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Prospecting Endophytic Bacterial Colonization and their Potential Plant Growth Promoting Attributes in Hybrid Maize (*Zea mays* L.)

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A B S T R A C T

Maize, a crop cultivated worldwide, was investigated for colonization endophytic bacteria at different growth stage in different plant parts. Such bacterial interactions have high potential to enhance maize growth and development by means of plant growth promoting activities. 82 morphologically different endophytic bacterial isolates were isolated from hybrid maize variety, Pusa Extra Early Hybrid Maize (PEEHM-5) from root, stem and leaf tissues on different nutrient media at vegetative, flowering and maturity stages of growth. Among growth stages, the maximum population of endophytic bacteria was found at flowering stage followed by vegetative and maturity stage. Among plant tissues, root was harboring higher bacterial population followed by stem and leaf. Upon screening of those 82 endophytic bacterial isolates for plant growth promoting attributes 52 isolates exhibited one or more attributes for plant growth promotion. P, K, Zn solubilization was shown by 17, 8 and 21 isolates respectively. 10 isolates tested positive for phytohormone production, whereas 18 isolates were producing siderophore. Few isolates produced ACC deaminase (2), HCN (2) and biological nitrogen fixation (1). Biocontrol activity was shown by 5 isolates against *Exerohilum turcicum* whereas 3 isolates against *Rhizoctonia solani.* Primarily 59.6 % isolates were having single PGP trait, 23 % having double, 9.6 % triple and 7.7 % having four PGP traits. Careful selection from the group with multiple characters may lead to development of an effective bioagent.

Introduction

Various types of microorganisms, including bacteria, fungi and actinomycetes are found inside plants and are designated as endophytes and they live in plant tissues without causing substantive harm to the host. Endophytic bacteria exist within the living tissues of most plant species in form of symbiotic to slightly pathogenic. These have been recovered from a variety of plants including rice, tomato, sweet corn, citrus and potato (Ulrich *et al.*, 2008). They have significant influence on plant growth and development. The main reason for the interest in endophytes is the realization that if these bacteria can be reintroduced in the endophytic stage, a more stable relationship can be established between beneficial endophytic bacteria and plants, than for rhizospheric or epiphytic bacteria and plants. These constitute a great reservoir of bacterial diversity with a remarkable biotechnological potential (Malfanova *et al.*, 2011). Endophytic bacteria have been implicated in supplying

biologically fixed nitrogen in non-legumes, and these associations can increase the nitrogen economy of a crop and thus reducing the requirement for nitrogenous fertilizers (Sturz and Nowak, 2000). Bacterial endophytes might intimately interact with cells of the host, taking up secreted metabolites and releasing plant-growth-promoting (PGP) compounds, indole-3-acetic acid (IAA), siderophores and 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase production, nitrogen fixation, phosphate solubilization (Hardoim *et al.,* 2011 and Ma *et al.,* 2011), cyanide production (Flaishman *et al.,* 1996) and have capacity to control plant pathogens (Krishnamurthy and Gnanamanickam, 1997). The entry of these endophytic bacteria from rhizosphere is facilitated by passive mode via cracks, wounds and by active mode via hydrolytic enzymes by degradation of the wall for entry. Therefore, endophytes with the plant beneficial traits are potentially excellent plant growth promoters and/or biological control agents for sustainable crop production (Natalia *et al.,* 2011).

Maize (*Zea mays* L.) is one of the most important grain crop in terms of world production, together with rice and wheat is cultivated in many areas of the world. As world cereal consumption tends to increase due to a constantly growing population, productivity should be significantly improved through different strategies that allow an optimization of yields without implicating an increased sown area (von Braun, 2010). Hybrid varieties of maize are being researched for meeting the crop production targets worldwide and in a scenario of climate change, short duration and resilient varieties of grain crops are being emphasized. To obtain high yields in most crops, as is particularly true in maize, it is necessary to apply huge quantity of mineral fertilizers to the soil (Arruda *et al.,* 2013), that have less than 50% use efficiency of the applied N fertilizer by plants (Halvorson *et al.,* 2002). It also contributes to contamination of soils and ground water supplies, leading to health hazards and compromising agricultural sustainability. So there is need to have environmentally friendly and conservative alternatives to protect biodiversity and sustainability of agro ecosystems. Endophytic bacteria are of agronomic interest in particular because they can enhance plant growth by improving nutrition of plants. Therefore, the present study was taken up to decipher such beneficial attributes of endophytic bacteria of hybrid maize.

