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Endophytic bacteria colonizing maize (*Zea mays* L.) exhibiting plant growth promoting traits and influence on plant growth

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Abstract
Endophytes are microbial symbionts residing within the plant for the majority of their life cycle without any detrimental impact to the host plant. Some endophytes are able to promote the growth of plants by various PGP mechanisms. This study envisages the isolation of endophytic bacteria, morphological characterization, *in vitro* screening for their plant growth promoting traits and *in planta* assay for their influence on plant growth. A total of 106 morphologically distinguishable bacterial endophytes were isolated from composite maize variety, Pusa Composite-4 (PC-4) from surface sterilized root, stem and leaf tissues on different growth media at vegetative, flowering and maturity stages of growth. The maximum population of endophytic bacteria was found at flowering stage in root. 62 isolates out of 106 isolates exhibited one or more plant growth promoting traits. P, K, Zn solubilization activity was found in 19, 3 and 30 isolates respectively; phytohormone IAA production tested positive in 15 isolates, whereas 27 isolates were siderophore producer. Five isolates produced HCN and 3 isolates were biological nitrogen fixer. Biocontrol activity was tested positive in 6 isolates against *Exerohilum turcicum* and one isolate against *Rhizoctonia solani.* Primarily 54.83% isolates were having single PGP trait, 29.03% double, 9.67% triple, 4.83% four and 1.61% six PGP traits. Selected PGP isolates showed positive effects on germination %, shoot dry weight and root dry weight ranging from 91 to 96%, 1.9 to 4.2 gm plant−1 and 0.5 to 1.98 gm plant−1respectively, as compared to the un-inoculated control. Application of such PGP endophytes appears to be one of the best options in enhancing biomass yield and improving crop productivity.

Keywords: Endophytic bacteria, plant growth promotion (pgp), growth stages, composite maize

Introduction
Maize (*Zea mays* L.) crop cultivated worldwide, broadly used for human and animal consumption and for the production of high yields require large quantities of nitrogen (N) fertilizer. Fertilizer demand is increasing as world population is growing exponentially which is demanding increased food production. This has resulted not only in price rise of fertilizer, but will lead to an unavoidable world shortage of, e.g., phosphate, within decades (Vance 2011) [41]. On the water front also we face similar situation, and global warming making the situation even worse. These problems are demanding innovative agricultural management systems to fulfill the future global demand for food, feed, and fiber products while lowering negative impacts on, soil, water quality and atmosphere (Hayati *et al.* 2011; Lambin and Meyfroidt 2011) [12, 20].

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) [40]. Exploitation of bio-inoculants based on PGP bacteria seems to be a promising alternative to chemicals. Even partial decrease in N fertilizer application because of application of rhizospheric and endophytic bacteria would have economic and environmental relevance. These microbes ensure the provision of essential nutrients to plants along with increases nutrient use efficiency (Khalid *et al.* 2009) [17]. Proposed mechanisms by which PGPB may stimulate plant growth and nutrition include increasing availability of nutrients like phosphorus, iron and other microelements, biological nitrogen fixation (BNF), phytohormones production, environmental stress relief, synergism with other plant–microbe interactions, inhibition of plant ethylene synthesis by producing ACC deaminase enzyme which breaks down ACC precursor of ethylene, and growth

Enhancement by volatile compounds (Hardoim *et al*. 2008; Compant *et al.* 2010) [10, 7]. Colonizing capability of endophytic bacteria in ecological niche similar to that of phytopathogens, makes them suitable as biocontrol agents (Berg *et al.,* 2005) [5]. Numerous studies have reported that endophytic microorganisms can control plant pathogens (Krishnamurthy & Gnanamanickam, 1997) [19] and insects (Azevedo *et al.,* 2000) [3]. Therefore, beneficial endophytic bacteria can be a potential source for plant growth promotion and/or biological control agents for sustainable crop production (Natalia *et al.,* 2011) [27].

Characterization of endophytic bacteria with composite maize variety may provide valuable information, given the poor insights of the plant-bacteria interaction. The purpose of the present study was, thus, isolation of endophytic bacteria from root, stem and leaf of maize plant at different growth stages and *in vitro* assays for various plant growth promoting traits and to evaluate the effect of most promising isolates on maize plant biomass.

