Antifungal Activity of Pseudomonas fluorescens Against Different Plant Pathogenic Fungi

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Abstract

Antifungal activity of different strains of Pseudomonas fluorescens were tested against some plant pathogens such as Alternaria cajani, 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 spore germination of fungus was inhibited at 4000 and 5000 µg/mL. The result indicated that all the strains of Pseudomonas fluorescens presented a most significant value against Alternaria cajani and Curvularia lunata. Out of the five strains studied, the best result was shown by A-5, which showed almost complete inhibition against pathogenic fungi such as Curvularia lunata and Fusarium sp. at 4000 and 5000 µg/mL while strain L-5 was resistant against Fusarium sp. and Helminthosporium sp. at 5000 µg/mL. Among the fungus tested, bacterial strains C-03 and Pf4-1 were found to be more sensitive to Fusarium sp. and Helminthosporium sp.

INTRODUCTION

The surfaces of aerial plant parts provide a habitat for epiphytic micro-organisms, many of which also influence the growth of pathogens. Bacteria are generally the predominant initial inhabitants of newly expanded leaves, while yeasts and filamentous fungi dominate later in the growing season [1]. A large body of information has been accumulated regarding antagonism between bacteria and fungi on the leaf surface, and its possible role in the biological control of pathogenic fungi [2]. Biological control may be an alternative to chemicals in the control of some pathogenic fungi, or in order to reduce environmental pollution. Saprophytic organisms play an important part in reducing the incidence of foliar diseases from fungi and bacteria on crops in the field [3, 4].

The genus Pseudomonas has been heterogenous since Migula first named it in 1984 [5]. He designated and described the species associated with the genus in 1985 [6]. 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 anatagonistic activity plant pathogens, such as antibiosis (the production of inhibitory compounds) [7, 8], siderophores production (iron-sequestering compounds) and nutrition or site competition [9]. Some species of Pseudomonas can also produce levels of HCN that are toxic to certain pathogenic fungi [10]. These characteristics make Pseudomonas species good candidates for used as seed inoculant and root dips for biological control of soil-borne plant pathogen.

Pseudomonas fluorescens has the ability to grow at 4^{O}C and hydrolyse gelatin. These characteristics help explain its frequent environment in spoilage of refrigerated food. The main property that conspires against its becoming important opportunistic pathogen is the inability to grow at body temperature. It is rarely pathogenic for humans, even though they have been found associated with empyema, urinary tract infections and septicemia. Some Pseudomonas have been recognized as antagonists of plant fungal pathogens and antibiotic producers [11]. This is probably due to the abundance of this diverse group of bacteria and their obvious importance in the soils. Pseudomonas plasmids confer resistance to many antibiotics and antibacterial agents. Emmerich and Low [12] 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 [13].

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 [14], but one of the most important mechanism responsible for the suppression of plant pathogens for Pseudomonas spp. is siderophore-mediated competitions for iron [15]. An inhibitory effect against pathogenic and spoilage bacteria by Pseudomonas species isolated from fish was also demonstrated [16]. 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 [17].

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. Siderophore production in Pseudomonas strains inhibited the growth of Staphylococcus, Escherichia coli and Aeromonas hydrophila to 96.7% [18]. Pseudomonas spp. isolates from Tuber borchii ascocarps, known to be able to produce phytoregulatory and biocontrol substances in pure culture, were used to perform studies on their possible physiological role in nature. Antimycotic activity was confirmed against fungal contaminants isolated from the ascocarps, suggesting that populations associated with Tuber borchii fruit bodies may play a role in the maintenance of ascocarp health. On the contrary, growth of the arbuscular mycorrhizal fungus Glomus mosseae and the ectomycorrhizal fungus Laccaria bicolor, putative competitors of Tuber for mycorrhizal infection sites on roots, was not influenced by the presence of any bacterial strain.The possibility that these bacteria, which show antifungal activity and fungal growth modulation activities, might be incorporated in the developing ascocarp by means of their preferential adhesion to Tuber mycelium [19].

The purpose of this study was to examine the antifungal activity of Pseudomonas fluorescens against different plant pathogenic fungi viz. Alternaria cajani, 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 cajani, Curvularia lunata, Fusarium sp., Bipolaris sp. and Helminthosporium sp. was isolated from different disease suppressive soils of King's B medium. The bacterial strains was cultured in nutrient agar at 28±2^{O}C. For long-term maintenance the strains was preserved in nutrient broth containing 15% v/v glycerol at -70^{O}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^{O}C on PDA slants. 7-10 days old culture were used in the experiment. The fungi included in the present study are Alternaria cajani, Curvularia lunata, Fusarium sp., Bipolaris sp. and Helminthosporium sp.