Materials and Methods

Experimental site and plant sampling

Plant samples of hybrid maize variety Pusa Extra Early Hybrid Maize-5 (PEEHM-5) were collected from the farm of Indian Agricultural Research Institute, New Delhi. The samples were collected from the site where uniform growth of healthy plants was seen**.** Sampling was done at three different stages of plant growth *viz.* vegetative, flowering and maturity of maize crop. At each sampling event five healthy plants were carefully deep uprooted in a zigzag sampling pattern from the field.

Isolation of maize bacterial endophytes and growth conditions

Adhering soil particles from the plant samples were removed by several washing of tap followed by sterilized water. Each plant was separated into root, stem and leaf by sterilized scissors. The composite sample from 5 plants was prepared. 10 g of different tissue samples were sequentially surface sterilized using 70 % ethanol and sodium hypochlorite 2 % (v/v) for 1 and 3 min, respectively. Surface sterilization was ensured by plating aliquots (100 µl) of the final rinse DW onto Trypticase

Soy Agar (TSA) growth medium plates. The inoculated plates were incubated for 24 hr at 30^0C to check for any surface microbial contaminant growth. 10 gm of surface sterilized plant material were macerated in a sterilized pestle mortar for 10 min and were suspended in 90 ml (0.9 %) saline blank in 250 ml flask to make 10^{-1} dilution.

For the exudation or detachment of endophytic bacteria in suspension, samples were shaken on rotary shaker at 30 °C for 1 hr at 120 revolutions per minute (rpm). Further serially diluted samples (100µl) from different dilutions were spread plated on Nutrient Agar (NA), Trypticase Soy Agar (TSA) plates. Inoculated plates were incubated at 30 °C for 24-72 hrs.

Selection and purification of different bacterial morphotypes

Each plate was examined critically and selection of bacterial morphotype was done on the basis of morphological parameters viz. size, shape, colour, margin and texture of bacterial colonies.

All morphotype were purified by quadrant streaking on respective growth medium plates. Purified bacterial morphotypes were preserved both in respective medium slants at 4 °C as working culture and as 25 % glycerol stock at -20 °C for future use.

Functional annotation of endophytic bacteria

Screening of endophytic bacteria for plant growth promoting traits

Liquid suspension of the purified bacterial isolates was prepared in respective medium broth to an approximate titre of 10^6cfu/ml. The purified isolates were functionally screened for following plant growth promoting attributes:

Phosphate solubilization

Phosphate solubilizing activity of the isolates was screened on Pikovskaya Agar, (Pikovskaya, 1948). Each plate was divided in sectors and each sector was inoculated with a spot of 10 µl bacterial suspension containing $\approx 10^4$ bacterial cells. Incubation of the plates was done for 48-96 h at 30 °C for the observation of clearing zones which is an indicator of P-solubilization.

Potassium solubilization

Potassium solubilization by bacterial isolates was screened on modified Aleksandrov agar medium plates (Hu *et al.,* 2006). Each plate was divided in sectors and each sector was inoculated with a spot of 10 µl bacterial suspension containing $\approx 10^4$ bacterial cells. The plates were incubated at 30 °C for 48-96 h for observation of clearing zone which is an indicator of K-solubilization.

Zinc solubilization

Zinc solubilizing ability was screened on nutrient agar medium plates supplemented with 0.1 % insoluble zinc oxide (ZnO) (Saravanan *et al.,* 2004). Each plate was divided in sectors and each sector was inoculated with a spot of 10 µl bacterial suspension containing $\approx 10^4$ bacterial cells. The plates were incubated at 30 °C for 48-96 h for observation of clearing zone known as an indicator of Zn-solubilization.

Siderophore production

Isolates were checked for the production of siderophore using specified chrome azurol-S agar medium (CAS blue agar) according to Schwyn and Neilands (1987). The CAS plates were prepared using 100 ml dark blue CAS mixture and nutrient agar medium (300 ml). It was autoclaved separately. The CAS plates

were inoculated with a spot of 10 µl bacterial suspension containing ≈10^4 bacterial cells and incubated at 30 °C for 7-10 days. Development of a deep yellow to orange colour surrounding the colony was a positive indication for siderophore production.

