Screening for Antifungal Activity of Pseudomonas Fluorescens Against phytopathogenic fungi

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Abstract

Antifungal activity of different strains of Pseudomonas fluorescens were tested against some plant pathogens such as Alternaria solani, Curvularia lunata, Fusarium sp., Bipolaris sp. and Helminthosporium sp. in in vitro. Different concentrations (1000, 2000, 3000, 4000 and 5000 µg/mL) of Pseudomonas fluorescens were used and maximum inhibition of spore germination of fungus starts from 2000 µg/mL. The result indicated that all the strains of Pseudomonas fluorescens presented a most significant value against all the phytopathogenic fungi. Out of the five strains studied, the best result was shown by PUR-46, which showed almost complete inhibition against Fusarium sp., Curvularia lunata and Bipolaris sp. followed by R1 and PSB-1 against fungus Helminthosporium and Curvularia lunata respectively at concentration of 5000 µg/mL. The lowest inhibition at 5000 µg/mL was shown by PSB-1 and R-1 against Bipolaris.

INTRODUCTION

Fungicides requirements are excellent potency against a variety of plant pathogens and safety, not only for humans, animals, and host plants but also for the ecosystems. Fungicides of microbial origin, which are synthesized biologically, have been demonstrated to be not only specifically effective on the target organisms but also inherently biodegradable [1] . Microbial products are so complex in their chemical structures that one compound may have two or more totally different chemical moieties which can interact with different receptors [2] .

The genus Pseudomonas has been heterogenous since Migula first named it in 1894 [3] . He designated and described the species associated with the genus in 1895 [4] . Pseudomonas are gram-negative, strictly aerobic, polarly flagellated rods. They are aggressive colonizers of the rhizosphere of various crop plants, and have a broad spectrum antagonistic activity plant pathogens, such as antibiosis (the production of inhibitory compounds) [5,6] , siderophores production (iron-sequestering compounds) [7] and nutrition or site competition [8]

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Pseudomonas plasmids confer resistance to many antibiotics and antibacterial agents. Some pseudomonads have been recognized as antagonists of plant fungal pathogens and antibiotic producers [9] . This is probably due to the abundance of this diverse group of bacteria and their obvious importance in the soils [10] . Emmerich and Low [11] reported that the cell free culture of Pseudomonas aeruginosa, a concentrated to one tenth of its original volume, killed several kinds of bacteria. Due to the lytic action of culture broth on suspensions of some kinds of bacteria, they ascribed the inhibition to an enzyme termed pyocyanase. It has been used extensively in the therapy of diphtheria, influenza and meningitis [12] .

Antagonistic activity was also observed for Pseudomonas spp. in the rhizosphere has been recognizes as major factor in the suppression of many phytopathogens. Several antibiotic –like substance have been identified, including bacteriocins and phenazine antibiotics [13] . Bacteria of the genus Pseudomonas comprise a large group of the active biocontrol strains as a result of their general ability to produce a diverse array of potent antifungal metabolites. These include simple metabolites such as 2,4-diacetylphloroglucinol, phenazine-1-carboxylic acid and pyrrolnitrin [3-chloro-4-(2'-nitro-3'-chlorophenyl)-pyrrole], as well as the complex macrocyclic lactone, 2,3-de-epoxy-2,3-didehydra-rhizoxin. Pyrrolnitrin is active against

Rhizoctonia spp, Fusarium spp, and other plant pathogenic fungi, and it has been used as a lead structure in the development of a new phenylpyrrole agricultural fungicide [14] . Strains of Pseudomonas fluorescence showed known biological control activity against certain soil-borne phytopathogenic fungi and has the potential to produce known secondary metabolites such as siderophore, HCN and protease that showed antagonistic activity against Macrophomina phaseolina, Rhizoctonia solani, Phytophthora nicotianae var. parasitica, Pythium sp. and Fusarium sp. [15] .