Stock solution (5000 µg/mL) of the Pseudomonas strains was prepared by dissolving 5 mg 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 serile filter papers each on the lid and base of the petriplates. The slides with spores were then incubated at 25±2^{O}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 cajani, Curvularia lunata, Fusarium sp., Bipolaris sp. and Helminthosporium sp. in vitro. The Pseudomonas strains showed antifungal activity against all tested strains (Fig. 1 and 2). Table 1 presents the different concentrations obtained for each strain tested.

Figure 1

Table1. Effect of on spore germination of some fungi

S.No.	Fungus	Host	Strains	Concentration (µg /ml) Spores inhibition (%)					
				Control	1000	2000	3000	4000	5000
1	*Alternaria cajani*	*Cajanus cajan*	A-5	33.33	50.00	78.57	86.21	87.50	92.86
			C-03	31.25	75.53	80.72	85.00	90.48	94.00
			CRM-3	23.81	57.78	82.14	85.25	86.67	94.29
			L-5	28.57	35.29	73.68	76.81	79.31	81.82
			Pf4-1	33.93	64.76	65.33	73.91	87.84	90.28
2	*Curvularia lunata*	*Sorghum vulgare*	A-5	47.31	53.13	76.92	91.67	100.00	100.00
			C-03	27.27	73.68	80.88	91.21	91.46	94.64
			CRM-3	31.75	67.65	70.00	83.78	84.21	91.11
			L-5	37.14	48.28	53.33	63.16	75.00	84.62
			Pf4-1	40.91	75.00	75.00	83.64	87.67	89.71
3	*Fusarium sp.*	*Cajanus cajan*	A-5	23.30	73.18	88.89	95.08	97.75	100.00
			C-03	8.89	17.07	18.37	21.57	22.54	26.67
			CRM-3	23.26	65.38	66.67	70.00	81.82	90.70
			L-5	50.61	66.67	86.89	88.14	89.47	97.94
			Pf4-1	14.89	15.79	17.54	18.18	20.69	22.73
4	*Bipolaris sp.*	*Oryza sativa*	A-5	49.57	71.79	85.19	87.50	95.92	96.43
			C-03	18.73	35.48	55.17	58.33	62.82	73.27
			CRM-3	10.64	25.71	30.77	35.14	54.29	59.26
			L-5	6.59	10.53	12.07	23.53	26.67	27.27
			Pf4-1	34.21	61.11	81.25	86.84	94.59	95.65
5	*Helminthosporium sp.*	*Saccharum officinarum*	A-5	10.27	35.71	69.64	71.43	72.22	73.68
			C-03	17.86	20.00	23.40	23.68	28.30	33.33
			CRM-3	12.12	25.37	28.89	39.39	41.38	50.00
			L-5	77.78	79.41	88.14	91.43	94.92	100.00
			Pf4-1	17.02	25.00	27.12	27.12	28.07	31.58