Hydrogen cyanide production

The production of hydrogen cyanide (HCN) was investigated by inoculating endophytic bacterial isolates in 5 ml nutrient broth containing 4.4 g L^{-1} glycine in 30 ml glass tubes. A strip of sterilized filter paper saturated with solution of picric acid (0.5%) and sodium carbonate (2%) was placed in cotton plug sealed tubes containing different bacterial isolates and incubated for 7-15 days at 30 °C. The change of filter paper colour from yellow to light brown or reddish brown was positive indication for the production of HCN (Bakker and Schippers 1987).

1-Aminocyclopropane-1-carboxylate deaminase production

ACC deaminase activity was checked by their ability to utilize 1-Aminocyclopropane -1-carboxylate (ACC) as sole source of nitrogen as given by Jacobson *et al.,* 1994. MDF (modified Dworkin and Foster medium) agar plates were used for checking ACC deaminase activity. MDF agar plates supplemented with 0.3 g of ACC L^{-1} were inoculated with a spot of 10 µl bacterial suspension containing ≈10^4 bacterial cells. Bacterial isolates was also spotted on plates of MDF medium containing 0.3 g L^{-1} ammonium sulphate as positive control and on plain MDF agar plate as negative control. Incubated plates for 72 h at 30 °C were observed for the growth.

Indole acetic acid

The qualitative analysis for production of IAA was carried out according to Bric *et al.,* (1991). For IAA production, 10 µl bacterial suspension containing ≈10^4 bacterial cells was spotted on Luria agar plates supplemented with 50 µg mL^{-1} tryptophan. Above mentioned plates were dried at ambient temperature and were covered with paper disc of sterile Whatman No. 1 filter.

The plates were incubated at 30 ^{0}C for 24 h. The filter paper disc removed was treated with Salkowski solution. The development of pink colour was an indication of IAA production.

Acetylene Reduction Ability (ARA)

Bacterial isolates were screened for ARA activity on N-free Jensen medium for isolates using gas chromatograph according to Hardy *et al.,* (1973).

Biocontrol activities against potential maize pathogens

Biocontrol activity of the isolated endophytic bacteria was assayed using dual inoculation technique against two maize pathogens, *Exserohilum turcicum (Turcicum* leaf blight) and *Rhizoctonia solani (*root and stalk rot) according to the method described by Sijam and Dikin (2005).

These test fungi were grown separately on potato dextrose agar medium and its 3 mm disc was placed in the center of each modified PDA plates (PDA:NA:1:1). After incubation of 6 h at 37 °C the same plates were inoculated with a spot of 10 µl bacterial suspension containing ≈10^4 bacterial cells and the plates were incubated for 5-7 days at 30 °C. Plates inoculated with fungal disc alone were used as a control.

Three replications were maintained for each isolate. The zone of inhibition by bacteria against fungal pathogen was observed after sufficient incubation period.

Results and Discussion

Isolation of maize bacterial endophytes

Maize plant parts at various growth stages housed variable counts of culturable bacterial endophytes. It varied from $7.2X10^3$ to $1.9X10^4$ cfu g^{-1} of plant tissue. The maximum counts were observed in flowering stage in all three tissues *viz*. root, stem and leaf. Amongst tissues the maximum counts were observed in roots at three growth stages viz. vegetative, flowering and maturity (Table 1). On the basis of morphological characters 82 diverse isolates were purified of which 20 morphotypes were from vegetative, 30 from flowering and 32 from maturity stage across different tissues (Table 1).

Qualitative screening for plant growth promoting attributes

Phosphate solubilization ability

All purified bacterial endophytic isolates were screened *in vitro* for P-solubilizing activity by spotting on Pikovskaya Agar medium plates. A clearing zone around the colony is an indication of P-solubilization (Fig. 1). A total of 17 isolates from PEEHM-5 were found to possess P- solubilization activity (Table 2), out of which 3 isolates belonged to vegetative stage, 10 to flowering and 4 to maturity stage (Fig. 2) and further it was found that across different growth stages there were 8 isolates from root, 5 from stem and 4 from leaves as P solubiliser. Verma *et al.,* (2015) has reported the phosphate solubilizing property of endophytic bacteria *viz. Azotobacter*, *Burkholderia*, *Citrobacter*, *Enterobacter*, *Pantoea* and *Pseudomonas* in wheat. There are reports of bacteria belonging to genera *Bacillus, Pseudomonas, Serratia, Enterobacter*, solubilizing the insoluble phosphate compounds and aid in plant growth (Hameeda *et al.,* 2008). Joe *et al.,* (2016) reported two salt tolerant endophytic and phosphate solubilizing bacteria ACMS25 and PVMX4 isolated from *Phyllanthus amarus* and got identified them based on 16s rRNA sequencing as *Acinetobacter* sp. and *Bacillus* sp.