The purpose of this study was to examine the antifungal activity of Pseudomonas fluorescens against different phytopathogenic fungi viz. Alternaria solani, Curvularia lunata, Fusarium sp., Bipolaris sp. and Helminthosporium sp. in vitro.

MATERIALS AND METHODS

ISOLATION OF CULTURES

Five strains of Pseudomonas which showed strong in vitro antifungal activity against some plant pathogens such as Alternaria solani, Curvularia lunata, Fusarium sp., Bipolaris sp. and Helminthosporium sp. was isolated from different disease suppressive soils of King's B medium. The bacterial cultures were collected from Germplasm of Insititute of Bioengineering and Biological Sciences, Varanasi, India. The bacterial strains was cultured in nutrient agar at 28±2° C. For long-term maintenance the strains was preserved in nutrient broth containing 15% v/v glycerol at -70 ° C.

TEST ORGANISMS AND CULTURE MEDIA

Test fungi were isolated on potato dextrose agar (PDA) (peeled potato 250 g, dextrose 20 g, agar 15 g, distilled water 1 L) medium from their respective hosts collected from experimental farm of Banaras Hindu University, Varanasi, India. The cultures were further purified by single spore isolation technique and maintained at 25±2 ° C on PDA slants. 7-10 days old culture were used in the experiment. The fungi included in the present study are Alternaria solani, Curvularia lunata, Fusarium sp., Bipolaris sp. and Helminthosporium sp.

Stock solution (5000 µg/mL) of the P. fluorescens strains was prepared by dissolving 5 mL of the culture in 1 mL of distilled water. Required concentrations (1000, 2000, 3000, 4000 and 5000 µg/mL) were prepared from each stock solution by diluting with distilled water. One drop (40 mL) from each concentration was placed on grease-free glass slides. Fungal spores (200-300) were picked up from 7-10 days old culture with sterilized inoculation needle and mixed in solution of the fraction of different concentrations separately. The slides were placed in moist chambers made by placing two sterile filter papers each on the lid and base of the petriplates. The slides with spores were then incubated at 25±2° C for 24 hr. Germination was observed after staining with cotton blue prepared in lactophenol under binocular microscope (Nikon, Japan Type 102). Spores mixed in sterile distilled water only served as control. All the experiments were conducted in triplicate.

RESULTS AND DISCUSSION

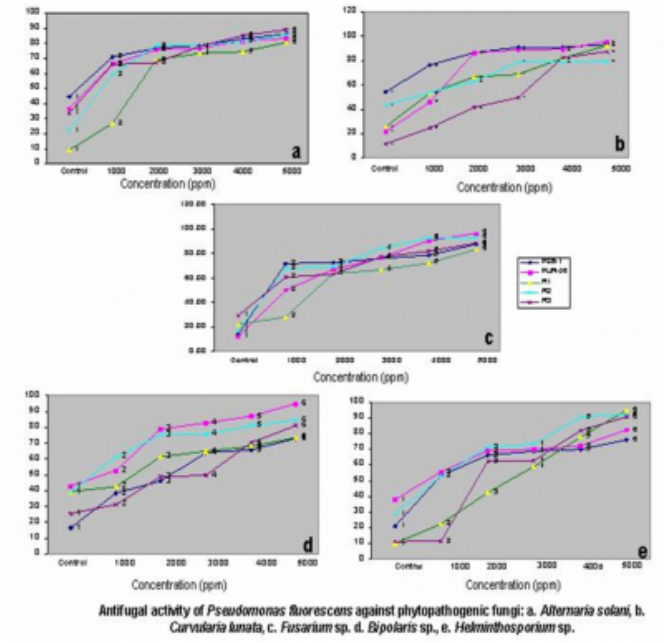
Five strains of Pseudomonas fluorescens were screened for their antimicrobial activity against different fungi viz. Alternaria solani, Curvularia lunata, Fusarium sp., Bipolaris sp. and Helminthosporium sp. in vitro. The Pseudomonas strains showed antifungal activity against all tested strains (Fig. 1 a, b, c, d & e). Table 1 presents the different concentrations (1000, 2000, 3000, 4000 and 5000 µg/mL) obtained for each strain tested. All the five strains showed varied levels of antifungal activity and maximum inhibition of spore germination of phytopathogenic fungi starts from 2000 µg/mL. At 5000 µg/mL, almost all the P. fluorescens strains (PSB-1, PUR-46, R1, R2 and R3) are significantly effective against all the phytopathogenic fungi. Out of the five strains studied, the best result was shown by PUR-46, which showed almost complete inhibition and maximum activity against Fusarium sp. (96.30%), Curvularia lunata (96.07%) and Bipolaris sp. (95.08%) followed by R1 that showed inhibition percentage of 94.55% against Helminthosporium at 5000 µg/mL. Also at concentration of 4000 µg/mL, all the P. fluorescens strains showed better activity against phytopathogenic fungi. The best result at 4000 µg/mL was shown by the strains of PSB-1 against Curvularia lunata and R2 against Fusarium sp. and Helminthosporium. The lowest activity was shown by the strain R3 against Curvularia lunata and Bipolaris sp. at concentration of 1000, 2000 and 3000 µg/mL. These data revealed that Pseudomonas strains exhibited significant antifungal activity. In testing, percentage of spores inhibition increased with increase in concentration and thus exhibiting concentration dependent activity

