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CHARACTERIZATION OF *PSEUDOMONAS FLUORESCENS* IN DIFFERENT MEDIA AND ITS ANTAGONISTIC EFFECT ON PHYTOPATHOGENIC FUNGI

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ABSTRACT
The present investigation was carried out to screen different media for growth and colony formation of test bacterium *Pseudomonas fluorescens* and to check its antagonistic activity on fungal plant pathogens by *in vitro* dual culture techniques. Out of the seven different media tested, King's 'B' media was best with an OD value of 2.50 and 6 X 10^8 cfu among the different media evaluated. As far as growth was concerned it was fast in King's 'B' medium, fair in Sim agar medium and slow in remaining media tested. In dual culture method, *P.fluorescens* on co-inoculation with fungal pathogens decreased their growth rate. Maximum inhibition was observed in *Sclerotium rolfsii* (63.15%) followed by *Fusarium oxysporum* (61.85%) *Rhizoctonia bataticola* (55.56%) and *R.solani* (53.15 %). study.

INTRODUCTION

The increasing demand for a steady food supply to the growing world population will require controlling of plant diseases that reduced crop yield subsequently. In order to control the plant diseases, biological control is gaining greater attention due to low cost and ecofriendly application. *P. fluorescens* is one such biocontrol agent with plant growth promoting ability coupled with antagonistic effect in phytopathogens. The synthesis of yellow-green, fluorescent, water-soluble pigments is a characteristic property of some *Pseudomonas* spp. (Stanier *et al.,* 1966). The effects of various minerals, growth factors, carbon and nitrogen source, pH, and temperature on antibiotic production of biocontrol strains of *P. fluorescens* in defined liquid media have been examined (Duffy *et al.,* 1999; Slininger *et al.,* 1992; 1995). Bacterial antagonists have the twin advantage of faster multiplication and higher rhizosphere competence hence, *P. fluorescens* have been successfully used for biological control of several plant pathogens (Ramamoorthy *et al*., 2002) and biological control using PGPR strains especially from the genus *Pseudomonas* is an effective substitute for chemical pesticides to suppress plant diseases (Compant *et al*., 2005). Their applicability as biocontrol agents has drawn wide attention because of the production of secondary metabolites such as siderophore, antibiotics, volatile compounds, HCN, enzymes and phytohormones (Nagarajkumar *et al*., 2004). The biocontrol mechanism to suppress fungal pathogens by *Pseudomonas* spp. normally involves the production of antibiotics and *P. fluorescens* possess gene cluster that produces a suite of antibiotics, including compounds such as 2,4-diacetylphloroglucinol (DAPG), phenazine, pyrrolnitrin, pyoluteorin and biosurfactant antibiotics (Angayarkanni *et al*., 2005). Several fungal (*Trichoderma* sp.) and bacterial (*Pseudomonas* sp. and *Bacillus* sp.) antagonists, have been successfully used as biocontrol agents in the control of seed and soil borne pathogens like *Sclerotium rolfsii, Rhizoctonia solani, Fusarium oxysporum* and *Sclerotinia sclerotiorum* in the various crops. (Sharma *et al.,* 1999; Mukhopadhyay *et al.,* 1992; Raguchander *et al.,* 1997; Sankar and Jeyarajan, 1996; Abrahm Mathew and Gupta, 1998; Kehri and Chandra, 1991).

In this context, investigation was carried out to screen simple and effective medium for Growth and colony formation of test bacterium *Pseudomonas fluorescens* and to check its antagonistic activity on fungal plant pathogens by *in vitro* dual culture techniques.

MATERIALS AND METHODS

The local isolate of *P. fluorescens* was evaluated in the following media and broth.