Potassium solubilization

All purified bacterial endophytic isolates were screened in vitro for K- solubilizing activity by spotting on Petri plates containing modified Aleksandrov agar medium. A clearing zone around the colony is an indication of K-solubilizers (Fig. 1). A total of 8 isolates from PEEHM-5 were found to possess K-solubilization activity (Table 2), out of which 7 isolates from vegetative and only 1 from flowering stage were K- solubilizer (Fig. 2) and across different stage 7 were from root and 1 from stem respectively. Yuan *et al.,* (2015) had done PGP characterization and the 16S rDNA sequence analysis of endophytic bacteria isolated from the root, rhizome, stem, and leaves of Moso Bamboo and showed that the 20 phosphorus- and potassium-solubilizing bacteria belong to 14 species from 10 genera, and mainly consist of *Alcaligenes* spp., *Enterobacter* spp. and *Bacillus* spp. The potential endophytic bacteria solubilizing K from seedling roots of date palm (*Phoenix dactylifera* L.) were *Bacillus endophyticus* strain 2DT, *Acinetobacter pittii*, *Achromobacter* sp. (Yaish *et al.,* 2015).

Zinc solubilization

All purified bacterial endophytic isolates were screened in vitro for Zn- solubilizing activity by spotting these on nutrient agar medium plates supplemented with 0.1 % insoluble zinc compounds as (ZnO). A clearing zone around the colony is an indication of Zn-solubilizers (Fig. 1). A total of 21 isolates (Nearly 25 % of total isolates) PEEHM-5 were found to possess Zn- solubilizing activity. Among 21

Zn- solubilizing isolates of PEEHM-5 (Table 2), 3 were from vegetative, 9 from flowering and 9 from maturity stage (Fig. 2) respectively and across different stage 11 isolates were from root, 6 from stem and 4 from leaves respectively. Yaish *et al.,* (2015) reported potential zinc solubilizing endophytic bacteria from seedling roots of date palm (*Phoenix dactylifera* L.) belonging to various genera of *Achromobacter, Acinetobacter, Bacillus, Chryseobacterium, Enterobacter, Klebsiella, Paenibacillus, Rhodococcus and Staphylococcus.*

Siderophore production

All purified bacterial endophytic isolates were screened in vitro for siderophore production. Overall 18 isolates from PEEHM-5 were found to possess siderophore production ability (Fig. 1 and Table 2). A single isolate from vegetative, 11 from flowering and 6 from maturity stage (Fig. 3) were found to produce siderophore respectively and out of these 8 isolates were from root, 6 from stem and 4 from leaves respectively across different stage. Hydroxamate-type Siderophore producing endophytic bacteria *Methylobacterium spp*., has been reported from citrus plants (Lacava *et al.,* 2008).

Hydrogen cyanide production

All purified bacterial endophytic isolates were screened in vitro for hydrogen cyanide production. Only 2 isolates from PEEHM-5 were found to possess hydrogen cyanide production ability (Fig. 1 and Table 2). Among 2 isolates, a single isolate each from vegetative and flowering stage was hydrogen cyanide producer (Fig. 3) and across different stage 1 was from root and another one was from leaves respectively. Rodrigues *et al.,* (2016) reported isolation of 136 endophytic bacteria associated with sugarcane with 83 of them presenting some plant growth mechanism: 47 % phosphate solubilizers, 26 % nitrogen fixers and 57 % producing IAA, 0.7 % HCN and chitinase, 45 % ammonia, 30 % cellulose and 8 % pectinase. The seven best isolates were tested for their ability to promote plant growth in maize. The isolates tested for plant growth promotion belong to the *Enterobacteriaceae* family and the *Klebsiella, Enterobacter* and *Pantoea* genera.

