Plant Growth Promoting Microorganisms (PGPMs) from Bamboo Rhizosphere

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Abstract

Three bacterial and one actinomycetes isolates were isolated from the Bamboo rhizospere . All the isolates inhibited the growth of *Fusarium.* The isolate-1 showed maximum inhibition of *Fusarium* followed by isolate 3 and isolate 2. All the isolates increased the plant growth. Seed treatment of Isolate - 3 showed highest plant height but isolate 1 recorded maximum number of leaves.

Key words: *PGPMs, Antagonist, Fusarium,*

Introduction

The plant growth promoting microorganisms (PGPMs), are defined by three intrinsic characteristics: (i) they must be able to colonize the root, (ii) they must survive and multiply in microhabitats associated with the root surface, in competition with other microbiota, at least for the time needed to express their plant promotion/protection activities, and (iii) they must promote plant growth (Lugtenberg *et al.,* 1999,2001; Rothballer *et al.,* 2003; Espinosa-Urgel, 2004; Gamalero *et al.,* 2004). The PGPR are known to participate in many important ecosystem processes, such as the biological control of plant pathogens, nutrient cycling, and/or seedling growth (Persello-Cartieaux *et al.,* 2003; Barea *et al.,* 2004; Zahir *et al.,* 2004).

Pathogenic microorganisms affecting plant health are a major and chronic threat to food production and ecosystem stability worldwide. As agricultural production intensified over the past few decades, producers became more and more dependent on agrochemicals as a relatively reliable method of crop protection helping with economic stability of their operations (Compant *et al.,* 2005). However, increasing use of chemical inputs causes several negative effects, i.e., development of pathogen resistance to the applied agents and their nontarget environmental impacts (Werger *et al.,*1995). Furthermore, the growing cost of pesticides, particularly in less-affluent regions of the world, and consumer demand for pesticide-free food has led to a search for substitutes for these products. There are also a number of fastidious diseases for which chemical solutions are few, ineffective, or nonexistent (Gerhardson, 2002.). Biological control is thus being considered as an alternative or a supplemental way of reducing the use of chemicals in agriculture (Welbaum, 2004). Baboo is a fast growing plant and can come up in water stress area.Considering these points antagonistic isolates from the bamboo rhizosphere soil was isolated and characterized for biocontrol and plant growth promotional property.

Materials and Methods

Pathogenic *Fusarium*: The isolates was obtained from Department of Agricultural Microbiology, UAS, GKVK, Bangalore.

Isolation of antagonistic bacteria: The antagostic actinomycetes and bacterial isolates were isolated by following serial dilution and plating technique. The bamboo rhizospere soil sample was used as source of these microorganisms. These serially diluted sample was plated on nutrient (Anon, 1957) and Kuster's agar (Kuster and Williams, 1964). These plates were incubated in an inverted position in an incubator at 28±2°C for three days. The colonies showing growth inhibition of other colonies growing nearby are purified and maintained on agar slants.

Characterisation of isolates

The bacterial isolates were subjected to some morphological and biochemical tests such as Gram staining and endospore staining (Anon, 1957), and catalase test (Blazevic and Ederer, 1975). The morphological characters of actinomycetes isolate was observed.

Determination of antagonistic property

The antagonistic acivity were determined by following two tests.

1) The isolates were further confirmed for antagonistic activity by inoculating them to PDA plate, incubating for 48 hrs at 28±2°C and finally inoculating mycelial bit of *Fusarium* nearby. The isolates showing

growth inhibition of *Fusarium* were retained for further study.

2) The antagonistic bacterial cultures were inoculated to nutrient broth and antagonistic actinomycetes culture was inoculated to liquid Kuster,s broth in separate 250 ml conical flasks and incubated at 28±2°C for five days. The culture filtrates were transferred to molten lukewarm PDA media @ 5ml filtrate per 100 ml medium and poured to the sterilized Petri dishes aseptically.

The mycelium of *Fusarium* was grown on PDA medium at ambient temperature. Agar discs of 0.7 cm were removed from the periphery of actively growing Petri dish culture and placed in the center of Petridishes containing PDA supplemented with filtrates. The growth rates were measured by averaging two perpendicular mycelial diameters subtracting 0.7 cm of the initial disc diameter. All the experiments were triplicated .The radial growth of *Fusaium* was measured on 5th day after inoculation.

Plant growth promoting activity of antagonistic isolates

All the antagonistic isolates were grown on Potato Dextrose Broth for ten days. The plant growth promoting activity was determined by Bengal gram seedling bioassay (Zehra and Okon, 1993).

Ten Bengal gram seeds were inoculated with 5 ml of culture filtrate. These seeds were placed in on sterile Petriplate lined with sterile blotting paper and incubated at 28±2°C for three days. The germinated seeds were transferred plastic cups containing 100 g sterile sand. Over the seed 1ml of culture filtrate was added and incubated at 28±2°C for three days. Uninoculated control, consisting of seeds treated with just potato dextrose broth filtrate was maintained. After seven days, the plant height and number of leaves were determined.

