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A Study on the Antagonistic Action of some Bacterial isolates against some common Soil Fungi

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ABSTRACT

Three bacterial species (*bbrd sp., bprd sp., bsrd sp.*) and five fungal species (*frd1 sp., ard2 sp., prd3 sp., rrd4 sp.* and *trd5 sp.*) were isolated from the soil sample collected from various place of garden of School of Environmental Science and agricultural field near B.B.A. University, Lucknow, India. Basic aim of the isolation of fungi and bacteria was to evaluate the antifungal capacity of some bacterial species. Bacterial species inhibit the growth of fungi by the process of antagonium. Antagonism is the phenomenon in which one microorganism destroys the other interacting partner to ensure its own survival. Three bacterial stains *bbrd sp., bprd sp., bsrd sp.* showed to be excellent producers of antifungal metabolites. The present data exhibit the antifungal activity of the bacterial strain indicate the possibility to use it as biological agents against some plant's pathogenic fungi by the antimicrobial activity of the microbial metabolites. Antagonistic interaction between microbes were studied by the measurement of the zone of inhibition on agar plate, that allow for repeated observation of numerous sites and inhibition with a minimum disturbance of the cells and soil particles.

1) INTRODUCTION

Antagonism is the phenomenon in which one microorganism destroys the other interacting partner to ensure its own survival. In other words, antagonistic effects of some microorganisms against others *in vivo* and *in vitro* have been reported by many investigators. On many raw foods, the bacterial micro-biota is often composed of mixed species. The activities of one of bacterial species may be influenced by the growth activities of others [1].

Several environmental factors affect the growth and survival of antagonists. They includes the growth of interacting microbes in soil, influenced by environmental factors like moisture contents, chemical nature of organic matter, volatile substances like ammonia and other nitrogenous gases, CO_2, methane etc. .The stalling products of microbes are preferably called antibiotic substances or metabolites. These antibiotic or stalling products are produced by the micro-organisms under sugar rich condition [2].

Under the given circumstances, the fungal mycelium or the bacterial cell is unable to stop absorbing sugar from the culture medium and the excess is excreted out as shunt metabolites having antibiotics properties. Biological control using antagonistic bacteria has been reported as an attractive alternative due to their ability to antagonize the pathogen by different modes of action, and to effectively colonize distinct plant habitats [3].Antagonistic effect of micro-organism is the result of interaction among the microbial populations. Its applied aspect is to control the disease caused by different pathogens [4].

Antagonism is grouped into:

1. Antibiosis which is the inhibition or killing of one microbe by the other one by producing antibiotics or toxic metabolites.
2. Parasitism where one micro-organism is a parasite on the other partner for nutrition.

Important feature of the antifungal microorganism includes:-

I. Production of antibiotic / toxins.
II. Production of enzyme.
III. Inhibition of growth.
IV. High competitive saprophytic ability.

Most suitable example of the antagonism is inhibition of growth of *Staphylococcus aureus* causing abscess or boil, generally growing in the wound by the metabolite of *Penicillium notatum* [5]. Antagonistic effects of some micro-organisms against others in vivo and in vitro have been reported by many investigators. On many raw foods, the bacterial micro-biota is often composed of mixed species. The

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activities of one of bacterial species may be influenced by the growth activities of others [1]. Bacterial antagonism could arise from the combined effects of several mechanisms during their growth in the media. For example, one group of micro-organisms may remove a growth factor required for the growth of another, synthesize a inhibitory substance to another or produce an adverse pH or Oxidation Reduction Potential (Eh) [7].

Microbial antagonist strains capable of producing both non-volatile compounds and volatile compounds (VOCs), which exhibit strong inhibitory activity against plant pathogens, have received much attention. These antagonists include bacteria, such as Pseudomonas spp. [8, 9] and non pathogenic fungi like *Trichoderma sp*. The release of VOCs by soil microbes has been reported to promote plant growth display nemati-cidal activity and induce systemic resistance in crops [10]. Antifungal agents produced by micro-organisms may be used as biocontrol agents. Some soil borne fungi, bacteria and actinomycetes have been identified and used as antagonistic microbes. A number of bacterial species have been tested as biocontrol agents. Antifungal metabolites produced by bacteria like *Pseudomonas sp., Bacillus sp., Serratia sp.,* have been well documented for their antifungal activity [11]. The mechanisms underlying these bacterial antagonisms for plant pathogens involve antibiosis, competition for nutrients or space, enhancement of root and plant development, induction of plant resistance and/or inactivation of the pathogen's enzymes [12]. Antibiosis, in particular, is the most important mechanism for control of plant disease.