1-Aminocyclopropane-1-carboxylate deaminase production

All purified bacterial endophytic isolates were screened in vitro for ACC utilizing ability as sole source of nitrogen. 2 isolates from PEEHM-5 were found to possess ACC utilizing ability (Fig. 1 and Table 2). In case of PEEHM-5, a single isolate each from flowering and maturity (Fig. 3) and across different stage one was from stem and other one from leaves as ACC utilizing isolate. ACC deaminase producing bacteria helps plant to relieve stress caused by ethylene by breaking down ACC into ammonia and a-ketobutyrate (Mayak *et al.,* 1999).

Table.1 Isolation of total endophytic bacteria (cfu g^{-1})* from different growth stages and tissues

Growth stages/ tissues	Root	Stem	Leaf
Vegetative	$1.6X10^4$ (7)	$1.0X10^4$ (8)	$8.8X10^3$ (5)
Flowering	$1.9X10^4$ (16)	$1.5X10^4$ (4)	$1.3X10^4$ (10)
Maturity	$1.2X10^4$ (7)	$8.1X10^3$ (11)	$7.2X10^3$ (14)

Figure in parenthesis indicate number of purified morphotypes

Table.2 Plant growth promoting activities of endophytic bacteria from PEEHM-5

Isolates	Solubilization			Production					Biocontrol	
	PO_4	K	ZnO	Siderophore	HCN	ACC	IAA	Biological N_2 fixation	*Exerohilum turcicum*	*Rhizoctonia solani*
PHM5-1	-	+	-	-	-	-	-	-	-	-
PHM5-2	-	+	-	-	-	-	-	-	-	+
PHM5-3	-	-	-	-	+	-	-	-	+	-
PHM5-5	-	+	-	-	-	-	-	-	-	-
PHM5-6	-	+	+	-	-	-	-	-	-	-
PHM5-7	-	+	-	-	-	-	-	-	-	-
PHM5-8	-	+	+	-	-	-	-	-	-	-
PHM5-10	-	-	-	+	-	-	-	-	-	-
PHM5-11	-	-	+	-	-	-	-	-	-	-
PHM5-12	+	-	-	-	-	-	-	-	-	-
PHM5-13	+	-	-	-	-	-	-	-	-	-
PHM5-17	+	-	-	-	-	-	-	-	-	-
PHM5-21	+	-	-	-	-	-	-	-	-	-
PHM5-22	-	-	-	+	-	-	-	-	-	-
PHM5-23	+	-	-	-	-	-	-	-	-	-
PHM5-24	-	-	+	+	-	-	-	-	-	-
PHM5-25	-	-	-	+	-	-	-	-	+	+
PHM5-26	-	-	+	-	-	-	-	-	-	-
PHM5-27	-	-	+	-	-	-	-	-	-	-
PHM5-28	-	-	+	-	-	-	-	-	-	-
PHM5-30	+	-	+	+	-	-	+	-	-	-
PHM5-31	-	-	+	+	-	-	-	-	-	-
PHM5-32	+	-	-	-	-	-	+	-	-	-
PHM5-33	+	-	-	-	-	-	-	-	+	+
PHM5-35	+	-	-	-	-	-	-	-	-	-
PHM5-36	-	-	+	+	-	-	-	-	-	-
PHM5-37	+	-	-	+	-	-	+	+	-	-
PHM5-38	+	-	-	+	-	+	+	-	-	-
PHM5-39	+	-	-	-	-	-	+	-	-	-
PHM5-40	-	-	+	-	-	-	-	-	-	-
PHM5-44	-	+	-	+	-	-	-	-	-	-
PHM5-46	+	-	-	-	+	-	-	-	-	-
PHM5-48	-	-	-	+	-	-	-	-	-	-
PHM5-49	-	-	+	-	-	-	-	-	-	-
PHM5-50	-	-	+	-	-	-	-	-	-	-
PHM5-54	-	-	+	-	-	-	-	-	-	-
PHM5-55	+	-	-	+	-	-	+	-	-	-
PHM5-56	+	-	-	-	-	-	-	-	+	-
PHM5-58	-	-	+	-	-	-	-	-	-	-
PHM5-59	-	-	-	+	-	-	-	-	-	-
PHM5-61	-	-	+	-	-	-	-	-	-	-
PHM5-63	-	-	-	+	-	-	-	-		-
PHM5-65	-	-	+	-	-	-	+	-	+	-
PHM5-67	-	-	-	+	-	-	-	-	-	-
PHM5-68	-	-	-	+	-	-	-	-	-	-
PHM5-69	-	-	+	-	-	-	-	-	-	-
PHM5-70	-	-	-	+	-	-	-	-	-	-
PHM5-72	-	-	-	-	-	+	-	-	-	-
PHM5-73	+	-	+	-	-	-	+	-	-	-
PHM5-74	-	-	+	-	-	-	+	-	-	-
PHM5-76	+	+	+	+	-	-	-	-	-	-
PHM5-80	-	-	-	-	-	-	+	-	-	-