Figure 2

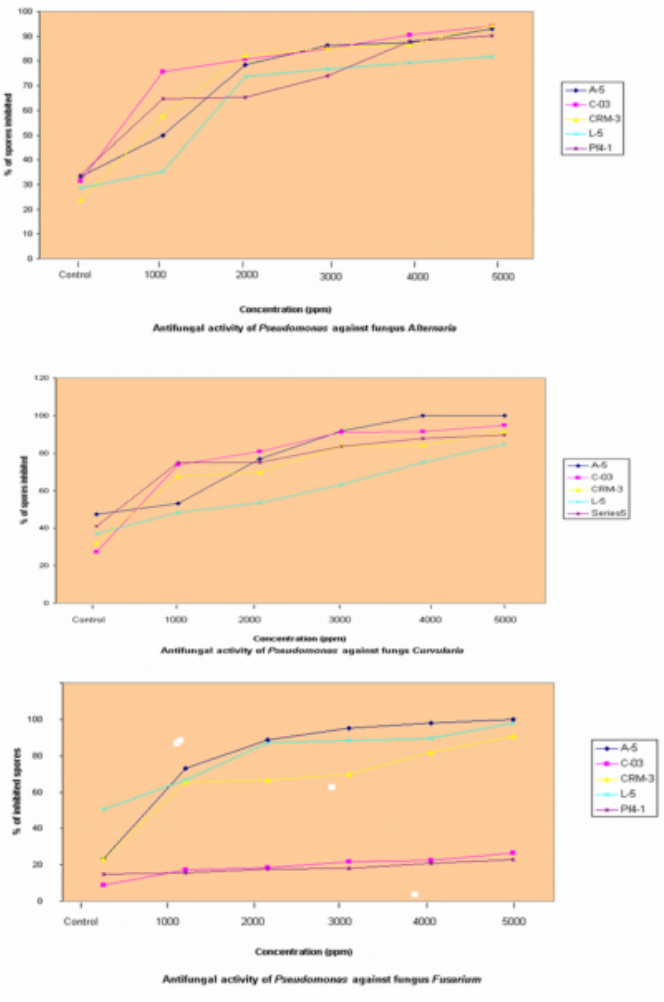
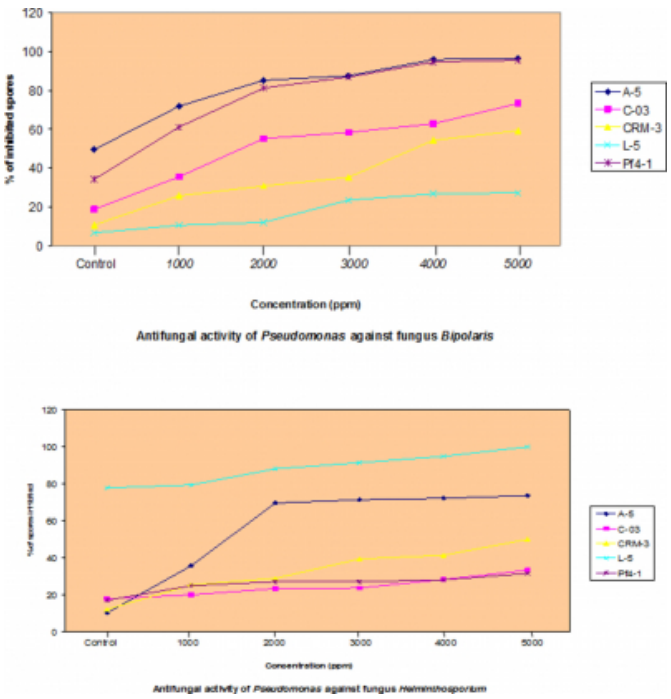


Figure 3



All the five strains showed varied levels of antifungal activity and the highest concentration of Pseudomonas strains were capable of inhibiting the growth of the pathogenic microorganism. At 5000 µg/mL, all the Pseudomonas strains (A-5, C-03, CRM-3, L-5 and Pf4-1) were highly resistant and showed highest inhibition percentage (81% to 100%) against fungus Alternaria cajani and Curvularia lunata. Out of the five strains studied, the best result was shown by A-5, which showed almost complete inhibition and maximum activity against Curvularia lunata and Fusarium sp. at 4000 and 5000 µg/mL while strain L-5 was resistant against Fusarium sp. and Helminthosporium sp. at 5000 µg/mL. The moderate activity was shown by the strains C-03 and CRM-3 against Bipolaris and A-5 and CRM-3 against Helminthosporium. The most sensitive strains were C-03 and Pf4-1 against pathogenic fungi Fusarium sp. and Helminthosporium sp. while strain L-5 against Bipolaris sp. at 5000 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.

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 [20]. When identifying potential biocontrol agents, antifungal metabolites produced by them or ability of these agents to induce antifungal compounds in plants are important factors to be taken into account. Many research groups are actively trying to find metabolites produced by biocontrol agents or induced by them in plants, and that will suppress particular diseases [21]. In earlier studies [22, 23] 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 [24]. Pseudsomonas, Bacillus and Stenotrophomonas maltophilia, showed antifungal activity against Verticillium dahliae var. longisporum in vitro and were evaluated as potential biocontrol agents by Berg et al. [25]. Rajappan and Ramaraj [26] 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. [27] 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 [28]. Experiments in vitro and in vivo by Rozsnyay et al. [29] showed that some strains of P. fluorescens, some epiphytic bacteria and some fungi inhibited canker and dieback diseases of apricot. Janisiewicz and Roitman [30] 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 [31], 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 [32].

Recent increases in fungal infections, the few available antifungal drugs, and increasing fungal resistance to the available antifungal drugs have resulted in a broadening of the search for new antifungal agents. Strains of Pseudomonas syringae pv. Syringae produce cyclic lipodepsinonapeptides that showed broad antifungal activity and fungicidal actions. Overall, the cyclic

lipodepsinonapeptides were more effective against yeasts than against the filamentous fungi [33]. Pseudomonads represnts 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 [34].

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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