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Studies on the isolation, identification and enzyme activity of bioagent *Pseudomonas fluorescens* used for controlling brown spot disease of rice caused by *Helminthosporium oryzae*

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Abstract

In the present investigation studies was carried out ontheIsolation, Identification and Enzyme activity of bioagent *Pseudomonas fluorescens* used for controlling Brown spot disease of Rice caused by *Helminthosporium oryzae*(Breda de Haan).This is a fungal pathogen causing major disease that causes enormous losses in grain yield (upto 90%) particularly when leaf spotting phase assumes epiphytotic proportions.

Keywords: Pseudomonas fluorescens; Bioagent; Disease; Helminthosporium oryzae; Enzyme; Rice

1. Introduction

Rice is a staple food to more than half of the world's population and to two third population of India [1]. This single most important cereals crop is inflicted by various diseases of fungus, bacteria, viruses, nematodes and mycoplasma pathogens.Losses due to rice diseases have been estimated to be 10-15% in general [2]. Among these diseases brown spot disease caused by *Helminthosporium oryzae* (Breda de Haan) a fungal pathogen is one major disease that causes enormous losses in grain yield (upto 90%) particularly when leaf spotting phase assumes epiphytotic proportions as observed in Great Bengal famine in 1942-43 [3]. In the present investigation studies was carried out on the Isolation, Identification and Enzyme activity of bioagent *Pseudomonas fluorescens* used for controlling Brown spot disease of Rice.

2. Methodology

2.1. Isolation, identification of bio-agent (*Pseudomonas fluorescens*) and in-vitro test

The rhizospheric soil loosely adhering to the roots of Paddy plant was collected from the experimental field of Sam Higgibottom University of Agriculture. Technology and Sciences (SHUATS), Prayagraj, (U.P.).The collected soil samples were shade dry and finely powdered, then 10 g of this powdered soil was added into 90 ml sterile distilled water to make 1:10 dilution (10^{-1}) and shaken vigorously then transfer 1 ml of the suspension (1:10 dilution) to another 9 ml sterilized distilled water to make 1:100 dilution 10^{-2}, likewise prepare serial solution from 10^{-3} upto 10^{-7} as earlier then a loopfull of this last dilution suspension was spread on plated King's medium B (KMB) agar and incubated at 37±1ºC for 24 hrs. [4] Pick up the individual colony with sterilized loop & transfer on to fresh King's medium then single colony of bacteria was transfered into King's medium B (KMB) slants to obtain pure culture and stored in refrigerator at 4ºC (Figure-1(a) & 1(b) and sub-cultured periodically at 15 days intervals on the same KMB medium.Morphological characteristics of *Pseudomonas fluorescens* are shown in below (Table 1) and Figure 1(a) & 1(b).

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Table 1 Characteristic of *Pseudomonas fluorescens* isolate

Sl. No.	Observed morphological characteristics
1	Cells are single, straight or curved and no helical
2	Gram negative (retains crystal violet stain during alcohol wash)
3	Culture produces diffusible fluorescent (Yellow-green) pigment on King,s medium B

Figure-1(a). Pure culture isolates of *Pseudomonas fluorescens from* paddy rhizosphere

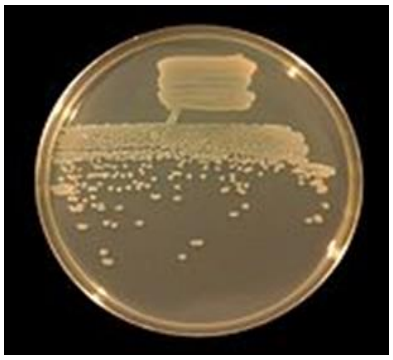

Figure-1(b). Colony of *Pseudomonas fluorescens* under white light

2.2. Extra-cellular enzymatic activities test for identification of non pathogenic nature of rhizobacteria (Pseudomonas fluorescens)

2.2.1. Cellulase activity test

This test was conducted to confirm the non pathogenic nature of the isolated bio-agent (*P. fluorescens*). For this test a medium (Carboxy Methyl Cellulose-5 g, agar-17 g, and water-1000 ml) was prepared and sterilized in conical flask by autoclaving and allow cooling down to 45ºC and then poured into petri plates and allowed it to solidify. Isolated rhizobacterial culture of 24 hours was spot inoculated just by stabbing with inoculating needle and kept in incubator for one week at 28ºC. If the bacterium hydrolyzed cellulose, the growth was surrounded by clear orange zone. [5]

2.2.2. Pectinase activity test

A medium containing pectin-5 g, yeast extract-5 g, agar-16 g, and water-1000 ml was sterilized in flask by autoclaving and allowed to cool up to 45ºC .

