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RESEARCH ARTICLE

ISOLATION OF ANTAGONISTIC BACTERIA AGAINST FUNGAL PHYTOPATHOGEN FROM PADDY FIELD OF KANCHEEPURAM DISTRICT

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ABSTRACT

The objective of this study was to isolate and test the efficacy of *pseudomonas aeruginosa* which produces antifungal substances, to inhibit plant pathogenic fungi. The soil bacterium *pseudomonas aeruginosa* was isolated from the Rhizosphere soil, showed high antagonistic activity against Rhizoctonia *solani*, isolated from Rhizosphere region of rice (*Oryzasativa*) plant using dual culture and volatile assay. The isolated bacteria were subjected to antagonistic activity against *Rhizoctonia solani*. The four (4) isolates were selected out of eleven. *Pseudomonas aeruginosa* was found to be highly effective in suppressing the growth of fungal plant pathogens *(Rhizoctonia solani),* hence it can be used as biocontrol agent.

INTRODUCTION

Owing to their shattering effects on plant health and crop yield, plant pathogenic microbes impose a major peril to food production as well as ecosystem stability. So far, the use of chemical pesticides has remained the method of choice to control plant pathogens due to their proficient and consistent feat along with the ease of application. However, in the light of developing sustainable agricultural practices and increasing public awareness about the adverse effects of agrochemicals, research directed towards the development of substitute and complementary pathogen control methodologies is highly defensible. According to Prashar 2013, the intrinsic inhibitory potential possessed by many microbes against the phytopathogens may prove to be the alternative and environment-friendly substitute of chemical pesticides (Prashar *et al.,* 2013). It has been disclosed in 2002 (Raaijmakers *et al.,* 2002) that, biological control using antagonistic bacteria have a striking alternative due to their capability to antagonize the pathogen by different modes of action, and to effectively colonize distinct plant habitats. Fungal phytopathogens are the causes of many plant diseases and much loss of crop yields, especially in subtropical and tropical regions. Many *fluorescent* pseudomonades species from the soil environment could produce secondary metabolites to inhibit phyto-pathogenic bacteria, oomycetes and fungi (Raaijmakers *et al.,* 2002; Haas and Défago, 2005). Control of phytopathogens by biological means is environmentally valuable in contrast with chemical control (Nautiyal and Mehta, 2001). Several Pseudomonas species have been broadly used for biological control against many soil-borne plant pathogens (Weller *et al.,* 1988). The biocontrol properties of the bacteria belonging to the genus Pseudomonas are considered a better-quality because of their adaptive metabolism and their potential to produce a range of compounds inhibiting the growth of several fungal pathogens (Thomashow *e al.,* 1990). Microbial populations in the rhizosphere may benefit the plant in a variety of ways, including:

- Increased recycling, solubilisation and uptake of mineral nutrients.
- Synthesis of vitamins, amino acids, auxins and gibberellins which stimulate plant growth.
- Antagonism with potential plant pathogens. The objective of this study was to isolate *fluorescent Pseudomonades* from rhizosphere soils in South Tamil Nadu and assess their antagonistic potential against target pathogenic fungi *in vitro*.

MATERIALS AND METHODS

Material: NA-PDA media, King's B media, Glass Wares, *Pseudomonas aeruginosa,* Rhizosphere soil, *Rhizoctonia solani.*

Methodology

Sample collection: Three (3) different rhizospheric soil samples were collected from paddy field of different locations

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in kattupakkam, Tamil nadu, Chennai, India. Collected soil samples were sealed in sterile polyethylene bags and brought to the lab under ice box.

Isolation of antagonistic bacteria: Rhizobacteria were isolated from the collected soil samples according to the method of Bashan *et al*. 1993. Inoculation was carried out by spread plate method and the media used were King's B agar (selective media for *Pseudomonas*). One gram of each soil sample was weighed and transferred into 250 ml conical flask containing 100 ml sterilized water and then kept in shaker for about 20 min. 50 µl of this soil suspension was taken and spread on King's B agar medium to isolate the colonies. The plates were incubated at 28º C for 48 hr. The colonies showing yellow pigmentation on King's B medium from different soil were picked up based on the pigment formation and fluorescence under UV light. The isolated colonies of Rhizobacteria strains were selected based on their different colony morphology and further purified by sub-culturing on newer plates.