Media and broth used

Following media were used during laboratory studies on *Pseudomonas fluorescens*

King's 'B' medium (g/L)

Table 1: Effect of different media on growth and colony characters of *Pseudomonas fluorecsens*

No.	Media	Optical density*(HAI)**						Colony type	Colour of the colony	Type of growth	Reaction to UV light	No. of colony after 48hrs
		12	24	36	48	72	96					
1	King's 'B' Medium	0.72	0.80	0.96	1.27	1.57	2.50	Round	Greenish Yellow	Fast	Bright	608***
2	Nutrient Agar Medium	0.57	0.63	0.80	0.80	0.84	0.97	Round	Yellowish	Medium	Bright	183
3	Standard Nutrient Broth Medium	0.19	0.28	0.43	0.65	0.89	1.31	Round	Greenish Yellow	Medium	Bright	213
4	Urea Broth Medium	0.59	0.63	0.55	0.54	0.23	0.32	Round	Whitish	Slow	Bright	00
5	Sim Agar Medium	0.70	0.77	0.83	0.96	1.62	2.00	Round	Greenish Yellow	Fast	Bright	266
6	Beef Peptone Agar Medium	0.47	0.71	0.94	1.23	1.77	1.77	Round	Yellowish	Medium	Bright	227
7	Wakimoto's Semi Synthetic Medium	0.52	0.59	0.80	0.87	1.02	1.28	Round	Yellowish white	Medium	Bright	114
$SE_{(m)}\pm$	-	-	-	-	-	0.08						
CD (5%)	-	-	-	-	-	0.23						

* Average of four replications; **Hours after inoculation; ***Average of three replications

Protease peptone - 20.0 g, Dipotassium hydrogen phosphate - 1.5 g, Magnesium sulphate - 1.5 g, Glycerol - 8.0 mL, Agar-Agar - 20.0 g, Cyclohexamide - 75.0 mg, Novobiocin - 45.0 mg, Penicillin - 75.0 mg, Distilled water - 1000.0 ml.

Nutrient agar medium (g/L)

Peptone - 10.0 g, Beef extract - 5.0 g, NaCl - 3.0 g, Agar-Agar - 20.0 g , Distilled water - 1000 mL, pH - 7.6

Standard nutrient broth medium (g/L)

Sucrose - 18.00 g, Peptone - 5.00 g , Yeast extract - 2.00g , MgSO4 - 0.30g , K_2SO_4 - 0.50g, Distilled water - 1000 ml, pH - 7.20

Urea broth medium (g/L)

Yeast extract - 0.1 g, KH_2PO_4 - 9.1 g, Na_2HPO_4 - 9.5 g, Urea - 20.0 g, Phenol red - 0.01 g, Distilled water -1000 ml, pH - 6.8

Sim agar medium (g/L)

Peptone - 30 g, Beef extract - 3 g, Perrous-ammonium-sulphate -0.2 g, Thiosulphate - 0.025 g, Distilled water - 1000 ml, Agar-Agar - 18.00 g, pH - 7.3

Beef Peptone Agar medium (g/L)

Beef extract - 3.0 g, Peptone - 5.0 g, Dextrose - 10.0 g, Yeast extract - 5.0 g, Agar-Agar - 15.0 g, Distilled Water - 1000 mL, pH - 7.2

Wakimoto's Potato Semi-Synthetic medium (g/L)

Potato - 300 g, $Ca(No_3)_2.4H_2O$ - 0.5 g, $Na_2HPO_4.12H_2O$ - 0.2 g, Peptone 5 g, Sucrose - 20 g , Agar - 15 g, Distilled water -1000 ml Note: - All three antibiotics namely cyclohexamide, novobiocin and penicillin were dissolved in 3mL of 90 per cent ethanol and added after autoclaving when medium was cooled to 45ºC.

Measurement of growth and colony character on different media

Seven different media *viz*., King's 'B' medium, Nutrient agar medium, Standard nutrient broth medium, Urea broth medium, SIM agar medium, Beef Peptone Agar medium, Wakimoto's Potato Semi-Synthetic medium were used in the growth variation study. Hundred ml broth medium was taken in each Erlenmeyer flask and was inoculated with a loopful 42 h old culture and four replications of each medium were maintained and incubated at 25 ± 2ºC. Increase/ decrease in the turbidity of the broth was recorded as positive/ negative growth reaction on the basis of optical density through the spectro-photometer and number of colony were also recorded 48 h after inoculation

Table 2: Antagonistic effect of *Pseudomonas fluorecsens* against *Fusarium oxysporium* , *Sclerotium rolfsii*, *Rhizoctonia bataticola* and *R.solani (in vitro)*