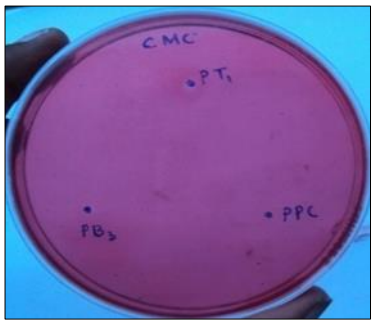

Figure-2(a) Cellulase

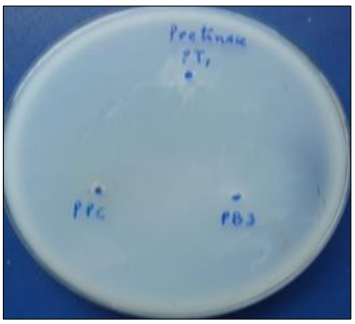

Figure-2(b) Pectinase

It was then poured to petri plate and allowed to solidify. The same bacterial isolate of 24 hours was spot inoculated and was then kept in the incubator upto 2-4 days at 28ºC. [6]

In the above cellulose enzymatic test there is no clear orange zone development around the inoculated spot of the rhizobacteria.Likewise in case of Pectinase test also no clear zone was found developed around theinoculated spot of the bacterial isolates thus it was well confirmed that the isolated rhizobacterial are non pathogenic in nature [Figure-2(a) & 2(b)]

2.3. Counting of cfu and preparation of different cfu concentration of bio-agent (*Pseudomonas fluorescens*)

The pure culture of *Pseudomonas fluorescens* isolates was grown in 50 ml of King's medium B (KMB) brothand waiting to reach $1x10^9$ cfu/ml. For determination of *P. fluorescens* cfu/ml from stock culture of King's B broth was done by following the formula [7]

$$\text{CFU/ml} = \frac{\text{Number of colonies x Dilution factor}}{\text{Volume of culture}}$$

Different cfu concentration was obtained by serial dilution technique [8]. 1 ml suspension *Pseudomonas fluorescens* from 250 ml of stock culture having reached cfu strength ($1x10^9$ ml^{-1}) was taken with the help of sterilized pipette and transfered into 9 ml sterile distilled water to make 1:10 or (10^{-1}) dilution, vigorously shake the dilution, now this first dilution will have $1x10^8$ cfu ml^{-1}.Then transfer 1 ml of suspension of this first dilution to another 9 ml sterilized distilled water to make second dilution of 1:100 or (10^{-2}) which will contain $1x10^7$cfu ml^{-1} likewise prepare serial, dilution of 10^{-3}, 10^{-4} etc., till the desire cfu concentration per millilitre (ml^{-1}) of dilution was obtained. For preparation of different bacteria cells concentration methods [9] was followed**.** *P.fluorescens* isolate was grown in 5 litres fermenters on nutritive broth and allow reaching $1x10^9$ cfu ml^1 concentration and then diluted with water to achieve the desired bacterial density. Foliar application was done with bacterial suspension of cell concentration@ $1.37x10^8$/ litre of water or 500 litres ha^{-1}.

2.4. *In-vitro* evaluation of antagonistic effect of *Pseudomonas fluorescens* isolates

The *in-vitro* evaluations of antagonistic effect of *Pseudomonas fluorescens* isolates against the pathogenic fungi *H.oryzae* was done by dual culture technique. The petriplates were having 15 ml PDA without antibiotic and the fresh loop full of *P. fluorescens* stock culture ($1x10^9$) cfu/ml concentration was streaked leaving 1 cm from the margin. Then 5 mm mycelial disc of *H.oryzae* taken from 5 days old culture with the help of sterilized cork borer and placed at the centre of each petriplates and incubated at 28±1ºC for 4 days. The distance between fungal growth and bacterial colonies was recorded as inhibition zone. For each treatment four replication were maintained (Table 2).