Figure 1

Table 1: Effect of on spore germination of some phytopathogenic fungi

S.No	Fungus	Host	Strains	Concentration (µg / ml) Spores inhibition (%)						Coefficient of variation (CV)
				Control	1000	2000	3000	4000	5000	
1	*Alternaria solani*	*Solanum tuberosum*	PSB-1	44.44	70.97	76.92	78.79	83.13	86.67	0.21
			PUR-46	36.84	66.67	75.86	76.92	81.94	83.33	0.25
			R1	9.09	26.83	69.39	73.33	74.47	80.77	0.54
			R2	22.50	59.09	78.57	78.79	81.48	86.08	0.35
			R3	33.93	66.00	67.44	78.13	85.48	89.23	0.28
2	*Curvularia lunata*	*Oryza sativa*	PSB-1	54.29	76.67	86.67	90.82	91.11	93.33	0.18
			PUR-46	21.74	46.06	66.67	68.57	89.13	96.07	0.42
			R1	25.81	53.57	66.67	68.75	81.94	92.50	0.36
			R2	43.48	53.85	63.16	78.95	78.95	79.55	0.23
			R3	11.63	25.00	41.67	50.00	82.58	87.50	0.61
3	*Fusarium sp.*	*Cucurmis sativa*	PSB-1	14.29	71.93	72.73	76.00	78.65	87.50	0.39
			PUR-46	12.50	50.00	67.21	76.63	90.65	96.30	0.47
			R1	22.39	28.00	63.64	66.67	72.22	83.33	0.44
			R2	17.31	67.47	69.23	83.95	92.75	93.10	0.40
			R3	29.41	60.56	63.33	77.50	81.82	88.24	0.32
4	*Bipolaris sp.*	*Cocos nucifera*	PSB-1	16.67	38.46	46.03	64.00	85.71	73.97	0.42
			PUR-46	42.86	53.06	78.85	82.86	87.88	95.08	0.28
			R1	39.39	42.42	61.54	65.22	68.18	73.33	0.24
			R2	39.44	61.36	75.38	76.00	81.88	84.62	0.24
			R3	25.58	31.25	48.84	50.00	70.59	81.25	0.42
5	*Helminthosporium sp.*	*Zea mays*	PSB-1	21.05	53.45	66.07	68.75	69.77	75.86	0.34
			PUR-46	37.93	55.56	69.05	70.00	72.00	82.35	0.24
			R1	10.20	22.73	42.55	58.97	77.78	94.55	0.63
			R2	28.95	52.94	71.43	73.33	90.48	92.11	0.35
			R3	11.43	11.76	62.50	62.75	82.05	90.70	0.64

Figure 2



Antifugal activity of *Pseudomonas fluorescens* against phytopathogenic fungi: a. *Alternaria solani*, b. *Curvularia lunata*, c. *Fusarium* sp. d. *Bipolaris* sp., e. *Helminthosporium* sp.

