and *B.subtilis* for the treatment of foliar fungal pathogens. The selection of microbial antagonists used for biological control of plant pathogens is broadly based on their ability to compare with pathogens through production of antibiotic compounds growth and enzyme producing activities.Thus our findings may have small start.

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