S.N.	Pathogen	Radial growth(mm)	% Inhibition
1	*Fusarium oxysporum*	34.33*	61.85
2	*Sclerotium rolfsii*	33.17	63.15
3	*Rhizoctonia bataticola*	40.00	55.56
4	*Rhizoctonia solani*	42.17	53.15
5	Control	90.00	-
$SE_{(m)}\pm$	0.73		
CD (5%)	2.40		

* Average of four replications

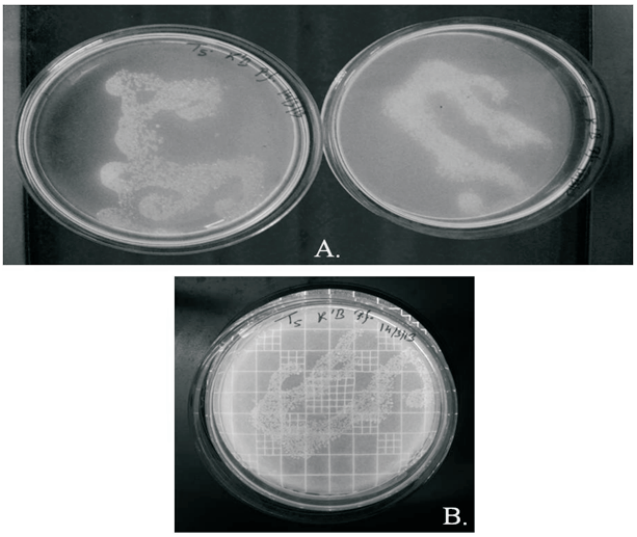

Plate 1: Enumeration of bacterial colonies of *P. fluorescens* grown on King's 'B' media using Colony counter

through colony counter which are inoculated in solid medium (Aneja, 2003).

Testing the antagonistic potential of *Pseudomonas fluorescens*

The antagonistic activity of *P. fluorescens* against *Sclerotium* spp., *Rhizoctonia* spp. *and Fusarium* spp. was studied by dual culture technique (Rabindran and Vidyasekaran, 1996). Bacterial isolate was streaked at one side of petri dish (1 cm away from the edge) containing King's 'B' medium. Five mm mycelial plug from seven-day-old PDA cultures of pathogens were placed at the opposite side of petri dishes perpendicular to the bacterial streak. Petri dishes were then incubated at 28 ± 2ºC for 5 days. Petri dishes inoculated with fungal discs alone were served as control. Each treatment was replicated 4 times. Observations on width of inhibition zone and mycelial growth of test pathogens were recorded and percent inhibition of pathogen growth was calculated (Vidyasekaran *et al.*, 1995).

The per cent growth inhibition was calculated by using the following formula given by Vincent (1947)

$$I = \frac{C - T}{C} \times 100$$

Where,

I = Per cent inhibition C = colony diameter in control (mm)

T = colony diameter in treatment (mm)

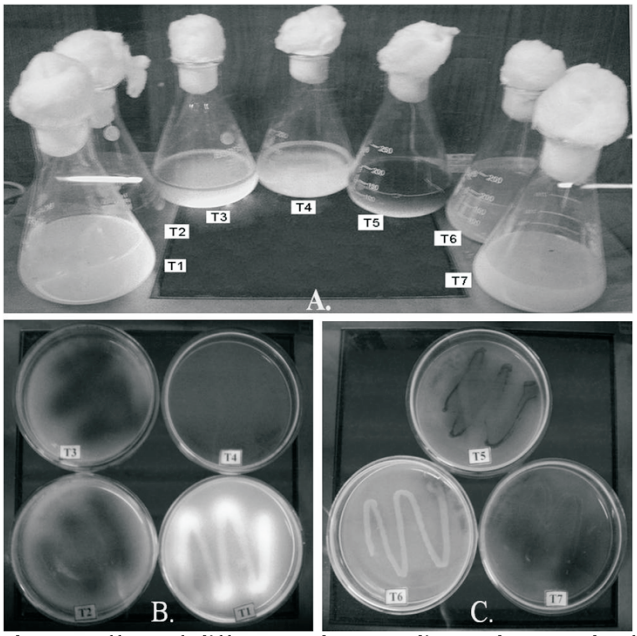

Plate 2: Effect of different culture media on the growth of *Pseudomonas fluorescens* (T); King's 'B' medium 2) Nutrient Agar Medium 3) Standard Nutrient Broth; Medium 4) Urea Broth Medium 5) Sim Agar Medium 6) Beef Peptone Agar Medium 7) Wakimoto's Semi Synthetic Medium; 1-7 broth media and B-C. 1-7 solid media

