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Effective Biocontrol of Leaf Rot Disease on *Aloe vera* Plant by PGPR in Green House Experiment

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ABSTRACT
Fusarium oxysporum f. sp. *lycopersici*, being a soil borne pathogen causes leaf rot disease in *Aloe vera* plant and pose serious threat for its production. In the present investigation, *Acinetobacter radioresistens* antagonistic to *F. oxysporum* was applied to manage leaf rot disease in *Aloe vera* plants. *Acinetobacter radioresistens* showed significantly higher fungal growth inhibition of 66%. Isolate showed positive result for root colonization. In green house *Acinetobacter radioresistens* was found significant in suppressing the incidence of *Fusarium* and also increased the plant growth promoting characteristics overcontrol.
KEY WORDS: *Fusarium oxysporum, Leaf rot, PGPR, Pathogen, Aloe vera*

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INTRODUCTION

PGPR may protect plants against pathogens by direct antagonistic interactions with the pathogen, as well as through induction of host resistance. Bacteria in the genera *Bacillus*, *Streptomyces*, *Pseudomonas*, *Burkholderia* and *Agrobacterium* are the biological control agents predominantly studied and increasingly marketed. They suppress plant disease through at least one mechanism; induction of systemic resistance and the production of siderophores or antibiotics. PGPB protects the plants from fungal, bacterial, and viral diseases and insect and nematode pests by several mechanisms. The mechanisms include the induction of systemic resistance in plants [1-2], production of antibiotic metabolites, siderophores [3] and cell wall lytic enzymes [4]. The use of PGPB as an inducer of systemic resistance to crop plants against various fungal, bacterial and viral pathogens [5] has been demonstrated under both the greenhouse and field conditions. Sonawane and Konde [6] studied the effect of diazatrophic and VAM fungi on grape vines and reported that there was a significant increase in leaf area and early sprouting of the cuttings due to *Azotobacter chrococcum* inoculation. Leaf rot disease caused by *Fusarium oxysporum* is one of destructive disease caused in *Aloe vera* plants.The presence of fungal infectionin *Aloe barbadensis* plant is concern for public health due to its use in medicine and cosmetics [7-8]. Some fungal pathogens and non-pathogens produce mycotoxins in their infected hosts and substrates on which they grow. *Fusarium* produces mycotoxin trichothecenes which is very toxic for human [9]. Alexoupolos*et al*. [10] reported that this toxin can cause cancer, hemorrhage, edema and immune deficiency. WHO [11] reported that mycotoxins are hazardous to human and animal health. There is need to control the fungal infections in order to reduce the losses and the risk of mycotoxins contamination.

In current scenario use of chemical fertilizer for biocontrol of soilborneplant pathogens including *F. oxysporum* has been shifted to the option of green technology that have an agriculture importance. PGPR can act as biocontrol agent and alternative of application of chemical fertilizers. In this study *Acinetobacter radioresistens* SMA4 isolated from *Aloe vera* rhizosphere has been used as a biocontrol agent against *Fusarium oxysporium* in green house experiment.

MATERIALS AND METHODS

Isolation

Isolate SMA4 used in this study has been isolated from *Aloe vera* rhizosphere. On the basis of morphological, biochemical and molecular characterization isolate SMA4 has been identified as *Acinetobacter radioresistens.* The 16S rDNA sequences of the isolates were deposited in NCBI GenBank under the accession numbers JQ618289. Phylogenetic tree showing its similarity with other closest relative is given in Figure 1. In earlier studies abilty of SMA4 to control *Fusarium oxysporum* in vitro using dual plate culture assay has already revealed [12]. Theobjective of current study is to assess the biocontrol ability of SMA4 against *Fusarium oxysporum* under greenhouse conditions.

Preparation of Talc based formulation

A loopful of *Acinetobacter radioresistens* was inoculated into nutrient broth and incubated in a rotary shaker at 150 rpm for 72 h at room temperature (28-72 °C). After 72 h of incubation, the broth containing 8×10^{9}cfu ml^{-1} was used for the preparation of the talc-based formulation. To the 400 ml of bacterial suspension, 1 kg of the purified talc powder (sterilized at 105 °C for 12 h), calcium carbonate 15 g (to adjust the pH to neutral) and carboxymethyl cellulose (CMC) 10 g (adhesive) were mixed under aseptic conditions [13]. The product was shade dried to reduce the moisture content below 20% and used for application. At the time of application, the population of bacterium in the talc formulation was checked to $2.5–3\times10^{8}$cfu g^{-1}.