Materials and Methods

Experimental material and isolation of bacterial endophytes

Composite maize variety PC-4 plant samples were collected from the farm of Indian Agricultural Research Institute, New Delhi, at three different plant growth stages *viz.* vegetative, flowering and maturity. Five healthy plants were sampled for further processing in the laboratory. After removing soil particles from roots by several washings with sterile water, each plant was divided into different parts and composite samples of root, stem and leaf were prepared. The samples (10 g) were surface sterilized using ethanol (70%) and sodium hypochlorite 2% (v/v) for 1 and 3 min, respectively, followed by washings with sterile water. The surface sterilization was ensured by plating the last wash on Trypticase Soy Agar (TSA) plates. The surface sterilized root, shoot and leaf samples 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). After serial dilutions samples were spread plated on different growth medium *viz*. Nutrient Agar (NA), Luria-Bertani Agar (LBA), Trypticase Soy Agar (TSA) plates. Inoculated plates were incubated at 30 °C for 24-72 hrs.

Bacterial morphotyping

Post incubation all plates were examined for total bacterial load. Based on morphological parameters each bacterial colony (morphotype) was examined for parameters *viz.* size, shape, colour, texture and margin of bacterial colonies. After purifications on respective growth medium, the purified bacterial endophytic isolates were preserved both in respective medium slants at 4 °C as working culture and as 25% glycerol stock at -20 °C for future use.

Bacterial mediated Direct Plant Growth Promoting mechanisms Availability of major and micronutrients

Nitrogen

Nitrogen fixation ability of all bacterial isolates was evaluated using Acetylene Reduction on N-free Jensen medium using gas chromatograph according to Hardy *et al.,* (1973) [11]. Around 105-106 cells (100 µl) was inoculated on Jensen medium slants in tubes and were incubated for 3 days at 30 °C. After incubation, the acetylene gas being an analogue of N2 gas was added in each tube (10% v/v) and kept incubated for 4 hr at 30 °C. Appropriate uninoculated controls were maintained. For measuring acetylene reduction, gas sample (1ml) was injected into preheated gas chromatograph (Nucon 5765 model) with FID detector and Porapak N column. Nitrogen gas was used as carrier gas. The operating temperature conditions were 110, 75, and 110 °C for injector, column and detector, respectively. Retention period of standard ethylene (100 vpm) was used to calculate the amount of ethylene produced by acetylene reduction. Protein content was estimated by using Lowry,s method (1951). The ARA was expressed in terms of nanomoles of ethylene produced per mg protein per hour.

Phosphorus

Phosphorus solubilization ability of all bacterial isolates was screened qualitatively on Pikovskaya Agar medium containing tricalcium phosphate (TCP) as a source of insoluble P (Pikovskaya 1948) using spot assay. For this bacteria (10 µl) of approximately 104 bacterial cells of different isolates were spotted on Pikovskaya agar plates and plates were incubated at 30 °C for 48-96 h. Colonies were observed for the clearing zone around the colony by dissolution of TCP. The positive bacterial isolates were further processed for quantification of P solubilization ability using NBRI phosphate medium broth (NBRI-PM) and ascorbic acid method as explained earlier (Mehta and Nautiyal 2001) [25].

Potassium

All bacterial isolates were screened for potassium solubilization activity on modified Aleksandrov agar medium plates containing potassium aluminium silicate 0.2% (Hu *et al.,* 2006) [14] using spot assay. Plates were spotted with 10 µl bacterial suspensions containing ≈104 bacterial cells. Inoculated plates were incubated at 30 °C for 48-96 h and were observed for the clearing zone around the colony which is an indicator of K-solubilization.

Zinc

All bacterial isolates were screened on nutrient agar medium plates supplemented with % insoluble zinc oxide (ZnO) for zinc solubilizing activity (Saravanan *et al.,* 2007) [33]. Plates were inoculated with 10 µl bacterial suspension containing ≈104 bacterial cells. Inoculated plates were incubated at 30 °C for 48-96 h and observed for the clearing zone around the colony which is an indicator of K-solubilization.

Siderophore production

All bacterial isolates were tested for the production of siderophore using specified chrome azurol-S agar medium (CAS blue agar) according to Schwyn and Neilands (1987) [34]. The CAS plates were prepared using 100 ml dark blue CAS mixture and nutrient agar medium (300 ml). It was autoclaved separately. A spot of 10 µl bacterial suspension containing ≈104 bacterial cells were inoculated in CAS plates and incubated at 30 °C for 7-10 days. Development of a deep yellow to orange colour after chelation of the bound iron, surrounding the colonies, was evaluated as siderophore production. The quantification of siderophores was done as explained earlier (Abirammi *et al*. 2018) [1].

