

Antifungal Activity of *Pseudomonas* *aeuroginosa* and *Bacillus subtilis* against Pathogens of Cucurbitaceous Fruits

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ABSTRACT: Soil-borne plant pathogenic fungi are of major concern problem in agriculture which affects yield and quality of agricultural products. In this study, antagonistic effects of two bacterial biocontrol agents, *Pseudomonas aeuroginosa* and *Bacillus subtilis* isolated from rhizosphere were evaluated against plant pathogenic fungi *Fusarium oxysporum*, *Rhizoctonia solani* causing soft cottony leak of cucurbitaceous fruits. The ability of *Pseudomonas aeruginosa* and *Bacillus subtilis* in antagonizing or inhibiting the growth of phytopathogenic fungi was tested by measuring the inhibition zone for the growth of the tested fungi using dual culture method. Both bacterial antagonist, *Bacillus subtilis* and *Pseudomonas aeuroginosa* had significantly inhibited the radial growth of tested fungal pathogens. Solubility of siderophore of two bacterial strains was examined.

KEYWORDS: Biocontrol agents, Pathogenic fungi, Dual culture method, siderophore solubility.

I.INTRODUCTION

There is an increasing awareness that pesticides and fertilizers cause damage to the environment and effect human health [1]. The use of chemical fungicides reduced the infection level [2], but chronic treatment with these fungicides may lead to the emergence of resistant fungal strains. In addition, the use of these chemical products is costly for farmers, human health and environment [3]. As a consequence, there is a trend toward finding ways to minimize the use of fungicides [4]. The use of biocontrol agents [5] and alternative treatments (e.g., cultural practices, cover crops, organic amendments) are perceived to be less harmful than conventional fungicides and may be an alternative in controlling plant diseases [6]. Thus, searching for biocontrol agents against fungal pathogens constitute a valuable alternative to chemical pesticides [7] which could allow the finding of natural drugs for the treatment of several diseases. Bacterial species like *Bacillus*, *Pseudomonas*, have been proved in controlling the fungal diseases. Several bacteria thrive on abundant nutrients in the rhizosphere and some of these possess antagonistic action, which safeguard plants from pathogens and stimulate growth [8]. The genus *Pseudomonas* has been heterogenous since Migula first named it in 1894 [9]. Some *Pseudomonas* has been recognized as antagonists of plant fungal pathogens and antibiotic producers [10]. *Pseudomonas spp* received great attention as biocontrol agent because of their catabolic versatility, excellent root-colonizing abilities and production of broad range antifungal metabolites such as 2,4-diacetylphloroglucinoal (DAPG), pyoluteorin, pyrrolnitrin and phenazines [11, 12]. Antagonistic bacteria, specially belonging to the *Bacillus* strains are well known for their ability in producing bioactive natural peptides which are potentially active against bacteria, fungi and enveloped viruses [13,14]. The diversity and beneficial activity of the plant-bacterial association and its understanding is important to sustain agro-ecosystems for sustainable crop production [15]. Siderophores are extracellular, small compounds, which selectively bind iron. The siderophores are generally produced by microorganisms, both aerobic and facultative anaerobic.

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II. MATERIAL AND METHOD

The two bacterial isolates which has high antagonistic activity were selected as follows,

- 1) *Bacillus subtilis*.
- 2) *Pseudomonas aeruginosa*.

Antifungal activity of Antagonistic bacteria:

The bacterial antagonists were tested against *Fusarium oxysporum*, *Rhizoctonia solani*. PDA was prepared, autoclaved and after cooling, 20ml was poured in each petriplate. After solidification, the respective bacterial bioagents were streaked on PDA plates. Four streaks were drawn on petriplates, in a square pattern. Each streak was drawn 3cm away from the center of the plate. After streaking, mycelial discs of 6mm was taken from the margin of 7 days old cultures, were inoculated in the center of PDA petriplates, separately. Each treatment was replicated thrice. The petriplates having targeted fungal Pathogens served as control. The petriplates were subsequently incubated at $37 \pm 1^\circ\text{C}$, till the complete growth was observed in control plates. Colony diameter of the targeted fungal pathogens in treated plates was recorded and Percent growth inhibition over control was calculated according to the formula given by [16].

$$I = \frac{R1 - R2}{R1} \times 100$$

Where, I = per cent inhibition of mycelia growth.

R1 = Mycelial growth in control.