Preparation of fungus inoculum

*Fusarium oxysporum*f. sp. *lycopersici* was collected from IARI PUSA, New Delhi, which was maintained on potato dextrose agar (PDA). Inoculum of this fungus was prepared by culturing the isolate for 15 days at 25 °C. Mycelium was collected on blotting paper, and excess water and nutrients were removed by pressing between 2 folds of blotting paper. Mycelium (100 g) was macerated in 1 L of distilled water and 10 mL of this suspension containing 1 g of fungal mycelium was poured around the roots.

Experimental design

The experiment was carried out in a completely randomized block design, with 2 main blocks: A) without fungus and B) with fungus. Each main block was tested with and without PGPR, i.e. a) without *Acinetobacter radioresistens*b) with *Acinetobacter radioresistens*. Four treatments were designed in all-T1 (Control), T2 (*Acinetobacter radioresistens*), T3 (*Fusarium oxysporum*) and T4 (*Acinetobacter radioresistens+ Fusarium oxysporum*)

Disease index

The disease index was computed by development of disease symptoms was observed and recorded. Percent disease index (PDI) was determined by using the formula:

$$\%\ \text{Disease Index:} = \frac{\text{No. of plants showing disease symptoms}}{\text{Total no. of Plants}} \times 100$$

Observations

Plants were harvested 90 days after inoculation. Plant biomass, root length, shoot length, gel weight, colonization of roots by rhizobacteria, and the wilting index were recorded; roots inoculated with rhizobacteria were partially collected 1month after sowing. One gramof roots was surface sterilized and crushed in sterile normal solution (NSS), and 0.1 mL of serially diluted extract was placed on nutrient agar plates and incubated at 37 ± 1 °C for 72 h. These plates were placed on a Quebec colony counter to count the bacterial colonies. Colonies falling within the 30-300 range were selected and multiplied by the reciprocal dilution factor to obtain the number of bacterial colonies represented as colony forming units (CFU) per gram of root.

RESULT

On the basis of nucleotide homology and phylogenetic analysis isolate SMA4 has been identified as *Acinetobacter radioresistens*. The 16S rRNA gene sequence of SMA4 comprised of 1431 bp. Phylogenetic tree showing the distance and its relationship with closest relative has been shown in figure 1.

Inoculation of plants with *F. oxysporum* caused a significant reduction in shoot dry weight, as compared to un-inoculated plants (Table 1). Inoculation of pathogen-inoculated plants with *Acinetobacter radioresistens,* caused a significant increase in shoot dry weight, as compared to plants inoculated with *F. oxysporum* alone. Inoculation of pathogen-inoculated plants with*Acinetobacter radioresistens* caused a greater increase in root dry weight than that ofplants infected with *F. oxysporum*alone. Gel is important component of *Aloe vera* plant which is used for medicinal purpose. Plants infected with *F. oxysporum* leads to reduction of gel weight of 36.66%. Moreover if gel from infected plants is used, mycotoxin present in it may cause negative impact on the health of the person using products obtained from these

infected plants. Plants treated with *Acinetobacter radioresistens* showed reduction in disease index of 65% as compare to control. More disease severity was observed in only pathogen (control) treated plants without PGPR.

DISCUSSION

The suppression of phytopathogens growth by *Acinetobacter radioresistens* SMA4 might be due to offensive root colonization and defensive retention of rhizosphere. The antifungal activity of the isolate might be due to the production of siderophore and HCN or synergistic interaction of these two or with other metabolites. In earlier studies SMA4 was found to have various biocontrol properties such as Antifungal activity, Siderophore production and HCN production [14]. Bacterial siderophores increase the availability of iron to the bacteria in the soil surrounding the roots, making it unavailable to pathogens and stimulate the biosynthesis of other antimicrobial compounds, thus inhibiting the growth of pathogens [15].The siderophore also involved in the induction of systemic resistance (ISR) in plants by enhancing the self-defense capacity against a broad spectrum of pathogens [16-17]. The enzymes such as β_1,3-glucanase,chitinase and cellulase degrade the cell walls of pathogenic fungi [18-19]. The disease suppressive nature of rhizobacterial isolates especially was due to their ability to produce AFSMs and lytic enzymes chitinase and cellulase.