Aspergillus flavus is a human pathogen, allergen and mycotoxin producer, while A. niger is generally involved in the etiology of otomycosis aside its major role as a plant pathogenic fungus [13]. *Fusarium moniliforme* is an important plant pathogenic fungus capable of producing different mycotoxins in food and agricultural commodities. *Penicillium marneffei* is a saprophytic fungus responsible for opportunistic invasive infections in immune compromised patients [14].

Objectives:

- Isolation of bacteria from soil with antagonistic properties.
- Isolation of fungi.
- To study the antagonism between bacteria and fungi.

2) MATERIAL AND METHOD

2.1 Collection of Soil Sample

Soil samples for isolation of bacteria and fungi were collected in sterile polythene bags from cultivated, uncultivated and rhizosphere soil of agriculture field near B.B.A. University & from garden of School of Environmental science building B.B.A. University, Lucknow, India.

2.2 Isolation of Bacteria

Cultivation medium: The Bacteria were cultured on simple nutrient agar medium.

Medium composition

Peptone	-	5.0 g
Beef extract	-	3.0 g
Nacl	-	250 g
Agar	-	18 g
Distil water	-	1000 ml
pH-	-	7.0

The pH of the culture media was adjusted to 7.0 with the help of 0.1N NaOH and 0.1N HCl.

Soil sample were serially diluted and 0.1 ml of diluted sample were spread on nutrient agar plate. The plates were incubated at 30°C in inverted position. The colonies appearing on plate were purified by repeated streaking. Pure colonies were preserved on Nutrient agar slant and stored under refrigeration.

Identification of isolation bacterial strains

The isolated bacterial strains were subjected to Gram's staining (Gram, 1884).

Reagents used: Crystal violet, gram's iodine solution, ethyl alcohol, safranin.

Procedure: Thin smears of individual strains of isolated bacteria were heat fixed on glass slide. The smear was covered with crystal violet for 30 seconds and after 30 seconds washed with distilled water and again washed with iodine solution, for 60 seconds. After 60 seconds ethyl alcohol was drop wise added and subsequently washed with distilled water. Finally safranin was applied to smear for 30 seconds & washed with distilled water. The slide was observed under the microscope.

Result: Those bacteria that appeared purple were referred to as gram positive & those appearing pink in colour were referred as gram negative.

2.3 Isolation of Fungi

Cultivation medium: The fungi were cultured on Gzapek Dox medium.

Medium composition

Sodium Nitrate	:	2.0g
Dipotassium Hydragen Phosphate	:	100g
Potassium Chloride	:	0.5g
Magnesium Sulphate	:	5.0g
Ferous Sulphate	:	0.01g
Sucrose	:	30g
Agar	:	15g
Distilled Water	:	1000 ml.

Serial dilutions of the samples were done and 10^{-3} dilution was taken for the isolation on the solidified petri plate. After 10 days fungal mycelia were identified based on microscopic observation of spores.

2.4 To Study the Antifungal Activity Bt Bacteria Strains

Nutrient agar plates, sterile filter paper disc of 0.8 mm diameter, broth medium were used to determine antifungal activity. The cell free culture fluids of bacteria strains were passed through filers 2.0m.

Sterilized filter paper discs of (0.8mn) diameter were then loaded with 20ml of the sterilized bacterial culture fluids which were placed on agar plate inoculated with fungi. After incubation at 37^0 C for 24h, zone of growth inhibition were measured.

Concentrated culture fluids were loaded on paper disc which were subsequently evaluated for their antifungal potential described above.

3) RESULT AND DISCUSSION

Three bacterial species (*bbrd sp., bprd sp., bsrd sp*.) and five fungal species (*frd1 sp., ard2 sp., prd3 sp., rrd4 sp.* and *trd5 sp*.) isolated from the agricultural field near B.B.A. University & from garden of School of Environmental science building B.B.A. University, Lucknow, India.

Only one fungi sp. (*frd1 sp*.) out of the five fungal species has shown better antagonistic action against *bbrd sp.* while *rrd4 sp. and trd5 sp.* shows very little antagonistic action and

others two fungal sp. (*prd3 sp., ard2 sp.)* did not show antagonism against the isolated *bbrd* sp.. *frd1 sp.* showed maximum growth inhibition zone around the growth of *bbrd* sp. after 24 hours of the incubation. The size of observed inhibition zone was 5mm (fig.1).