RESULTS AND DISCUSSION

Effects of different media on the growth of *Pseudomonas fluorescens:*

Seven media were tested to understand the cultural behaviour and to find out the best growth and colony forming supporting medium.

It is evident from the data presented in Table 1, that *Pseudomonas fluorescens* preferred King's 'B' medium for growth and colony formation. Optical density was observed significantly superior on King's 'B' medium (0.72, 0.80, 0.96, 1.27, 1.57 and 2.50 O.D.) and Urea broth medium showed least growth (0.59, 0.63, 0.55, 0.54, 0.23 and 0.32 O.D.) followed by Nutrient agar medium (0.57, 0.63, 0.80, 0.80, 0.84 and 0.97 O.D.) at 12, 24, 36, 48, 72 and 96 hours after inoculation, respectively.

There was variation in the colony characters of test organism. As far as growth was concern, it was fast in King's 'B' medium, fair in Sim agar medium and slow in remaining media tested. After forty eight hours highest numbers of colonies were observed in King's 'B' medium (608) followed by Sim agar medium (266), Beef peptone agar medium (227), Standard nutrient agar medium (213), Nutrient agar medium (183) and Wakimoto's semi synthetic medium (114). In urea broth medium growth (colonies) of *P. fluorescens* was not observed after 48 hrs (table 1).

The similar finding was also reported by Jagadish (2006) Li *et al.* (2009) and Kotgire *et al.* (2012) They found that King's 'B' medium was best for growth and colony formation of *P. fluorescens* supports the findings of present study.

Antagonistic effect of *Pseudomonas fluorescens* in dual culture technique:

The antagonistic activity of *Pseudomonas fluorescens* against *Fusarium oxysporum*, *Sclerotium rolfsii*, *Rhizoctonia bataticola* and *R.solani* under *in vitro* condition by dual culture technique was studied and data presented in table 2.

Pseudomonas fluorescens inhibited the mycelial growth of *Fusarium oxysporum*, *Sclerotium rolfsii*, *Rhizoctonia bataticola* and *R.solani* by the antagonistic activity. The maximum growth inhibition was recorded in *Sclerotium rolfsii* (63.15%) followed by *Fusarium oxysporum* (61.85%),