Results and Discussion

Three microbial isolates comprising of two bacterial and one actinomycetes isolates were isolated from bamboo rhizosphere. Both the bacterial isolates (Isolate 1 and 2) were Gram negative, rod shaped aerobic non endospore forming in nature (Table-1). The actinomycete isolate Produces leathery colony, and its vegetative mycelium is nonseptate. There are many reports indicating the role of Gram negative bacteria and actinomycetes in control of plant pathogenic organisms (Dobbelaere *et al.,* 2001;Vessey, 2003; Lucy *et al.,* 2004; Sahin *et al.,* 2004; Zahir *et al.,* 2004 and Lim *et al.,* 1999). All the isolates inhibited the growth of pathogenic Fusarium (Table 2 and plate 1 and 2) .The Culture filtrate of isolate-1 inhibited Fusarium to a maximum extent (with radial growth of 9.3 ± 0.5 mm) followed by Isolate -3 (11.0±2.0 mm) and 2 (20.0±1.0mm). The widely recognized mechanisms of biocontrol mediated by PGPB are competition for an ecological niche or a substrate, production of inhibitory allelochemicals, and induction of systemic resistance (ISR) in host plants to a broad spectrum of pathogens (Dobbelaere *et al.,* 2001; Hass, 2000; Hass, 2002) .The PGPMs isolated from the bamboo rhizosphere increased the growth of Bengal gram plants which is reflected in increased shoot length and number of leaves (Table 3 and Plate 3). There has been a large body of literature describing potential uses of plant associated bacteria as agents stimulating plant growth and managing soil and plant health (Bloemberg *et al.,* 2001; Glick,1995)

Table1. Morphological and biochemical properties of antagonistic bacteria

Isolate Number	Gram reaction	Shape of the cell	Endospore	CatalaseTest
1	__	rod	__	+
2	__	rod	__	+

Table2. Radial growth of *Fusarium* (mm) on antagonistic culture filtrate enriched medium

Isolate Number	Radial growth of *Fusarium* (mm)
Control	24.0 ±1.0
1	9.3±0.5
2	20.0±1.0
3	11.0±2.0

(Replication =3, mean± SD)

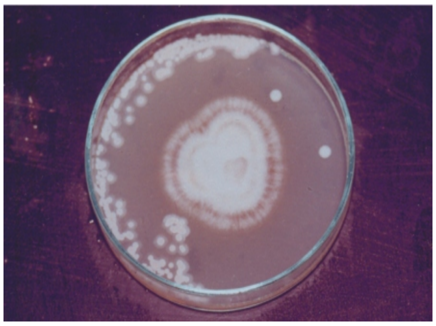

Plate 1. Pure culture of *Fusarium*

Table 3.Influence antagonistic culture filtrate on shoot length (mm) and number of leaves

Isolate Number	Shoot length (cm)	Number of leaves
Control	9.3±0.26	8.0± 0.78
1	14.5±0.90	10.6±1.58
2	13.6±1.30	9.3±0.5
3	15.3±1.10	8.3±0.7

(Replication =3, mean± SD)

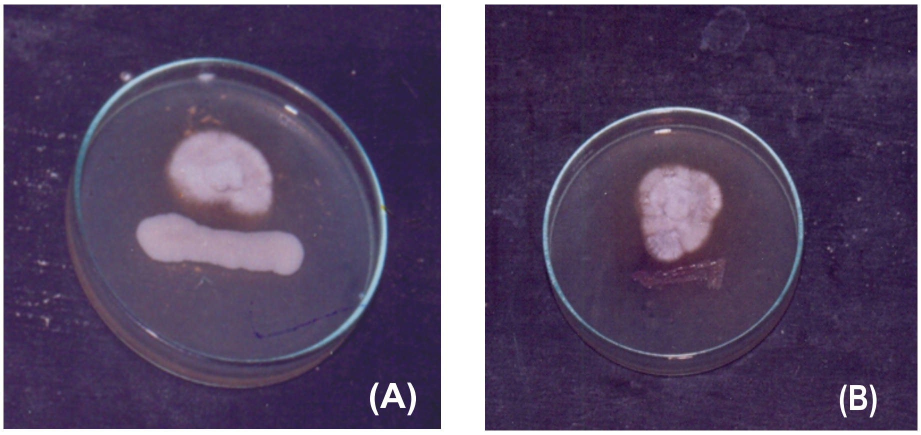

Plate 2. Inhibition of *Fusarium* brought about by antagonistic bacteria (A) Isolate-2 (B) Isolate-1

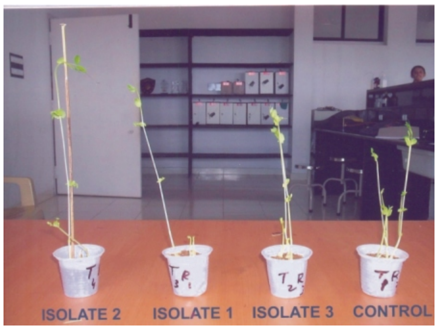

Plate3. Plant growth promoting property of antagonistic

Conclusion

The isolation of PGPMs from different sources open new doors to design strategies for improving the efficacy of biocontrol agents. Identification of key antimicrobials produced by superior agents, can be exploited for streamlining strain discovery by targeting selection of new isolates that carry relevant biosynthetic genes. Determination of the role of edaphic parameters favorable for disease suppression, particularly those that stimulate antibiotic production and activity, can be exploited by targeting inoculants for soils that are more likely to support biocontrol. Biocontrol with plant growth promotion helps increasing the vegetative yield and there by increasing crop yield.

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