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Isolation of Fluorescent Bacteria from different Maize Soils sample and Recognition of Antagonistic Property against Exserohilum turcicum

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Introduction

Exserohilum turcicum is the casual organism of maize crop which is also known as Northern Corn Leaf Blight. It is a major constraint with 70% yield losses of maize production in India. Plant diseases are creating adverse effects on maize plant leaf and disturb photosynthesis to which losses grain yields (Harlapur; 2005). *Exserohilum* is decline plant leaf quality and decrease photosynthesis rate. Plant defense mechanism is causing a complex ecology of processes disease lead more effective process and provides plant pathogen protection. Different types of microbial use are also uses plant defense mechanism and control maize crop pathogen. Soil microbes are natural enemy in maize crop production during field condition. These microbes are produces different secondary metabolites after interaction to each other in a surrounding. Pathological controls inhabit bacteria and microorganism and provide plant protection (Scott et al., 2009). Rhizobacteria is a bacterium that colonizes maize plant root. Plant pathogens and harmful microbes are produces some antibiotics, lytic enzyme, hydrogen cyanide and sidero-phore, that competing to nutrient and improve plant health (Ashrafuzzaman et al., 2009). Rhizobacteria is create a zone between soil and plant population in microbial biota to which provide a strong supplements in growth and development. These supplements provide a resistance in plant population (Verma et al., 2018). Antagonistic property is promotes seedling, vigor and better quality seed production under laboratories condition (Antoun and Kloepper, 2001). A fluorescent bacterium is a rod shaped gram negative bacteria which identified from motility and aerobic property of bacteria, and high G+C content (59.68%). The present study is carried out with the objectives to isolation of fluorescent bacteria and understanding antagonistic property against *Exserohilum turcicum* occurrence in maize crop and mechanism of phyto-pathogen suppression in new environment.

Materials and Method

Isolation and characterization of fluorescent bacteria

The rhizospheric soil samples are collected from four environment of Uttar Pradesh (Agriculture Field BHU Varanasi, IESD Field, RGSC field Mirzapur and Formers field). Three hundred sixty soil samples of maize crop were collected from different fields of Varanasi and Mirzapur region in Eastern Uttar Prades. Isolation of Fluorescent bacteria was completed under laboratory Molecular and Plant Breeding, Department of GPB, IAS, BHU, Varanasi (UP). The petriplates were taken after solidification of media, and incubated bacterial culture for 2-7 days at 28ºC. Fluorescent *Pseudomonas* isolates was prepared from collected maize soil samples and inoculation King's B medium. The kings medium was prepared from the provide protocol by Vidhyasekaran *et al.* 1997). With the help of UV rays fluorescence bacteria was identified in selected culture. Morphology a single colony is isolated from culture by serial dilution by Wang et al 2015).

Antagonistic test

1 gram soil sample was collected and weighed and dilute under 90 ml sterile double distilled water (SDDW) and shake it for 20 minute to create a suspension stock. With the help of pipette 1 mL of suspension dilution was transfer in flask that containing 9 mL of SDDW. Now a pattern was forming with dilutions, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, and $10^{-7}$of the test-tube. 0.2 mL of suspension was taken from last test tube and placed on nutrient agar. The inoculated petri-dishes were stored in the refrizrator under the control temperature 27^{o}C±2 for one week (Wang et al. 2015). Plate confrontation method was selected for the screening of antagonistic test on the PDA transferred plate (Miao et al. 2009). A control was used

as control for compare bacterial and fungal growth After 7 DAI bacterial culture was observed at 27°C and culture inhibition growth rate was calculated by following way;

$$\text{Inhibition Rate} = \frac{\text{Control} - \text{Colony diameter with treatment}}{\text{Control (colony diameter)}} X100$$

Biochemical analysis

After the identification of fluorescence bacteria selected some chemicals and performed biochemical test under the Molecular Biology and GPB laboratories BHU. The biochemical test is proofing of antagonistic properties of selected isolates.