Table 2 Antagonistic effect of bio-agent (*Pseudomonas fluorescens*) isolates on linear growth of *Helminthosporium oryzae in-vitro* test

Sl.No.	*P. fluorescens* isolates	Linear growth of *H. oryzae*at 120 hours (cm)*	Actual zone of inhibition (cm)*	% growth inhibition over control
1.	Pf1	5.69	1.5	55.29
2.	Pf2	4.37	1.3	50.95
3.	Pf3	4.29	1.2	39.80
4.	control	7.47	0.8	-
	CD (0.05%)	0.43	0.28	-

*Mean of four replication

The data presented in Table-2, Figure-3(a) & 3(b) is the result of *in-vitro*test of three rhizobacterial isolates (*Pseudomonas fluorescens*) *viz.* (1). Pf1, (2). Pf2 and (3). Pf3 showing antagonistic activities against *Helminthosporium oryzae,* an incitant of brown spot disease of rice. Among the different isolates, Pf2 and Pf3 was found most effective against the linear growth of *H. oryzae* giving maximum inhibition zone of 1.7 cm with 55.29% and 1.3 cm with 50.97% respectively over the control. The antagonistic nature of rhizospheric bacteria against *H. oryzae* was also reported by several workers. However, Rosales *et al.* (1999) reported rhizospheric bacteria reduced 70% disease incidence of *H. oryzae*.

Figure-3(a) Antagonistic studies of *P. fluorescens* isolate against the pathogenic fungi

Figure-3(b) Different *P. fluorescens* isolates againstthe linear growth of *H*. o*ryzae* Plate No: (1).Pf1, (2) .Pf2, (3). Pf3, (4).control

3. Conclusion

In the present investigation bioagent *Pseudomonas fluorescens* used for controlling Brown spot disease of Rice caused by *Helminthosporium oryzae* (Breda de Haan) and thereby increasing crop yield.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

Both the authors have contributed in the manuscript and both have interest for getting the research paper published for use of students and scientific community.

References

[1] Rout S, Tewari SN. Amalab, a formulated botanical product potential against rice blast incitant *Pyriculari agrisea. The Bioscan*. 2012; 7: 547-552.

[2] Kandhari J. Management of sheath blight of rice through fungicides and botanicals. *Indian Phytopathology.* 2007; 60: 214-217.

[3] Ghose RLM, Ghatge MB, Subramanian V. Rice in India (revised edn.), New Delhi, ICAR. 1960; 474.

[4] Rosales RA, Thomashow L, Cook RJ,Mew TW. Isolation and identification of antagonastic *Pseudomonas spp*. *Phytopathology*. 1995; 85: 1028-1032.

[5] Asoufi H, Hameed KM, Mahasneh A. The cellulose and pectinase activities associated with the virulence of indigenous *Sclerotinia sclerotiorum* isolates in Jordan valley*. Plant Pathol. J.* 2007; 3(4):233-238.

[6] Hagerman AE, Blau D, McClure AL. Plate assay for determining the time of production of protease, cellulose and pectinases by germinating fungal spores. *Ann. Biochem*. 1985; 151: 334-342.

[7] Aneja. Experiments in microbiology, Plant pathology and biotechnology. Fourth edition. New Age International (P) limited, Publishers, New Delhi. 2004; 430-458.

[8] Mahaveer B, Singh CP,Manoj KJ. Potential of *Pseudomonas fluorescens* as biocontrol agent of fungal pathogens. Crop Protection. 2012; 179-187.

[9] Lucas JA, Solano BR, Montes F, Ojeda M, Megias FJ, Gutierrez M. Use of two PGPR strains in the integrated management of blast disease in rice (*Oryza sativa*) in Southern Spain. Field Crop Research. 2009; 114: 404-410.