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Endophytes as biocontrol agents of plant pathogens and insects

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ABSTRACT

One hundred and fifty bacteria were isolated from internal root tissues (56 isolates) of rice and soil rhizospheres (94 isolates). All the bacterial isolates were screened for their plant growth promoting activities and antibiosis against selected fungal pathogens and insect pests. The present investigation shows the potent but varied bio-control activities of the bacterial isolates and provide an advantage as biological control agents for use in fields, due to their ability to colonize the internal tissues of the host plant.

Keywords: Endophytes, biocontrol, plant pathogens, insects

INTRODUCTION

Internal tissues of plants teem with microbial populations, and are far from sterile. Bacterial endophytes that is, bacteria that are present within plants, have been known for >120 years. Endophytic bacteria generally colonize the intercellular spaces, an ecological niche very similar to that of phytopathogens, which makes them suitable as biocontrol agents (Berg *et al*., 2005). Several reports have shown that endophytic microorganisms can have the ability to control plant pathogens and insects (Azevedo et al. 2000). These organisms can also accelerate seedling emergence and promote plant establishment under adverse conditions. Bacterial endophytes have been shown to check progress of disease through endophyte mediated *de novo* synthesis of novel compounds and antifungal metabolites like coronamycin, *p*-aminoacetophenomic acids, fusaricidineA-D etc. (Ryan et al 2008).

Antagonistic activity of endophytic bacteria has been reported against different phytopathogens such as fungi, bacteria and oomycetes (Lodewyckx et al. 2002). Antagonistic endophytic bacteria were isolated from xylem of lemon roots which were effective against root pathogens (Araújo *et al*., 2001). Endophytic actinobacteria isolated from healthy cereal plants were effective antagonists of the pathogenic fungi *Gaeumannomyces graminis*, *Rhizoctonia solani* and *Pythium* spp. (Coombs *et al*., 2004; Parmeela and Johri 2004). Potato endophytes exhibited antagonistic activity against fungal and bacterial pathogens (Sessitsch *et al*., 2004). Most commonly reported endophytes with antagonistic potential against phytopathogens are *Pseudomonas*, *Bacillus* and *Paenibacillus* spp. and strains of actinobacteria.

Webber (1981) for the first time demonstrated that the endophyte *Phomopsis oblonga* protected elm trees against the beetle *Physocnemum brevilineum* which additionally controlled the spread of the elm Dutch disease causal agent *Ceratocystis ulmi* by controlling its vector, the beetle *P. brevilineum*. Insect showed repellant behavior towards the toxic compounds produced by the fungi. Since then several reports have shown biocontrol of insect pests and nematodes through application of endophytes (Zehnder *et al*., 1997; Murphy *et al*., 2000; Siddiqui *et al.,* 2000). The present study is another attempt to showcase the bio-control potential of endophytic bacteria against selected fungal pathogens and insect pests.

MATERIALS AND METHODS

Bacterial isolation

For the isolation of endophytic bacteria, rice roots were collected from the reproductive stage of the plant growth. Fifteen grams of washed root tissue was surface sterilized using 0.1% $HgCl_2$, homogenized in phosphate buffer and centrifuged to collect the clear supernatant. The supernatant was serial diluted and plated on nutrient agar and King's B media. Different morphotypes were isolated and screened for antifungal activity along with other plant growth promoting traits like siderophore and HCN production following standard procedures (Schwyn and Neilands, 1987, Bakker and Schipper, 1987). For isolation of chitinolytic bacteria, IARI field soil samples were collected from different sites, enriched with chitin and incubated. These were then used as inoculum in broth

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for isolation of chitinolytic bacteria. These broth were kept on a shaker to allow bacterial growth and suitable dilutions were plated on media containing colloidal chitin. The bacterial colonies showing halo zones were picked up and purified.

In vitro bioassay

In vitro dual plate assay was carried out against three pathogenic fungi: *Sclerotium rolfsii, Fusarium oxysporum and Rhizoctonia bataticola* on PDA medium. An actively growing fungal agar plug (3mm diameter) was placed in the centre of PDA plate and each bacterial isolate was spot inoculated around the fungal plug. A control plate with only fungal plug was kept for each pathogen. Plates were incubated for 3-4 days at 28^{0}C. Inhibition radius was scored for each bacterial isolate against the fungal mycelia growth in control plate

Estimation of chitinase activity in different isolates

Bacteria showing chitinolytic activity on plates were inoculated in nutrient broth supplemented with 0.4% colloidal chitin and incubated on a shaker at 30$\pm$ 2^{0}C for 5 days. At the end of incubation, chitinase activity was quantified by the method Ohtakara *et al*., 1984.