The biological control of soil-borne pathogens with antagonistic bacteria, particularly Pseudomonas spp. belonging to plant growth promoting Rhizobacteria, has received prominent attention because of the dual role of these bacteria in plant-growth promotion and disease control [16] . Experiments in vitro and in vivo by Rozsnyay et al. [17] showed that some strains of P. fluorescens, some epiphytic bacteria and some fungi inhibited canker and dieback diseases of apricot. In earlier studies [18,19,20] a talc-based formulation of the P. fluorescens strains also used here reduced sheath-blight incidence under field conditions in different zones of Tamil Nadu state, India.

Pseudomonas strains were evaluated for their ability to control Sclerotinia homeocarpa and Bipolaris sorokiniana on the phylloplane of Ken- tucky bluegrass [21] . Pseudsomonas, Bacillus and Stenotrophomonas maltophilia, showed antifungal activity against Verticillium dahliae var. longisporum in vitro and were evaluated as potential biocontrol agents by [22] . Rajappan and Ramaraj [23] evaluated the efficacy in vitro of P. fluorescens against the cauliflower wilt pathogen Fusarium moniliforme. Fluorescent pseudomonad strains found to be effective against Sclerotium rolfsii were evaluated by Patil et al. [24] under greenhouse conditions for their effects on groundnut and on collar rot incidence. Trichoderma viride and Pseudomonas sp. controlled stalk rot (associated with Pythium aphanidermatum and Fusarium graminearum) at the seedling stage of maize [25] . Janisiewicz and Roitman [26] reported that blue mold and grey mold of apples and pears could be controlled by Pseudomonas. A bacterial strain identified ad Pseudomonas acidovorans NB-10II which has been renamed Comamonas acidovorans [27] , isolated from water pond in South Jordon was found to have an antifungal activity against filamentous fungi (Aspergillus niger SQ 40, Fusarium oxysporium SQ 11, Verticillium dahliae SQ 42), yeasts (Saccharomyces cerevisiae SQ 46, Candida albicans SQ 47). This bacterial isolate was found to accumulate the main portion of the antimicrobial substances in their cells [28] . The Pseudomonas fluorescens strain MM-B16 that showed significant antifungal and antioomycete activity against Colletotrichum orbiculare and Phytophthora capsici [29] .

Pseudomonads represents the major group of non-differentiating microorganisms that produce antibiotics such as phycocyanin, pyrolnitrin and pseudomonic acid, was investigated in in vitro and in vivo that showed anticandidal activity against Candida species [30] . Thomashow et al., [31] showed that the production of phenazine-1-carboxylic acid by P. fluorescens in the rhizosphere of wheat was correlated with take-all disease control. Antibiotics induced by P. fluorescens inhibit P. ultimum in the cotton spermosphere and rhizosphere [32] . Some species of Pseudomonas can also produce levels of HCN that are toxic to certain pathogenic fungi [33] . These characteristics make Pseudomonas species good candidates for used as seed inoculant and root dips for biological control of soil-borne plant pathogen. Studies by [34] reviewed the importance of the antibiotic phenazine-1-carboxylic acid, produced by strain 2-29 of P. fluorescens, to suppress take-all disease of wheat caused by Gaeumannomyces graminis f. sp. tritici. Leaf application of P. fluorescens effectively conferred resistance against leaf

pathogens [35] .

CONCLUSION

The presented data exhibit the antifungal activity of Pseudomonas strains and indicate the possibility of using Pseudomonas fluorescens as a biological control agent of some plant pathogenic fungi. However, this requires further screening of a large number of Pseudomonas strains from different regions of India. The antimicrobial activity of Pseudomonas may be attributed to the various phytochemical constituents have even more potency with respect to the inhibition of microbes.

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