Several studies demonstrated that production of siderophores, other secondary metabolites and lytic enzymes by*Pseudomonas*strains were effective in controlling the plant root pathogens including*F. oxysporum*and*R. solani* [20-21]. Application of *Pseudomonas*sp. strain WCS 417r protected plants systemically against *Fusarium* wilt caused by *Fusarium oxysporum* f. sp. *Dianthi* [22]. PGPR strains applied as a seed-treatment resulted in a significant reduction in anthracnose disease caused by *Colletotrichum orbiculare* in cucumber [23-24]. Similarly, induction of systemic resistance by *Pseudomonas putida* strain 89B-27 and *Serratia marcescens* strain 90-166 reduced *Fusarium* wilt of cucumber incited by *Fusarium oxysporum* f. sp.*cucumerinum* [25]. Foliar spray with *P. fluorescens*strains Pf1 and FP7 showed higher induction of ISR against the sheath blight pathogen, *Rhizoctonia solani* [26]. Similarly, in sugarcane, Viswanathan and Samiyappan [27] established PGPR-mediated induced systematic resistance against *Colletotrichum falcatum* causing red rot disease.

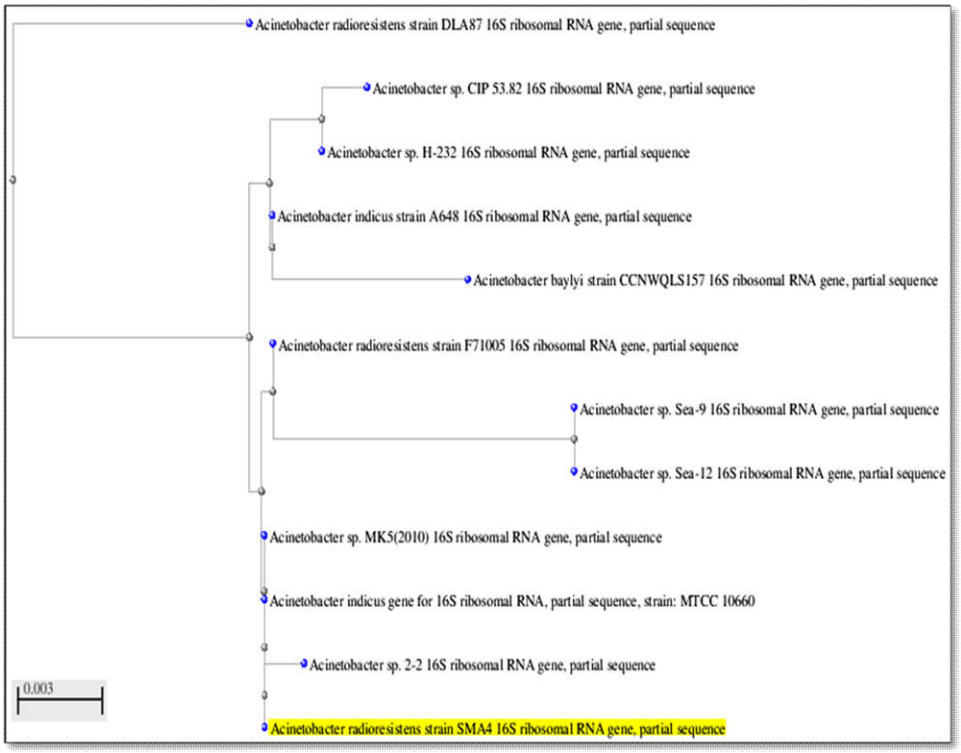

Fig.1: Phylogenetic tree of *Acinetobacter radioresistens* SMA4

Table 1: Efficacy of SMA4 on growth parameter and disease incidence in *Aloe vera* infected with *Fusarium oxysporum*

Treatment(s)	Plant biomass (g pot^{-1})	Root wt. (g pot^{-1})	Shoot wt. (g pot^{-1})	No. of leaves	Leave wt. (g pot^{-1})	Gel wt (g pot^{-1})
T1	180.40	40.24	120.78	8	26.60	8.52
T2	270.99	60.55	210.06	14	46.37	19.10
T3	128.70	36.28	98.5	10	22.80	5.40
T4	210.58	45.68	160.80	12	32.80	14.68

CONCLUSION

In conclusion the results of this study suggest that simultaneous screening of rhizobacteria for growth and yield promotion under greenhouse experiment is a good tool to select effective PGPR for biofertilizer development biotechnology.*Acinetobacter radioresistens* SMA4 strongly inhibited growth of *Fusarium oxysporum*. It enhanced growth parameter in *Aloe vera*and also suppressed leaf rot disease. Strategic and applied research has demonstrated that certain co-operative microbial activities can be exploited, as a low-input biotechnology, to help sustainable, environmentally-friendly, agro-technological practices.

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