Indole acetic acid

For qualitative IAA production, 10 µl bacterial suspension containing ≈104 bacterial cells was spotted on Luria agar plates supplemented with 50 µg mL-1 tryptophan. Upon drying of spots, a filter paper disc was placed in the plates. The plates were incubated at 30 °C for 24 h. The filter paper disc was removed and treated with Salkowski solution (2% solution of 0.5 M FeCl3 in 35% perchloric acid). Pink colour development on filter paper disc was taken as IAA production. For quantitative IAA estimation ccentrifuged culture supernatant was used using protocol of Bric *et al.* (1991) [6] and pellet for protein estimation (Lowry's method 1951) [25].

Bacterial mediated Indirect Plant Growth promoting mechanisms Hydrogen cyanide production

Hydrogen cyanide (HCN) production was determined by inoculating endophytic bacterial isolates in 5 ml nutrient broth containing 4.4 g L-1 glycine in 30 ml glass tubes. Soaked strip of sterilized filter paper in picric acid (0.5%) and sodium carbonate (2%) was placed in cotton plug sealed tubes having different bacterial isolates and incubated for 7-15 days at 30 °C. Colour change of filter paper from yellow to light brown or reddish brown was examined for HCN production (Bakker and Schippers 1987) [4].

Biocontrol activities against potential maize pathogens

Endophytic bacteria were evaluated for biocontrol activity using dual inoculation technique against against two maize pathogens, *Rhizoctonia solani (*root and stalk rot) and *Exserohilum turcicum (Turcicum* leaf blight) according to the method described by Sijam and Dikin (2005) [36]. After growing these test fungi on potato dextrose agar medium, its 3 mm disc was kept the center of each modified PDA plates (PDA:NA:1:1). Upon incubating for 6 h at 37 °C the same plates were inoculated with a spot of 10 µl bacterial suspension containing ≈104 bacterial cells, plates were re-incubated for 5-7 days at 30 °C. Plates inoculated with fungal disc alone were used as a control. The dual culture was performed in three replicates. The zone of inhibition by bacteria against fungal pathogen after sufficient incubation period was taken as positive indication of biocontrol activity.

Plant growth promotion assay

Selected 15 potential PGP endophytic bacterial isolates were tested for their effect on germination, shoot and root biomass of maize plant in a pot house experiment. Plastic pots of 6.5'' were filled with 2 kg of sterilized IARI farm soil. IARI farm soil is fine, loamy and noncalcareous and found to contain organic C 0.52%, total N 0.21%, available N 162 kg ha-1, available P 17.4 kg ha-1, available K 183 kg ha-1 and pH 7.2. Seeds of maize cultivar PC4, taken from Division of Genetics, IARI, were surface sterilized and soaked in the individual bacterial inoculum suspension in nutrient broth containing 108 cells ml-1 for 3 hrs. Control seeds were soaked in uninoculated nutrient broth. Bacterial primed and control seeds were sown in the pots and each treatment in triplicate were arranged in completely randomized design (CRD).

Germination was recorded at 10 days and sterile water was used for irrigating the pots to maintain near 60% water holding capacity (WHC) in soil. After germination three plants were maintained in each pot till harvest at 60 DAS. At harvest the plants were uprooted for measuring shoot and root. Samples were oven dried at 70 °C for 4 days for dry weight measurements.

Statistical analysis

The data was analyzed using MS-Excel data analysis software for Critical difference and standard errors. Principal Component analysis to correlate PGP traits of endophytic bacteria and plant biometric parameters on inoculation was done using trial version of XLSTAT.

Result and Discussion

Maize bacterial endophytes

The cultural bacterial endophytic population varied from 0.2× 103 to 1.63 × 104 cfu g-1 of plant tissue from different parts at different growth stages, with maximum in roots at flowering stage. Competent rhizobacteria first colonize on the root surface, forms micro-colonies and eventually enter inside the root systems either passively through cracks, wounds or actively by producing hydrolytic enzymes which breaks down plant cell wall component. Subsequently in plant growth cycle, the entered rhizobacteria multiply during vegetative and flowering stage with decline at maturity stage, partially due to factors like nutrient deficiency, water stress, free radical formation. Similar reports are there in crops maize, rice, cotton, potato (Hallmann *et al.* 1997; Sessitsch *et al*. 2004) [9, 35]. *Gluconacetobacter diazotrophicus* population decline with the advancement of age in sugarcane plant as it is linked with the physiological and metabolic changes occurring during sugarcane growth or plant defense system activation (Munoz-Rojas and Caballero Mellado 2003) [26]. Based on morphological characteristics 106 diverse isolates were purified of them 27 morphotypes were from vegetative, 49 from flowering and 30 from maturity stage across different tissues.