R2 = Mycelial growth in treatment.

Detection of siderophore production :

For detection of siderophore production by *B. subtilis* and *P. aeruginosa*, plate culture technique was used, in this universal Chrome Azurol S (CAS) agar medium was prepared as described by Schwyn and Neiland (1987) [22]. ME agar medium with Chromo Azurol S (CAS) dark-blue solution was autoclaved, cooled upto $50/60^\circ\text{C}$ and was poured into the petriplates. Then after these plates was inoculated with 24 hour old bacteria and kept for incubation for 72 hr at 30°C . Formation of yellow orange zone around the colonies from dark blue indicated positive result.

III.DISCUSSION

Due to increasing awareness of negative effects of synthetic fungicides on human, animal health and to the agro-ecosystem, research efforts on alternative and more environmentally friendly methods of controlling pests and diseases have proliferated. Microbial antagonists are widely used for the biocontrol of fungal plant diseases. Rhizospheric bacteria have proved as excellent agents to control soil-borne plant pathogens. Bacterial species like *Bacillus*, *Pseudomonas*, have been proved in controlling the fungal diseases. Bacteria identified as plant growth promoting rhizobacteria and biocontrol strains often belong to the following genera (i) *Bacillus* [18], (ii) *Pseudomonas* [19]. Antagonistic bacteria, specially belonging to the *Bacillus* genus are among the most used biological agents to fight against many plant pathogens [20] including *Rhizoctonia* [21]. Biological control of plant pathogens by soil bacteria is a well established phenomenon and chitinase production has been shown to play an important role in the suppression of various diseases [22].

Therefore the antifungal activity of two bacterial isolates *B. subtilis* and *P. aeruginosa* were tested against fungal pathogens of Cucurbitaceae. In addition, bacteria from the *Bacillus* genus are able to colonize endophytic plants [23] and to produce metabolites that affect bacterial and fungal growth [24]. In the present study, among the two tested antagonistic bacterial cultures, *P. aeruginosa* was most effective and showed 75.23% fungitoxic activity followed by *B. subtilis* with 71.84% inhibition of mycelial growth averagely. Similar findings were reported by [25] that *P. aeruginosa* showed significant results in controlling the infection of *M. phaseolina*, *R. solani* and *Fusarium* spp. [26] reported that the application of *L. nudicaulis* with the PGPR *P. aeruginosa* and microbial antagonist *P. lilacinus*, significantly suppressed infection by *M. phaseolina*, *R. solani* and *F. solani*.

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Many investigators have suggested the rhizospheric bacteria *Pseudomonas* spp. as very interesting sources for the identification of antimicrobial compounds and their practical use as biopesticides [27]. *Pseudomonas* strains exhibited significant antifungal activity with inhibitory effects against different fungi viz. *Alternaria cajani*, *Curvularia lunata*, *Fusarium sp.*, *Bipolaris sp.* and *Helminthosporium sp.* *in vitro* by [28].

IV. EXPERIMENTAL RESULTS

The antagonistic effect of bacterial isolates was screened by dual culture method. The two bacterial cultures namely, *Pseudomonas aeuroginosa* and *Bacillus subtilis* were tested against *F.oxysporum* and *R. solani*. After a week of incubation, the growth of targeted fungal pathogens towards and away from the bacterial isolate was recorded. The percent inhibition of mycelial growth over control was tabulated.

Both bacterial isolates *Pseudomonas aeuroginosa* and *Bacillus subtilis* significantly inhibited the mycelial growth of tested fungi. The highest inhibition of mycelial growth was recorded in *R. solani* as 90.31% when treated with both bacterial isolates. Whereas *F. oxysporum* showed average inhibition of 60.15% and 53.38% when treated with *Pseudomonas aeuroginosa* and *Bacillus subtilis* respectively. On average, *R. solani* was found to be most sensitive with 90.31% and *F. oxysporum* 56.76%.

