Original Research Article

Antifungal activity of pseudomonas aeruginosa against various plant pathogenic fungi

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Abstract

Biocontrol using bacteria that shows antagonistic activity has been considered as an alternative approach to agrochemicals that are harmful to environment as well as human health. Antifungal activity of Pseudomonas aeruginosa was tested against some plant pathogens like Fusarium sp., Rhizopus sp., Ustilago sp. and Macrophomina sp. in vitro. The Pseudomonas aeruginosa isolate was grown in King's B medium. The cell free culture supernatant was separated by ultracentrifugation and tested for siderophore production as well as for antifungal activity on dual media by disc diffusion assay. The results showed good siderophore production i.e 158 mg/l and remarkable antifungal activity of Pseudomonas aeruginosa against Fusarium sp., Rhizopus sp., Ustilago sp. and Macrophomina sp. with 77.2, 57.7, 51.1 and 35.5 % inhibition respectively. This study showed that Pseudomonas aeruginosa is a potential isolate and could be further used as biocontrol agent.

Key Words: Pseudomonas aeruginosa, Antagonistic, Biocontrol, Agrochemicals

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INTRODUCTION

Agrochemicals such as pesticides and chemical fertilizers cause great harm to environment and to the human health[1]. Biocontrol using bacteria that shows antagonistic activity has been considered as an alternative approach to agrochemicals. Numerous studies describe the use of fluorescent pseudomonads promising owing to their versatile physiological growth promotory and biocontrol activities[2,3,4]. Fluorescent pseudomonads such as *Pseudomonas aeruginosa* have been shown to have varying degrees of antagonistic activity against various pathogenic fungi causing diseases in plants.[5] Different species of Fluorescent pseudomonads produce siderophores[6,7] and antibiotics[8,9] such as pyocyanin[10], 2-acetamidophenol[11], phenazine-1-carboxylic acid (PCA)[12,13,14], pyrrolnitrin[15], pyoluteorin[16], phenazine-1-carboxamide (PCN)[17], 2,4-diacetylphloroglucinol[18], viscosinamide[19] and tensin[20]. In this study *Pseudomonas aeruginosa* was tested against four plant pathogenic fungi viz. *Fusarium sp.*, *Rhizopus sp.*, *Ustilago sp.* and *Macrophomina sp*. Soil borne fungus *Macrophomina sp.* cause charcoal rot disease in soybean, corn and peanut. *Fusarium sp.* the main soil borne phytopathogen in terms of economical damage in agricultural productions all over the world[21,22,23] cause dry rot on potato, wilting on bean or pea, crown rot and head blight on wheat, bakanae disease on rice etc. *Rhizopus sp*. has been implicated in causing head rot in vegetables, fruits and ornamental plants like sunflower. Smut disease is caused by *Ustilago sp.* in grasses including corn (maize), wheat, sugarcane, and sorghum.

MATERIALS AND METHODS

Fungal strains and Growth Conditions: The strains used in this study were *Fusarium sp.*, *Rhizopus sp.*, *Ustilago sp.* and *Macrophomina sp.* was provided by Department of Biotechnology, Career College, Bhopal. The fungal strains were grown in Petri plates containing Potato Dextrose Agar medium (PDA) The plates were maintained at 28°C for 8 days, and then kept at 4 °C.

Bacterial strain and Growth Conditions: *Pseudomonas aerugninosa* strain was also procured from Department of Biotechnology, Career College, Bhopal and was grown in

King's B media at 28°C. Loopful culture of bacteria was transfered to Standard succinate broth and incubated in a rotary shaker at 120 rpm at 28 °C for 24 h, and was maintained in 10% glycerol at −25 ° C.

Production of antifungal metabolite (Siderophore): For qualitative test, the culture was spot inoculated onto Chrome Azurol Medium (Chrome Azurol Test)[24]; the plates were then incubated at 28±2 °C for 3 days. The formation of orange halo around the bacterial culture indicated positive test for siderophore production. Quantification of siderophore produced in culture broth was carried in Standard succinate medium using extinction coefficient of siderophore (E= 16500, pH 7.2)[25]. Pure culture of *Pseudomonas aeruginosa* was grown in standard succinate broth for 24 h. One ml active culture was inoculated in 100 ml standard succinate broth and flasks were incubated at 28±2 °C (120) rpm. After 72 h of incubation, 10 ml of culture was withdrawn and centrifuged at 10,000 rpm for 5 min. The optical density of the supernatant was read at 400 nm and siderophore was quantified based on the extinction coefficient of pyoverdine as under:

$$\text{Siderophore (mg/l)} = \frac{A_{400} \times \text{Mol weight of Pyoverdine}}{E\lambda}$$