Plant growth promoting (PGP) traits of endophytes

All isolates were screened in vitro for direct and indirect PGP traits viz. Nitrogen fixation, solubilization of P, K, Zn, production of Indole Acetic Acid (IAA), siderophore, HCN and Biocontrol activity against maize pathogens

Nitrogen fixation (Acetylene reduction activity)

Amongst 106 isolates only three isolates were found to be nitrogen fixers as measured using acetylene reduction assay (ARA) (Table 1). The ARA activity ranged from 11 ±1.1 to 47.7±1.9 nanomoles of ethylene produced mg-1 protein hr-1 (Table 2). Ji *et al.* (2014) [15] described diazotrophic endophytic bacteria from the leaves, stems, and roots of 10 rice cultivars belonging to various genera *viz. Bacillus*, *Klebsiella*, *Microbacterium* and *Paenibacillus*. Suman *et al. (2005)* [37] reported 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).

Solubilization of nutrients

On screening all 106 endophytic isolates for solubilization of P, K and Zn, 19 isolates were found to be P solubilizer, 3 as K solubilizer and 30 as Zn solubilizer (Table 1). Phosphorus is the second most important macronutrient after nitrogen. But in environment most phosphorus is in insoluble form that cannot be utilized by plants and P solubilising endophytic bacteria is helpful for making it available. Joe *et al.* (2016) [16], reported endophytic bacteria *Acinetobacter* sp. and *Bacillus* sp. from *Phyllanthus amarus* as salt tolerant and phosphate solubilizer. Matos *et al.* (2017) [24] reported P solubilizing endophytic bacteria from banana trees *viz.*

Aneurinibacillus sp. and *Lysinibacillus* sp. and isolate EB. 78 (*Bacillus* sp.) showed P solubilization capacity in solid media when Ca3(PO4)2 and soy lecithin were used as P sources. In this study the bacteria could solubilize P ranging from 1.18±0.5 to 23.41±0.3 µg mg-1 protein, with maximum in ML-96 (Table 2).

Yuan *et al.* (2015) [45] reported 20 phosphorus- and potassium-solubilizing endophytic bacteria belonging to 14 species from 10 genera mainly consisting of *Alcaligenes* spp., *Enterobacter* spp. and *Bacillus* spp. from root, rhizome, stem and leaves of Moso Bamboo. Yaish *et al. (*2015) [44] reported K solubilizing potential of endophytic bacteria *Acinetobacter pittii*, *Achromobacter* sp, *Bacillus endophyticus* strain 2DT, from roots of date palm (*Phoenix dactylifera* L.).Ahmad *et al*. (2016) reported role of K solubilizing microbes in plant growth promotion. Yaish *et al.* (2015) [44] described zinc solubilizing potential of 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.*

Production of IAA, siderophore, HCN

Qualitative analysis indicated 15 isolates to be IAA producer, 27 as putative siderophore and 5 isolates as HCN producer (Table 1). On quantification 5 isolates produced IAA ranging from 69.41±1.1 to 190.03±1.4 µg mg-1 protein and 3 isolates produced hydroximate / catecholate siderophores (2.33±0.1 to 8.78±0.5 µg ml-1) (Table 2). Implication of microbial produced IAA has been well known in stimulation of plant growth. Hernández-Rodríguez *et al*. (2008) [13] reported *Pseudomonas fluorescens* potential of IAA production and increasing length in corn plants. Naveed *et al.* (2015) [29] reported the L-tryptophan–dependent biosynthesis of IAA by strain of *Burkholderia phytofirmans* PsJN and its role in improving plant growth and colonization. HCN plays role in biocontrol of several soil-borne pathogenic fungi (Ramette *et al*., 2003) [32]. Ngoma *et al.* (2013) [30] isolated 50 endophytic bacteria from the roots *Amaranthus hybridus*, *Solanum lycopersicum* and *Cucurbita maxima* and shown HCN production in six isolates *viz. Achromobacter xylosoxidans* KC010530, *A. xylosoxidans* KC010531, A. *xylosoxidans* KC010532, *Pseudomonas putida* KC010526, *P. putida* KC010527, and *P. putida* KC010528. Bacterial siderophores ability in enhancing iron availability *vis-à-vis* plant growth has been described by Kloepper *et al*. (1989) in root rhizosphere. Abirammi *et al*. (2018) [1] have shown the production of siderophores and their iron chelation efficiency from bacteria associated with different crops. Many reports have shown the ability of both gram positive group e.g. *Bacillus* and *Rhodococcus* and gram negative bacterial isolates *Pseudomonas* sp producing siderophores and their role in biocontrol activity and iron uptake (Gardner *et al*. 2004, Tian *et al.* 2009, Wilson *et al*. 2010) [8, 38, 43].