The Indole production, IAA, Methyl red, Voges proscauer test was performed from prepared broth according to Bhati et al 2015).

Results and Discussion

Identification and characterization of bacteria

There are 122 isolates were observed from out of 360 isolates of maize soil samples. These are showing property of fluorescent bacteria. The bacterial isolates were referred as VBAF, VBIESD, VFFJ, and MRGSC, where "V" stands for Varanasi, "M" stands for Mirzapur and last part showing field location. Selected isolates investigation was carried out under the molecular breeding laboratories on the King's B media.

Table 1. Details of the isolated bacteria isolated from rhizospheric soil of different plants.

S.No.	Strains	Location	No. of Isolates
1.	VBAF	Agriculture form BHU Varanasi	38
2.	VBIESD	IESD Research field BHU Varanasi	42
3.	VFFJ	Farmers field Jayapur Varanasi	23
4.	MRGSC	RGSC Research field Mirzapur	19

Antagonistic Test

The dual culture analysis on agar plates was adopted for this test, this procedure given by (Yoshida et al. 2001). In this way one hundred twenty two isolates of bacterial cultures were identified from different culture that recognized from different soil samples. These isolates were screened antagonistic property of *E. turcicum* and tabulated in register. Eleven isolates were showing antagonistic property against TLB pathogen. The bacteria VBAF-13showing highest percentage (76.3%) of inhibition followed by other isolates that showing in table 2. Among all the one hundred twenty two strains, twenty seven is selected for future study. Pandey and Chaube (2003) are also reported earlier similar studies in which identified *P. fluorescens* zone. Shanmugam et al. 2003) evaluated antagonistic property and stored under laboratories condition for future use in agricultural field condition.

Table 2. Observed *E. turcicum* isolates for antagonistic property from radial growth and percent inhibition test

S.No.	Isolates	Radial growth (*E. turcicum*)	Percent inhibition (mm)
1	VBAF-6	29	73.5
2	VBAF-13	30	76.3
3	VBAF-21	27	75.7
4	VBAF-27	24	74.9
5	VBIESD-7	23	71.3
6	VBIESD-23	26	69.5
7	VBIESD-9	29	73.7
8	VFFJ-13	27	74.2
9	VFFJ-17	24	75.6
10	MRGSC-5	25	66.5
11	MRGSC-16	23	68.5

The biochemical study recorded on the basis of methyl red, Voges Prosekauer, Gram Staining and Indole production test in which selected isolates showing –ive by methyl red

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and Gram staining test while Voges Proskauer and Indole production test indicating +ive test in culture. Indole acetic acid production test was performed to different concentration of tryptophan (gm/ml) during 1 weak of incubation period (Table 3).

Table 3 Indole acetic acid production from different concentration of tryptophan (mg/ml)

S.No.	Isolates	1mg/ml Tryptophan	5mg/ml Tryptophan
1	VBAF-6	16.56	97.86
2	VBAF-13	17.83	103.97
3	VBAF-21	18.13	106.54
4	VBAF-27	17.23	104.83
5	VBIESD-7	18.61	107.19
6	VBIESD-23	16.89	96.85
7	VBIESD-9	17.54	103.56
8	VFFJ-13	18.11	105.23
9	VFFJ-17	15.97	87.96
10	MRGSC-5	16.66	89.54
11	MRGSC-16	17.55	91.67

Conclusions

The plant pathogen and microbial interactions are plays an important role in pest management. A number of antifungal aspects observed for selected isolates of bacterial leaf blight disease in biological control. The actinomycetes, Bacillus, and Pseudomonas were act as phosphate solublization and produces Indole Acetic Acid hormones that react to antifungal activity. VBAF-13 and VBAF-21 isolates were observed as highest antagonistic activity against *E. turcicum*. Hence these isolates are stored in -20 for further experiment in Maize.

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