Where,
Mol weight of Pyoverdine = 1,500 Da
Extinction coefficient (Eλ) = 16,500

Antifungal activity of the cell free supernatant: Antifungal compound was extracted using Standard Succinate broth. In 250ml flask, 100ml growth medium was prepared, autoclaved and cooled. It was then inoculated with 1ml bacterial suspension (24 hours old). The pH 7±0.2 was maintained. The flask was incubated in shaker at 28°C for 48 hours. The incubated culture was centrifuged at 10,000 rpm for 10 minutes to obtain cell free supernatant. *Pseudomonas aeruginosa* was tested for their inhibitory activity against the four phytopathogens using disc diffusion method[26]. Each fungal pathogen was grown on PDA plate till it covered the whole surface of the agar. With the help of a sterile borer, a disc of fungal pathogen was placed at the centre of dual media (PDA and NAM in equal ratio) plate. Then, filter paper discs (about 6 mm in diameter), containing 10µl bacterial supernatant was placed on the dual media surface. The Petri dishes were kept in refrigerator for 1 hour and then incubated at 28°C for 96 hours. Dual media plate simultaneously inoculated with only the fungal pathogen[27] which served as a control. The diameters of inhibition growth zones were measured. Zone between the bacteria and fungus was used as an indication for the extent of antagonism. The percentage inhibition of mycelia growth of the fungus was calculated using the following formula[28].

$$\text{Inhibition Percentage} = \frac{(A - B)}{A} \times 100$$

where, A and B are the average diameter of fungal growth on control medium and diameter of fungal growth with bacterial supernatant disc on medium respectively.

OBSERVATIONS AND RESULTS

The plates of CAS agar medium inoculated with *Pseudomonas aeruginosa* showed the presence of orange halo around the bacterial colony. The presence of halo indicated positive test for siderophore production. Further the quantification of siderophore produced by bacterial isolate was performed and the isolate produced about 158 mg/l of siderophore under iron limiting condition. A zone of inhibition of varying sizes was produced against *Fusarium sp., Rhizopus sp., Ustilago sp.* and *Macrophomina sp.* by *Pseudomonas aeruginosa* (Table: 1). The plates were observed and the growth of pathogens in the treated plate and control was measured. *Pseudomonas aeruginosa* showed good activity against phytopathogens in study. The best antifungal activity was observed against *Fusarium sp.*

Table 1:

S. No.	Test phytopathogen	*Antagonistic activity of Pseudomonas aeruginosa*
1	*Fusarium sp.*	20.5 ± 0.2mm (77.2%)
2	*Rhizopus sp.*	38 ± 0.3mm (57.7%)
3	*Ustilago sp.*	44± 0.2mm (51.1%)
4	*Macrophomina sp.*	58 ± 0.4mm (35.5%)

Note: Mean values of triplicates ± S.D, values given in parentheses indicate % maximum activity

DISCUSSION

Most strains of bacteria, actinomycetes and fungi produce siderophores under iron limiting conditions. Siderophores chelate, the ferric ions with a high specific activity and serve as vehicles for the transport of irons (Fe3+) into the cell.Several soil microorganisms are known to improve the plant growth directly through nutrient mobilization and production of plant hormones and indirectly through suppression of plant pathogens or by inducing systemic resistance in plants. Siderophores enable bacteria to take

up iron under conditions of limited availability of the element in the environment[29]. They are responsible for the dissolution, chelation and transport of iron (III) into the cell. Siderophore-mediated and antibiotic-mediated suppression of soilborne plant diseases are the two most studied mechanisms involved in biocontrol by *Pseudomonas*. Our study showed good production of siderophore by *Pseudomonas aeruginosa i.e.* 158mg/l. In the current study *Pseudomonas aeruginosa* inhibited *Fusarium sp.* by a diameter of 20.5 ± 0.2 mm, inhibition was 77.2%. This is in accordance with the study report of Jenifer *et. al*. 2013[29] and Rini *et. al*., 2007 that showed fluorescent pseudomonad inhibited *Fusarium* by 83.5 % and 60% respectively. Inhibition of *Rhizopus sp.* by *Pseudomonas aeruginosa* was about a diameter of 38 ±0.3mm which is about 57.7%. In the similar way Fluorescent pseudomonad inhibited *Rhizopus sp.* as proved by P.G. Brisbane *et. al*., 1989[30] and also showed improved wheat germination. Arya P., 2017[31] showed the efficacy of *Pseudomonas sp.* against *Macrophomina phaseolina* with 48.32% inhibition and suppression of dry root rot disease of groundnut and in our study *Pseudomonas aeruginosa* inhibited *Macrophomina sp.* by a diameter of 58 ± 0.4mm and inhibition was 35.5%. Our study showed the inhibition of *Ustilago sp.* which causes Smut disease by a diameter of 44± 0.2mm and 51.1 % inhibition.

CONCLUSION

This research exhibits the antifungal activity of *Pseudomonas aeruginosa* and indicates the possibility of using *Pseudomonas aeruginosa* as a biological control agent owing to their versatile physiological growth promotory and biocontrol activities. In this study *Pseudomonas aeruginosa* showed good antifungal activity against some plant pathogenic fungi like *Fusarium sp., Rhizopus sp., Ustilago sp.* and *Macrophomina sp.* The results obtained can further be used for the design of new environmental friendly methodologies using bioformulations of *Pseudomonas aeruginosa* for the control of phytopathogens as an alternative of chemical compounds that contaminate agricultural soils.

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