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Isolation and Evaluation of Antagonistic Bacteria Towards Turcicum Leaf Blight and Maydis Leaf Blight and their Growth Promotion Ability in Maize

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ABSTRACT

Turcicum leaf blight and Maydis leaf blight are among the important fungal diseases of maize in the indo-gangatic plains. The aim of this work was to evaluate the possibility of exploiting antagonistic bacteria in the biological control of Turcicum Leaf Blight and Maydis leaf blight. Among the collections of the bacterial strains isolated from various plants in the different regions of Eastern Uttar Pradesh, eight and eleven antagonists were selected against *E. turcicum* and *Bipolaris maydis* respectively. These strains were identified as actinomycetes, pseudomonas and bacillus strains.The isolates BMBA64 and BMBK8 showed strong antagonistic activity against *E. turcicum*(85.5%) and *Bipolaris maydis*(87.2%) respectively.BMBK8 also showed strong plant growth promotion and phosphate solublization abilities. These results indicated that both the strains are promising candidates for biological control agents of Northern and Southern Corn Leaf Blight in the indo-Gangatic plains.

Keywords: Antagonistic bacteria, MLB, TLB

Introduction

Maize (*Zea mays* L; 2n=20) belongs to tribe maydeae of the grass family Gramineae (Poaceae). It is the third most important crop worldwide, after rice and wheat and is grown mainly as a high energy feed for human and animal consumption, besides diversified end use for industrial and pharmaceutical purposes(Doebley *et al*,2004) India ranks third in the total world production(18.54 million tons). Maize crop of the Indo-Gangatic Southern Indian plains, suffer from many diseases like Brown spot, Downy mildews, maize rust, carbonum leaf spot, leaf blotch, Maydis Leaf Blight and Turcicum leaf blight. Maydis Leaf Blight and Trucicum Leaf Blight are most prevlent in Uttar Pradesh, Karnataka and Himanchal Pradesh. Fungi side application and the use of resistant cultivars are the main means of disease control. With the application of fungicides there is a high risk of developing resistance to these compounds. The possibility of controlling plant diseases with antagonisti microorganisms is appealing in an age of concern with pesticides(Baker *et al*,1974). Biological control using antagonistic bacteria has been reported as an attractive alternative due to antagonize the pathogen by different modes of action, and to effectively colonize distinct plant habitats (Raaijmakers *et al*. 2002).

Most attention has been focused on the use of Gram-negative bacteria belonging to genera Pseudomonas (Brun-Kiewnick *et al.* 2000; Cartwright *et al*.1995; Costa *et al.* 2001; Sodha 2000; Slininger *et al*. 2000). These bacteria are prominent in the rhizosphere, rhizoplane and ectorhizosphere because of their siderophore complexex (Baker *et al.* 1986) and production of antibiotic complonds. Several Bacillus species are also good candidates for biological control agents as they have the ability to solubilize insoluble phosphate by producing various organic acids, siderophores, mineral acids, humic substances (Yadav and Dadarwal. 1991).

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The purpose of this study was to (i) isolate flurescent bacteria, actinomycetes and phosphate solublizing bacteria from the rhizosphere of different plants from Eastern Uttar Pradesh (ii) Screen antagonistic bacteria against the fungus *Bipolaris maydis* and *Exserohilium turcicum* (iii) Biochemical and molecular characterization of the antagonists (iv) determine the growth promoting ability in maize.

Material and Method

Test organism and culture conditions

Two different basediomycetes fungi *Bipolaris maydis* and *Exserohilium turcicum* were used to test the antifungal activity of the bacterial strains isolated in the study. The isolate *Bipolaris maydis* ITCC NO-6028 was provided by the Plant Pathology division of IARI, Pusa and *Exserohilium turcicum* was isolated from an infected maize leaf collected from B.H.U. farm. These phytopathogenic strains were maintained on potato dextrose agar plates (PDA) plates at 22 for 6-8 days as required.

Isolation and characterization of bacterial strains

Potential antagonistic bacteria were isolated from the rhizospheric soil samples collected from varied regions of Eastern Uttar Pradesh. For bacterial isolation, samples were processed according to the protocols described previously by Aneja (2005).Bacterial strains were routinely grown on nutrient agar(NA) at 28 C for 24 h. Bacterial strains showing a broad spectrum of antifungal activity on PDA plate assays were identified according to biochemical tests.

The tests carried out for preliminary characterization included Gram staining, Methyl Red test, Voges Prosayar test, Indole-3-acetia acid production test and indole production test. Further characterization was performed using 16S-rDNA sequence. To obtain 16S-rDNA PCR of the isolated DNA (minikit method) (Vogelstein, B. and Gileespie, D., 1979) was performed on selected isolates using primers 16S27F (5’ AGA GTT TGA TCC TGG CTC AG 3’) AND 16S1115R(5’AGG GTT GCG CTC GTT G3’) (Geneaid). The resulting PCR products were purified and used directly for sequencing. Homology studies were carried out using the NCBI program BLAST.

Antifungal activity assay

All bacterial isolates were scaeened for their ability to inhibit fungal growth on PDA using the technique of dual culture analysis (Haung and Hoes, 1976). Bacterial inocula were prepared from cultures grown on NA plates for 24 h whereas fungal cultures were grown on PDA plates for 8 days. A plug of mycelium of each fungus was placed at the center of the dual PDA plates and single bacterial colonies were patched parallely. Plates were incubated for 15 days at 22 C and the inhibition of the fungus growth was assessed by measuring the percentage growth inhibition.