Biocontrol activities against potential maize pathogens

The *in vitro* screening of biocontrol activity against two maize pathogens, *Rhizoctonia solani* (root and stalk rot) and *Exserohilum turcicum* (*Turcicum* leaf blight), indicated 6 isolates possessed antagonistic activity against *Exserohilum turcicum* and one isolates against *Rhizoctonia solani* (Table 1). Some of these antagonistic cultures were producing siderophore and HCN which can be implicated in biocontrol activity among others. White *et al.* (2014) [42] have shown the role of vanilla orchid endophyte *B. amyloliquefaciens* in protection of plant seedlings from pathogens. Marag and Suman (2018) have shown that maize endophyte *Bacillus licheniformis* inhibited *Exerohilum turcicum* and *Bacillus amyloliquefaciens* inhibited both *Exerohilum turcicum* and *Rhizoctonia solani.* Many species of genera *Bacillus, Exiguobacterium, Micrococcus, Pseudomonas* and *Psychrobacter* have shown antagonistic activity against fungal pathogens *Fusarium graminerum, Rhizoctonia solani* and *Macrophomina phaseoli* as reported by Verma *et al*. (2015) [41]. Zhang *et al.* (2011) [46] reported the potential of *Bacillus subtilis* isolated from roots of banana plant, against fungal pathogen *Fusarium* wilt.

Table 1: Plant growth promoting activities of endophytic bacteria from PC-4

Tissue	Growth stages of isolation	Isolates	Solubilization			Production			Biocontrol	
			PO4	K	ZnO	IAA	Siderophore	HCN	*Exerohilum turcicum*	*Rhizoctonia solani*
Root	Vegetative	VR-2	-	-	-	-	-	+	-	+
		VR-5	-	-	-	-	+	-	-	-
		VR-6	+	-	-	-	-	-	-	-
	Flowering	FR-29	-	-	-	-	+	-	-	-
		FR-30	-	-	-	-	+	-	+	-
		FR-31	-	-	+	-	+	-	-	-
		FR -34	-	-	+	-	+	-	-	-
		FR -35	-	-	+	+	+	-	-	-
		FR -36	-	-	+	-	+	-	-	-
		FR -37	+	-	+	+	-	-	-	-
		FR -38	-	-	-	-	+	-	-	-
		FR -39	-	-	+	-	-	-	-	-
		FR -40	-	-	+	-	-	-	-	-
		FR -41	-	-	-	-	+	-	+	-
		FR -42	-	-	-	-	+	-	-	-
		FR -43	-	-	+	+	-	-	-	-
		FR -45	-	-	+	-	-	-	-	-
		FR -47	-	-	+	-	-	-	-	-
		FR -48	+	-	+	+	-	-	-	-
		FR -49	+	-	+	+	+	+	+	-
		FR -103	-	+	-	+	-	-	-	-
		FR -105	-	-	-	+	-	-	-	-
		FR -106	+	-	-	+	-	-	-	-