Fig.1 Plant growth promoting traits; Solubilization of phosphorus, potassium and zinc; HCN, siderophore and ACC; biocontrol against *Rhizoctonia solani*

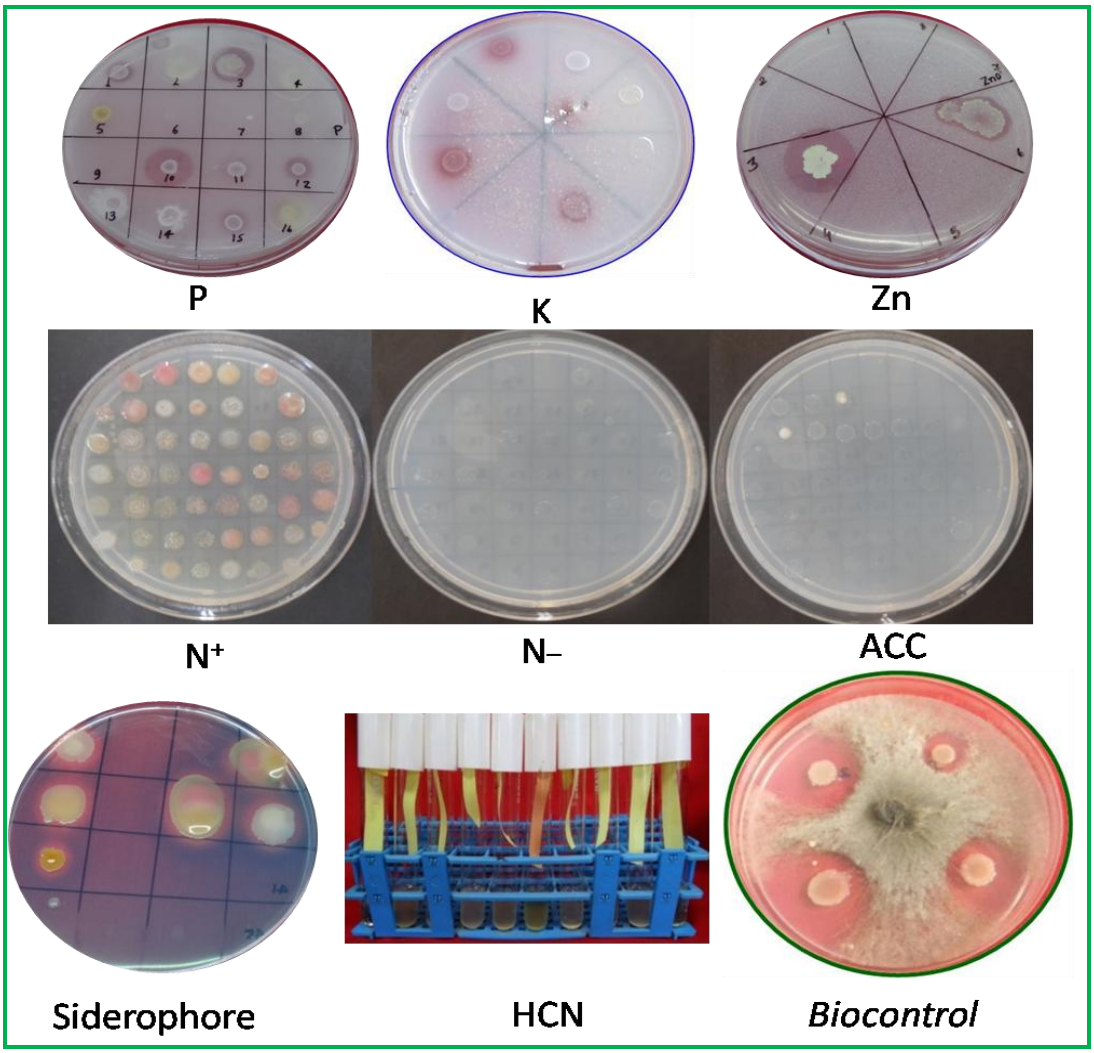