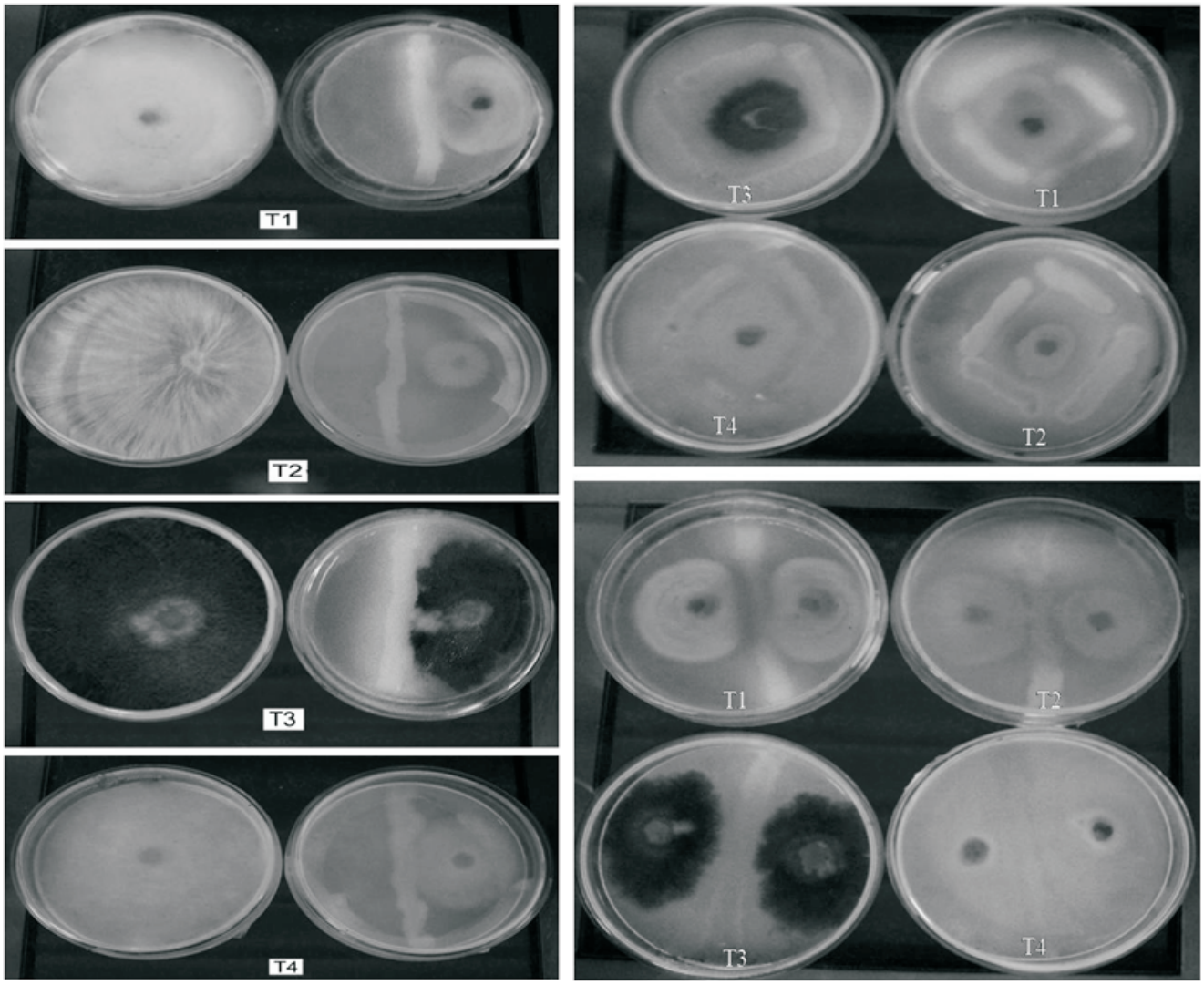

Plate 3: Antagonistic effect of *Pseudomonas fluorescens* in dual culture technique when streaked differently in T1 -*Fusarium oxysporum*, T2 - *Sclerotium rolfsii*, T3 - *Rhizoctonia bataticola* and T4 - *R.solani*

Rhizoctonia bataticola (55.56%) and *R.solani* (53.15%) 168 hrs after inoculation. Thus the result showed that *P. fluorescens* can efficiently inhibit the mycelial growth of all the fungal pathogens taken in the study.

P.fluorescens was an important antagonist inhibiting the growth of *F. oxysporum* and *Rhizoctonia solani* was reported by various workers (Vidhyasekaran *et al*. (1995); Saikia *et al*. (2003); Singh (2003); Wang *et al*. (2003); Shalini and R. Srivastava (2008); Rini *et al*. (2007); Reddy *et al*. (2009); Singh Narinder (2011); Rajeswari and Kannabiran (2011); Singh *et al*. (2011); Kapoor *et al*. (2012); Adhikari *et al*. (2013) Purohit *et al*. (2013) and Koche *et al*. (2013) also found that *P.fluorescens* was an important antagonist inhibiting the growth of *S. rolfsii* was reported by various workers Sarma *et al*. (2002); Dey *et al*. (2004); Sharma *et al*. (2005); Rakh *et al*. (2011). *P.fluorescens* showed inhibitory effect under *in vitro* condition against *Rhizoctonia bataticola* was also reported by Nautiyal (1997, 2002); Kumar *et al*. (2002). The findings of present study are in agreement with the findings of earlier workers.

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