Maintenance of isolates

All the isolates were maintained at 4°C in equal volumes of nutrient broth and 20% glycerol. The King's B agar slants were kept under 8°C for regular use.

Antagonistic activity of bacterial isolates against fungal pathogen

The fungal pathogen used in this study i.e. *Rhizoctonia solani* MTCC 104 was obtained from Microbial type culture collection (IMTECH, Chandigarh, Punjab).

The obtained culture was re-cultured on potato dextrose agar. All the rhizobacterial isolates obtained were evaluated for their antagonistic activity against mycelial growth of *Rhizoctonia solani* using dual culture technique (Gupta *et al.,* 2001).

The assay was carried out by streaking the bacterial isolate at both ends of the petri plate, and placing 5 mm disk from 7 days old culture of the fungal pathogen at the centre of the petri plate of 1:1 potato dextrose agar: nutrient agar (PDA : NA). Other plate was inoculated with the only pathogenic fungi as control treatment as shown in figure 3. The culture was grown at 28°C, and inhibition of fungal growth was recorded after four days. The efficiency of bacterial isolate in suppressing radial growth was calculated and recorded when the control plates were filled by growth of the pathogenic fungi using the formula below:

$(C–T)/C \times 100$

RESULTS

Figure 1. Soil Samples Collected

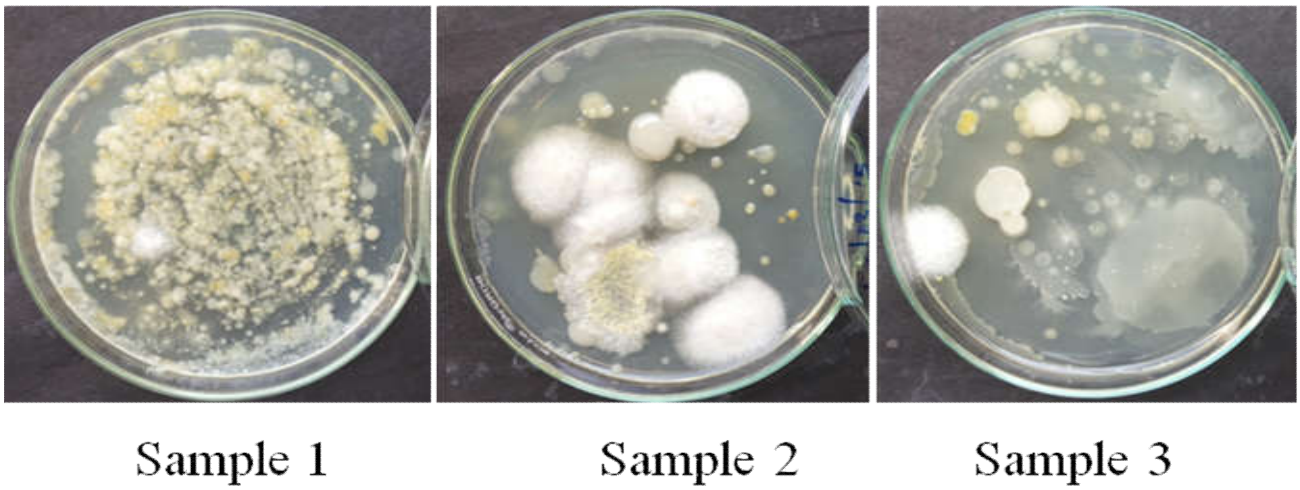
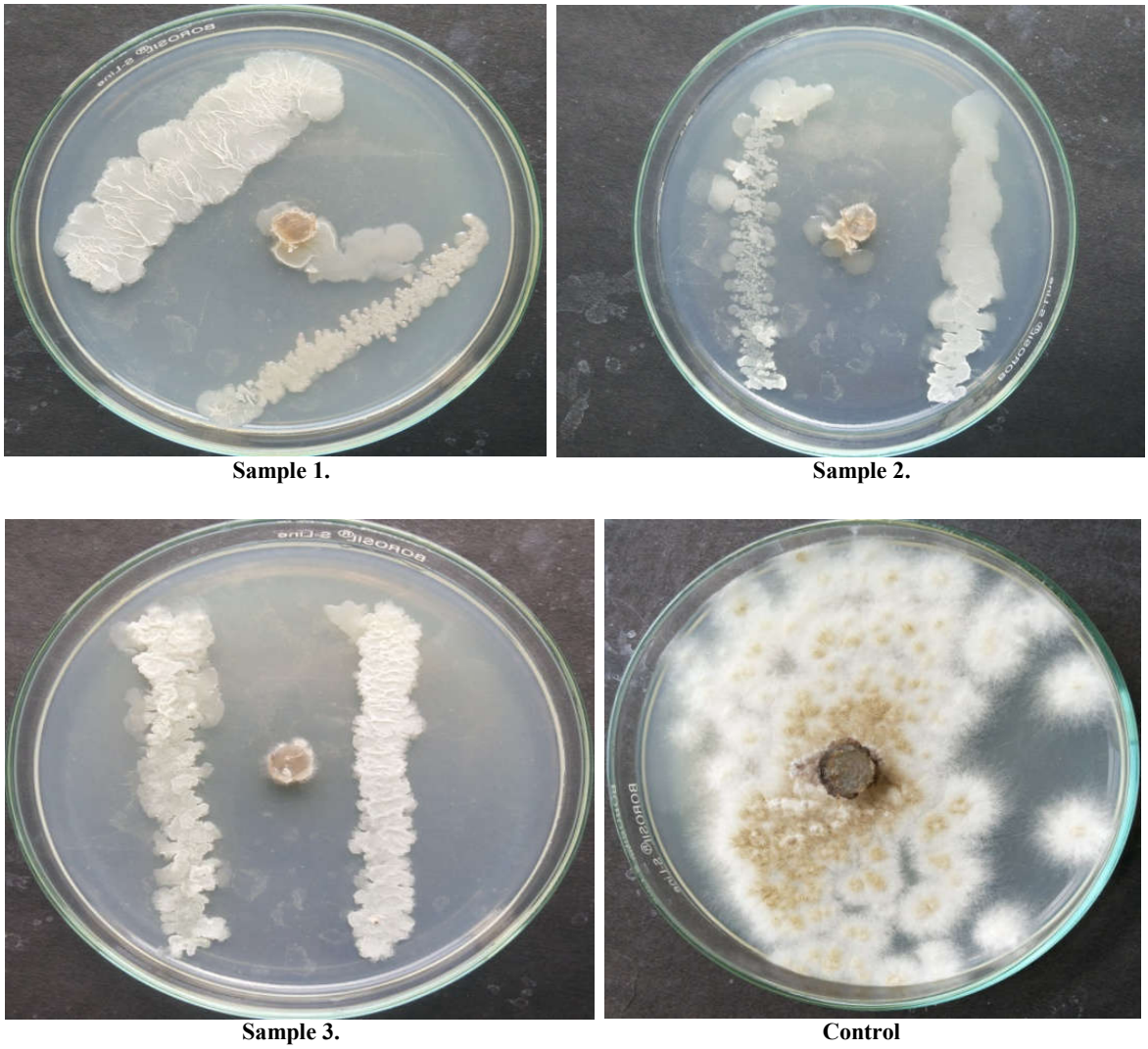