Fig.2 Nutrient solubilization by endophytic bacteria from PEEHM-5

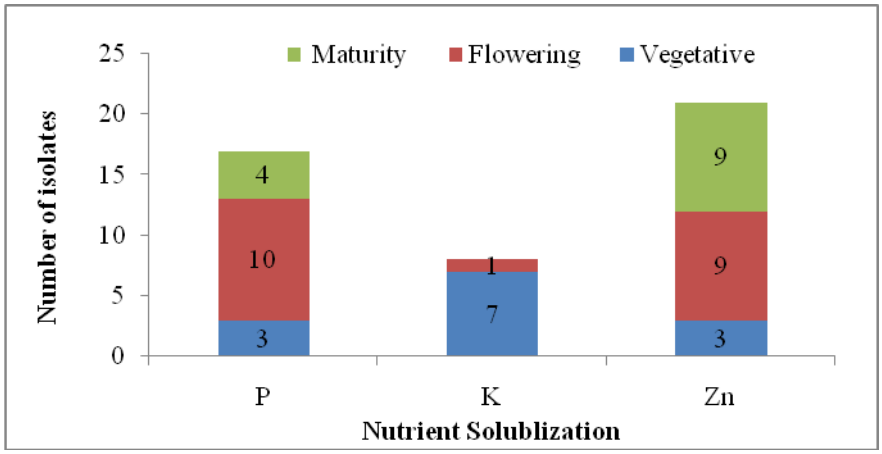


Fig.3 Production of compounds by endophytic bacteria from PEEHM-5

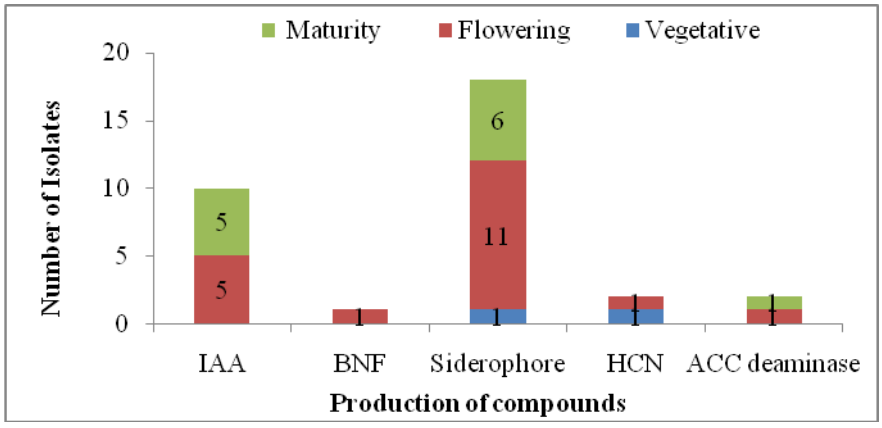
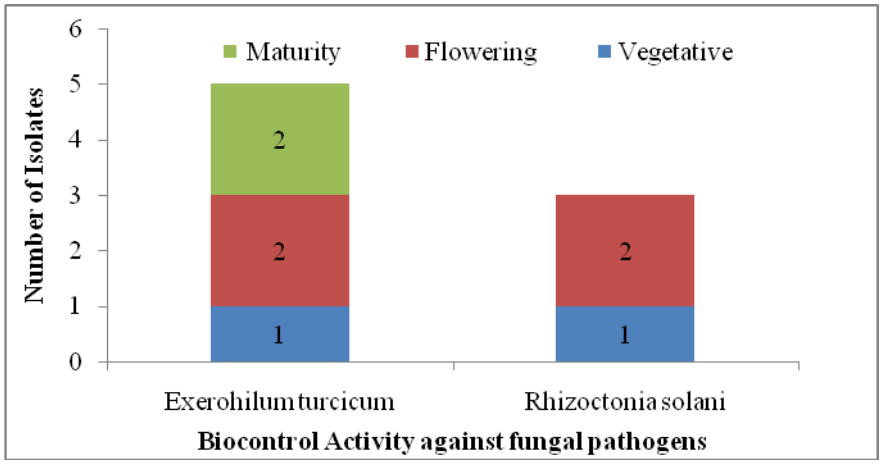