Estimation of activity of different chitinases in the promising isolate

The exo-chitinase activity was measured in broth cultures by using the method of Zaldívar. *et al.* (2001)**.** The endo-chitinase activity was measured on the basis of reduction in turbidity of a suspension of colloidal chitin (Tronsomo and Harman, 1993). Chitobiosidase activity was determined by measuring the release of *p*-nitrophenol from *p*-nitrophenyl-b-D-*N,N* -diacetylchitobiose by the method of Roberts and Selitrennikoff (1988).

Insect bioassay

The selected chitinolytic cultures were evaluated for their potential against 1st instar larvae of *Spodoptera litura*. Bioassay experiments were conducted in a climate-controlled room at 25^{0}C ± 0.5 with a photoperiod of 14/10 (light/ dark) and 70% ± 15 relative humidity. Larvae were fed 1 g of artificial diet treated with 100 ìl of bacterial broth cultures. Five replicates containing 10 larvae per treatment were maintained. Mortality was scored every 24 h for 7 days.

RESULTS AND DISCUSSION

About 50% of the 150 bacterial isolates showed inhibitory activity against three fungal pathogens (*S. rolfsii, F. oxysporum and R. bataticola*). Many isolates were able to inhibit more than one fungal pathogen (Fig 1). However, only 16 and 8 isolates were able to produce siderophore and HCN respectively. Fifty five isolates were observed to possess chitinolytic activity as indicated by presence

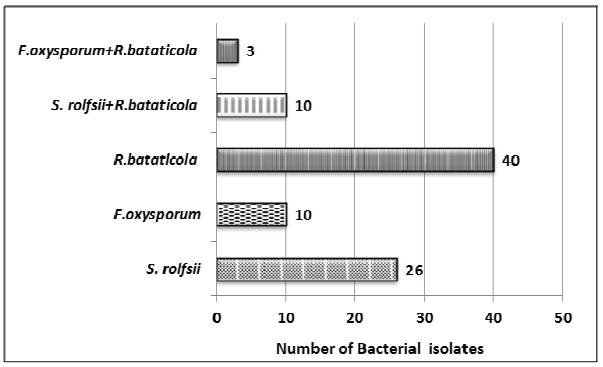

Fig 1 **Bacterial isolates showing inhibitory action against pathogenic fungi**

of halo zone around the colony (Fig 2). Of these, 8 isolates possessed chitinase activity more than 1000 U/mg protein

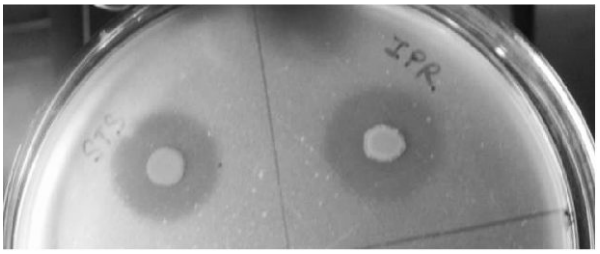

Fig 2: **Clear zone of chitin hydrolysis around the bacterial colony showing positive chitinase activity**

and were selected for insect bioassay. Larval mortality was observed to range between 30 to 100% (Table 1). Maximum mortality (100%) was observed for STS which was identified as *Serratia marcescens*. It was observed to possess all the three chitinase enzyme activities.

Table:1 Chitinase activity of bacterial isolates and their potential against *Spodoptera litura*

Culture	Chitinase activity (U) /mg of protein	Insect mortality(%)
S-19	2100	70
PN-22	1609.1	40
F-2	1918.6	70
F-4	1530.3	35
S-23	1087.9	30
KBY-1	1054.5	85
STS	1055.5	100
IPR-1	1074.4	75

Exochitinase activity was 0.40 nmol *p*-nitrophenol released/min, endochitinase activity was 33.86 U and chitobiosidase activity was 1.73 Nanokatals/min.

The criteria for putative plant growth promoting (PGP) traits related to plant protection are siderophore, HCN and chitinase production besides production of antibiotics (Cattelan et al. 1999; Adesina et al. 2007). Siderophore producing microorganisms protect plants at two levels; first by limiting growth of pathogenic microorganisms by competing for Fe and secondly, triggering plant's defensive metabolism. Chitin, an insoluble linear polymer, is a major structural component of most fungal cell walls and insects, therefore, many species of microorganisms and plants produce chitinolytic enzymes to protect themselves against fungi and insects, constituting good bio-control agents (Adesina et al. 2007; Nicho et al. 2010). HCN production has been postulated to play an important role in biological control of pathogens as it inhibits the electron transport, disrupting the energy supply to the cells, ultimately leading to death of the pathogen.

It has been shown that some of these endophytes can cause induced systemic resistance (ISR), very similar to systemic acquired resistance (SAR) with the result that plants withstand the pathogen attack by activating their defense mechanisms. Prior inoculation with endophytes has reduced the disease incidence and damage caused by fungal, bacterial and even nematode s and insects (Ryan et al. 2008). The present study has shown the potential of endophytes against selected fungal pathogens and has generated bacterial germplasm for biocontrol of fungal and insect pests.

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