Plant growth promotion ability in Maize plants

For the plant growth promotion ability test the seed of the variety HUZ-M60 was taken .The seeds were sterilized with Sodium hypochlorite solution by dipping for 30 sec and then washing with distilled water three times. Five ml of sucrose solution was added to the seeds.

Then each seed was inoculated with selected bacterial strains and were kept in the incubator for 5-6 h for incubation. Then each inoculated seed was swon and were kept in the plant growth chamber set at 25 C, 60% humidity and 12 h day/night cycle. After 21 days the grown plants were uprooted and the length of root and shoot were measured.

Result

Selection and identification of isolated bacteria

A total of ninety three bacteria were isolated from the rhizosphere of Rose, Kamini (wheat), Brassica, Maize, and Bathua collected from collected from B.H.U., Mirzapur, Gazipur, and Azamgarh districts of

Eastern Uttar Pradesh. With the screening of bacteria on three different media, 33 fluroscent pseudomonas, 30 phosphate solublizing bacteria and 30 actinomycetes bacteria were obtained. All the actinomycetes were found to be Gram positive while the phosphate solublizing bacteria and flurescent bacteria were Gram negative. All the isolates gave a posotive indole production test. The phosphate solublizing bacteria and actinomycetes gave a positive Methyl Red test and flurescent bacteria tested negative. While for Voges Proskaur test the flurescent and actinomycetes bacteria generally gave a positive test. All the isolates were found to produce IAA for 1, 3 and 6 mg/ml of tryptophane.DNA homology carried out with the sequences corresponding to 16S-rDNA also showed that the bacterial strains were related to *P.putida, P.licheniformis, Bacillus spp.*

Antagonistic bacteria

Out of the ninty three isolated bacteria 8 isolates (BMBK1, BMBK9, BMBK11, BMBK18, BMBP36, BMBP37, BMBA64, and BMBA7) showed ntagonistic activity against *E.turcicum* with BMBA64 showing the highest percentage inhibition (85.5%) Table 1 antagonists were obtained against *Bipolaris maydis*. (BMBA71, BMBK5, BMBP41, BMBA68, BMBA44, BMBA72, BMBK8, BMBA73, BMBA69, BMBP36, BMBA76). Out of these BMBK8 showed the highest percentage inhibition (87.2%) Table 2.

Plant growth promotion ability in Maize plant

Out of the nineteen antagonists only 8 were used to determine the plant growth promotion abilities as shown in Table 3. The bacterial strain BMBA64 was found to show the maximum growth promoting ability.

Discussion

As used by several authors (Andrews 1991; Wather *et al* 1998; Yoshida *et al*, 2001), the technique of dual culture analysis on agar plates was an easy assay with which to select antagonistic bacteria from a random group of bacterial isolates and to compare these selected strains for their fungal growth inhibition capabilities. In this way eight and eleven strains displaying a wide range of antifungal spectrum against *E.turcicum* and *Bipolaris maydis* respectively were selected as the most intresting bacteria to test for Biological control of Turcicum leaf blight and Maydis leaf blight. The characteristics of Pseudomonas, actinomycetes and Bacillus species as phosphate solublizers, and producers of IAA, Siderophores and exihibitors of antifungal activities are well known(Park *et al* 2005; Ahmad 2008; Mehalawy *et al* 2004). In the antagonistic activity assay the strain BMBA64 and BMBK8 showed the highest antagonistic activity against E. turcicum and *Bipolaris maydis* respectively.

Several reports have shown to control Southern corn leaf blight in vitro (Sleesman and Leban 1976; Haung *et al* 2010; Mohamed 1993). It has been amply demonstrated that the nature of the test strains (e.g. strains of phytopathogens and strains of antagonists) as well as several abiotic factors like composition of culture media significantly influnce antagonistic action against pathogens and affect the size of inhibition zone (Xu and Gross 1986).

The reason for this difference is unclear. In the test for plant growth promotion abilities, isolate BMBA64 was found to show the maximum plant growth promotion abilities. Strains of Pseudomonas, actinomycetes and Bacillus species have been found to promote plant growth abilities. (Tarabily *et al* 2006; Kaunat 1969; Haung *et al* 2010; Mehalawy *et al* 2004). The actinomycetes and Pseudomonas strains BMBA64 and BMBK8 isolated in this work have proved to be strong antagonists of E. turcicum and *Bipolaris maydis*. Hence these strains can be further used in the field experiments to note the level of disease control and their plant growth promotion abilities.

Table 1 Against *E. turcicum.*

Isolate	% of inhibition
BMBK5	77
BMBA68	70.9
BMBP44	63.9
BMBA69	67.1
BMBA71	72.2
BMBK8	87.2
BMBP41	80
BMBA72	85
BMBA73	69.2
BMBP36	61.2
BMBA76	64.8

Table 2 Against *B. maydis.*

Isolate	% of inhibition
BMBK1	78.8
BMBK9	77.7
BMBK11	74.4
BMBK18	66.6
BMBP36	82.2
BMBP37	68.8
BMBA64	85.5
BMBA74	72.2

Table 3 Plant growth promotion ability in maize.

Isolates	Root length (cm)			Avg.	Shoot length (cm)			Avg.
K1	23.5	24.5	24.5	24.16	35	39	34	36
K9	17.5	17	19	17.83	22	25	24	23.66
K11	20.5	19	20	19.83	28.5	30	29	29.16
K18	19	18.5	18	18.5	24	29	27	26.66
P3	20	21	22.5	21.16	24	24.5	24	24.16
P4	17	16.5	15.5	16.33	31	33	29.5	31.16
A1	18.5	19	19	18.83	27.5	28	26	27.16
A11	13	14	12.5	13.16	25	27	25	25.66
Control	26.5	27	27	26.83	24.5	25	24.5	24.66

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