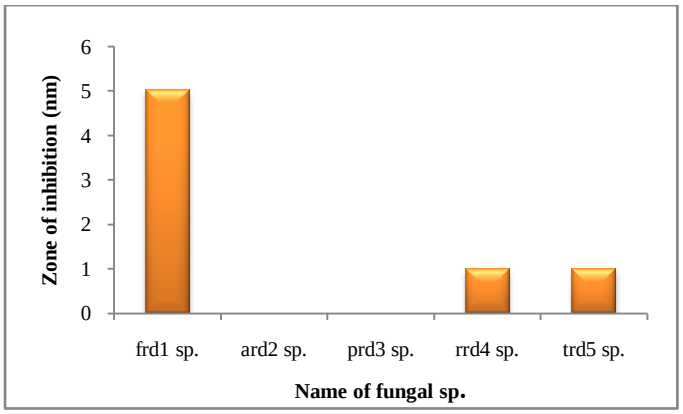

Figure 1 Growth inhibition of fungi by Isolated *bbrd sp.*

Out of the five fungal sp. four isolated sp (*frd1 sp., prd3 sp., rrd4 sp.* and *trd5 sp*.) have shown positive antagonism while only one fungal sp. (*ard2 sp.)* did not show antagonism against the isolated *bprd* sp.. *frd1 sp., prd3 sp., rrd4 sp.* and *trd5 sp*.) have showed growth inhibition zone around the growth of *bprd sp.* after 24 hrs. of incubation. The size of observed inhibition zone was 5mm, 3mm, 7mm, & 3mm. respectively for the isolated (*frd1 sp., prd3 sp., rrd4 sp.* and *trd5 sp.*).

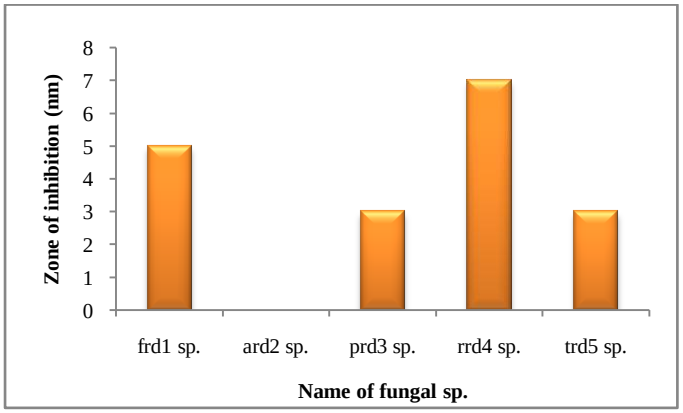

Figure 2 Growth inhibitions of fungi by Isolated *bprd sp.*

frd1 sp., ard2 sp., prd3 sp. and *trd5 sp*. have shown positive antagonism while one fungal sp. (*rrd4 sp.*) did not show antagonism against the isolated *bsrd sp.*. *frd1 sp., ard2 sp., prd3 sp.* and *trd5 sp*. have shown growth inhibition zone around the growth of *bsrd sp.* after 24hrs of incubation .The size of observed inhibition zone was 3mm, 4mm, 4mm & 4mm respectively for the isolated *frd1 sp., ard2 sp., prd3 sp.* and *trd5 sp*.

4) CONCLUSION

The aim of this study was to evaluate the antifungal capacity of some bacterial species. Three bacterial stains *bbrd sp., bprd sp.* and *bsrd sp*. showed to be excellent producers of antifungal metabolites. The present data exhibits the antifungal activity the bacterial isolated strains and indicates the possibility of using it as biological agents against some plant pathogenic fungi. Antagonistic interaction between microbes were studied by measurement of the zone of inhibition on agar plate, that allow for repeated observation of numerous sits and inhibition with a minimum disturbance of the cells and soil particles.

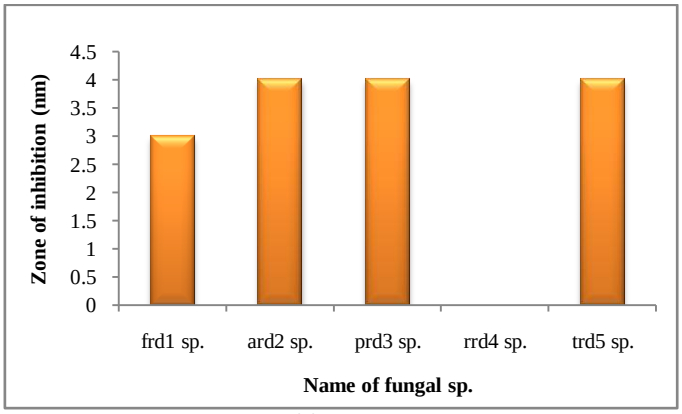

Figure 3 Growth inhibitions of fungi by Isolated *bsrd sp.*

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