Fig.4 Biocontrol activity against fungal pathogens of endophytic bacteria from PEEHM-5



Xu *et al.,* (2014), reported that endophytic bacteria *Bacillus subtilis* (HYT-12-1) isolated from tomato seeds had PGP traits along with ACC deaminase activity (112.02 nmol *α*-ketobutyrate mg^{-1} protein h^{-1}). Verma *et al.,* (2014) also reported psychrotolerant and drought tolerant endophytic bacteria from wheat producing ACC deaminase by various genera *viz. Arthrobacter, Flavobacterium, Bacillus, Pseudomonas, Methylobacterium* and *Enterobacter.*

Indole acetic acid production

All purified bacterial endophytic isolates were screened in vitro for Indole acetic acid production. A total of 10 isolates from PEEHM-5 were found to possess Indole acetic acid production ability (Table 2). Among 10 isolates, 5 from flowering and 5 from maturity stage (Fig. 3) while across different stage 3 were from root, 4 from stem and 2 from leaves respectively were IAA producer. Szilagyi-Zecchin *et al.,* (2014) reported six endophytic bacteria of corn roots which were identified by sequencing of the 16S rRNA gene as *Bacillus* sp. and as *Enterobacter* sp. Four of the strains, CNPSo 2476, CNPSo 2477, CNPSo 2478 and CNPSo 2480 were shown to have nitrogen fixation ability evaluated through the acetylene reduction assay and amplification of *nifH* gene. Two *Bacillus* strains (CNPSo 2477 and CNPSo 2478) found to possess outstanding

skills for the production of IAA, siderophore.

Acetylene reduction activity (ARA)

PMH5-37 was the single isolate from PEEHM-5 isolated at vegetative stage from stem and had nitrogenase activity (Table 2). Ji *et al.,* (2014) reported diazotrophic endophytic bacteria from the leaves, stems, and roots of 10 rice cultivars belonging to various genera *viz*. *Penibacillus, Microbacterium, Bacillus* and *Klebsiella.* Suman *et al.,* (2005) screened seven *Gluconacetobacter diazotrophicus* strains isolated from sugarcane roots for their efficiency to promote growth and nutrient uptake in sugarcane at three levels of urea N (0, 75, and 150 kg N ha^{-1}). Following inoculation by these strains improvement in germination, tiller number and plant height was observed.

Biocontrol activities against potential maize pathogens

All purified bacterial endophytic isolates were screened in vitro for biocontrol activity against two maize pathogens *Exserohilum turcicum* (*Turcicum* leaf blight) and *Rhizoctonia solani (*root and stalk rot). The zone of inhibition by bacteria against fungal pathogen was observed after sufficient incubation period. 5 isolates from PEEHM-5 were found to possess antagonistic activity against *Exserohilum turcicum* (Table 1). A single isolate was antagonistic against *Exserohilum turcicum* from vegetative, 2 from flowering and 2 from maturity stages (Fig. 4) respectively of which 4 were from root and only 1 from stem respectively across different stage. Three isolates from PEEHM-5, were found to possess antagonistic activity against *Rhizoctonia solani* (Table 1). A single isolate from vegetative stage and 2 isolates from flowering stage were antagonistic against *Rhizoctonia solani* (Fig. 4) of which all the three were from root across different stage. White *et al.,* (2014) reported the antifungal activity of *B. amyloliquefaciens* an endophyte from vanilla orchids which gave protection to plant seedlings from pathogens. Verma *et al.,* (2015) reported that many species of genera *Bacillus, Exiguobacterium, Micrococcus, Pseudomonas* and *Psychrobacter* showe antagonistic properties against fungal pathogens *Fusarium graminerum, Rhizoctonia solani* and *Macrophomina phaseoli*.

The present study provides baseline information on effect of plant developmental stage on differential colonization of culturable endophytic bacterial diversity in different plant tissue and their plant probiotics functions in a hybrid maize variety. Primarily 59.6 % isolates were having single PGP trait, 23 % having double, 9.6 % triple and 7.7 % having four PGP traits. Multi-PGP trait isolates selected in this study need to be further investigated under pot and field conditions for commercialization among farmers for enhancing maize growth and productivity.

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