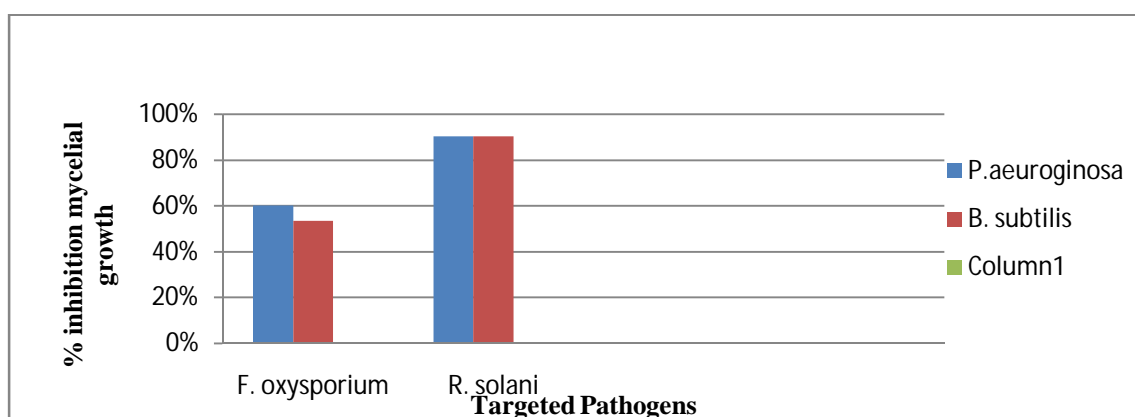
Solubility of siderophore produced by two strains was also checked and satisfactory results were found. The siderophore of both *B. subtilis* and *P. aeuroginosa* showed strong water solubility; it is also soluble in methanol and acetone but both the biocontrol agents show very poor solubility in ethyl acetate and not soluble in HCl.

Table1 : Antagonistic effect of *Pseudomonas aeuroginosa* & *Bacillus subtilis* against vegetable pathogens:

Biocontrol agents	Pathogens	Mycelial growth in control (mm)	Mycelial growth of Pathogen in presence of Biocontrol agents (mm)	% inhibition of mycelial growth over control
<i>P. aeuroginosa</i>	<i>F. oxysporum</i>	88.6	35.3	60.15±1.43
	<i>R. solani</i>	82.6	08	90.31±0.00
	C.V			85.8
<i>B. subtilis</i>	<i>F. oxysporum</i>	88.6	41.3	53.38±0.27
	<i>R. solani</i>	82.6	08	90.31±0.00
	C.V			92.7

Value expressed in mean ± S. E. M of triplicates

Fig : Antagonistic effect of bacteria against fungal pathogens of cucurbitaceae.



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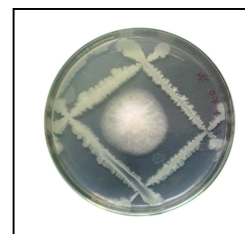
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F. oxysporum control



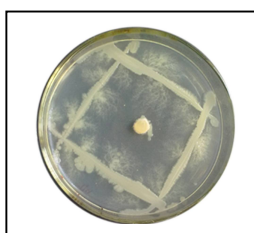
Growth inhibition of *F. oxysporum* by *P. aeruginosa*



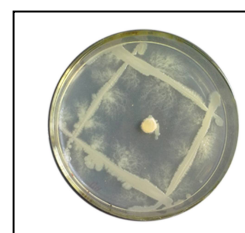
Growth inhibition of *F. oxysporum* by *B. subtilis*



R. solani control



Growth inhibition of *R. solani* by *P. aeruginosa*



Growth inhibition of *R. solani* by *B. Subtilise*

Table 2: Solubility of siderophore

Bacterial strains	solubility of siderophores in different solvents				
	Water	Acetone	Methanol	Ethyl acetate	HCl
<i>P. aeruginosa</i>	+++	++	++	+	-
<i>B. subtilis</i>	+++	++	+++	+	+

+++ Highly soluble, ++medium soluble, + poorly soluble, - insoluble.

V.CONCLUSION

The biological functioning agents in the present study were viz., *Bacillus subtilis*, *Pseudomonas aeruginosa*. From the farmer's point of view, the treatment which gives maximum return is more important than a mere control of the disease. The production of siderophore by biocontrol agents (BGA) and plant growth promoting microbes (PGPM) is one of the important mechanisms for plant growth promotion and disease suppression. Thus, the necessity to obtain vegetable yield of high quality, the concern for the environment and the human safety, the possibility to reduce and control the environmental pollution, the request to safeguard that impose reduction of chemical treatments, all that should induce the farmers to choose biological interventions. According to our experiment, it is concluded that *B. subtilis* is the best Biocontrol agent against fungal pathogens of cottony leak of cucurbitaceous fruits.

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