Figure 2. Cultured plates of rhizobacteria from rhizosphere soil



Sample 1. **Sample 2.**

Sample 3. **Control**

Figure 4. Antagonistic Activities of the Selected Bacterial Isolates

DISCUSSION

In figure 2 above, the yellowish colonies was observed, which indicates the presence of fluorescence pseudomonas bacterial species. This corresponded with the research conducted in 2002 by Meyer et al, that other characteristics that tend to be associated with *Pseudomonas* species include secretion of pyoverdine, a fluorescent yellow-green siderophore under iron-limiting conditions (Meyer *et al.,* 2012). However, samples 1, 2 and 3 shows zones of inhibition which results from *Pseudomonas* activities which in turn hampered the growth and development of *Rhizoctonia solani.* It has been reported that *Rhizoctonia solani* established the pathogenicity on most vegetable under green house conditions*. Pseudomonas* species have been applied to cereal seeds or applied to soil as way of preventing growth or establishment of crop pathogens.

Conclusion

The isolated organism, *Pseudomonas species* have potential of controlling the devastating fungal phytopathogens *Rhizoctonia solani.* Many *fluorescent* pseudomonades species from the soil environment could produce secondary metabolites to inhibit phyto-pathogenic bacteria, oomycetes and fungi. Hence, it could be used as a biocontrol agent (BCA).

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