	Maturity	MR-75	-	-	-	-	+	-	-	-
		MR -77	-	-	+	-	-	-	-	-
		MR -79	+	-	-	-	-	-	-	-
Stem	Vegetative	VS-9	-	-	+	-	-	-	+	-
		VS-11	+	-	-	-	-	-	+	-
		VS-12	+	-	-	-	-	-	-	-
	Flowering	FS-50	-	-	+	-	+	-	-	-
	Maturity	FS-51	-	-	-	-	+	-	-	-
		FS-102	-	+	-	+	-	-	-	-
		MS-83	-	-	-	-	+	-	-	-
		MS-84	+	-	+	-	-	-	-	--
		MS-86	+	-	-	-	+	-	-	-
		MS-87	+	-	+	+	+	-	-	-
		MS-88	+	-	-	-	-	-	-	-
		MS-89	-	-	+	-	-	-	-	-
		MS-91	+	-	-	-	-	-	-	-
		MS-92	+	-	-	-	-	-	-	-
Leaf	Vegetative	VL-15	-	-	+	-	-	-	-	-
		VL-16	-	-	-	-	-	+	-	-
		VL-18	-	-	+	-	-	-	-	-
		VL-19	-	+	-	-	+	-	+	-
		VL-21	-	-	+	-	-	-	-	-
	Flowering	FL-59	-	-	+	+	+	+	-	-
		FL-60	+	-	+	-	-	-	-	-
		FL -62	-	-	-	-	+	-	-	-
		FL -65	-	-	-	-	+	-	-	-
		FL -66	-	-	-	-	+	-	-	-
		FL -67	-	-	-	-	-	+	-	-
		FL -68	-	-	-	-	+	-	-	-
		FL -69	+	-	+	+	+	-	-	-
		FL -71	-	-	-	-	+	-	-	-
		FL -104	-	-	-	+	-	-	-	-
	Maturity	ML-94	+	-	+	+	-	-	-	-
		ML-95	+	-	+	-	-	-	-	-
		ML-96	+	-	+	-	-	-	-	-
		ML -98	-	-	+	-	-	-	-	-
		ML-99	-	-	+	-	-	-	-	-
		ML-100	-	-	-	-	+	-	-	-
		ML-101	-	-	+	+	+	-	-	-

Table 2: Quantitative estimation of plant growth promoting traits of endophytic bacteria

Isolates	ARA (nmoles of ethylene mg-1 protein hr-1)	P- solubilized (µg mg-1 protein)	IAA (µg mg-1 protein)	Siderophore (µg ml-1)
VS11	-	22.90±1.6	-	-
VS12	-	22.35±0.3	-	-
FR 35	11±1.1	-	69.41±1.1	8.78±0.5
FR 43	27±1.6	-	93.27±1.1	-
FR 48	-	21.64±1.3	98.70±0.3	-
FL 69	47.7±1.9	5.12±1.1	190.03±1.4	6.45±0.3
MR 79	-	5.82±1.6	-	-
MS 84	-	7.56±1.8	-	-
MS 86	-	1.18±0.5	-	2.33±0.1
MS 88	-	11.84±1.6	-	-
MS 91	-	7.56±1.1	-	-
MS 92	-	6.97±1.6	-	-
ML 94	20±1.4	12.14±1.5	141.02±1.2	-
ML 95	-	19.84±0.4	-	-
ML 96	-	23.41±0.3	-	-

***In planta* evaluation of selected endophytic bacterial isolates**

To analyze the effect of endophytic bacterial isolates (VS11, VS12, FR35, FR43, FR48, FL69, MR79, MS84, MS86, MS88, MS91, MS92, ML94, ML95, ML96) on maize plant biomass, a pot study was conducted with variety PC4 at 50% NPK level of recommended fertilizer dose (120:60:60). Plant biomass produced at 60 days was assessed for dry weight of shoot and root. The isolates showed positive effect on germination of maize seeds compared to control seeds with germination percentage from 91 to 96% with maximum in FL69 isolate (Fig. 1). Shoot biomass increase was recorded from 1.9 to 4.2 gm plant−1compared to 1.7 gm plant−1incontrol. The isolate ML94 inoculation increased maximum shoot biomass followed by FL69 isolate. Similarly the root dry weight ranged from 0.5 to 1.98 gm plant−1compared to 0.43 gm plant−1 in control. The maximum increase was recorded in ML94 followed by FL69

and ER43 (Fig. 1). Naveed *et al.* (2014) [28] reported the positive effects of *Enterobacter* sp. strain FD17 on maize with regard to plant biomass, number of leaves plant−1, leaf area, and grain yield up to 39%, 14%, 20%, and 42%, respectively, in omparison to the un-inoculated control. López- Ortega *et al.* (2013) [21] reported positive effects of diazotrophic phosphate-solubilizing bacteria upon inoculation on maize in increasing plant biomass up to 39% and accumulation of phosphorus by 10%.

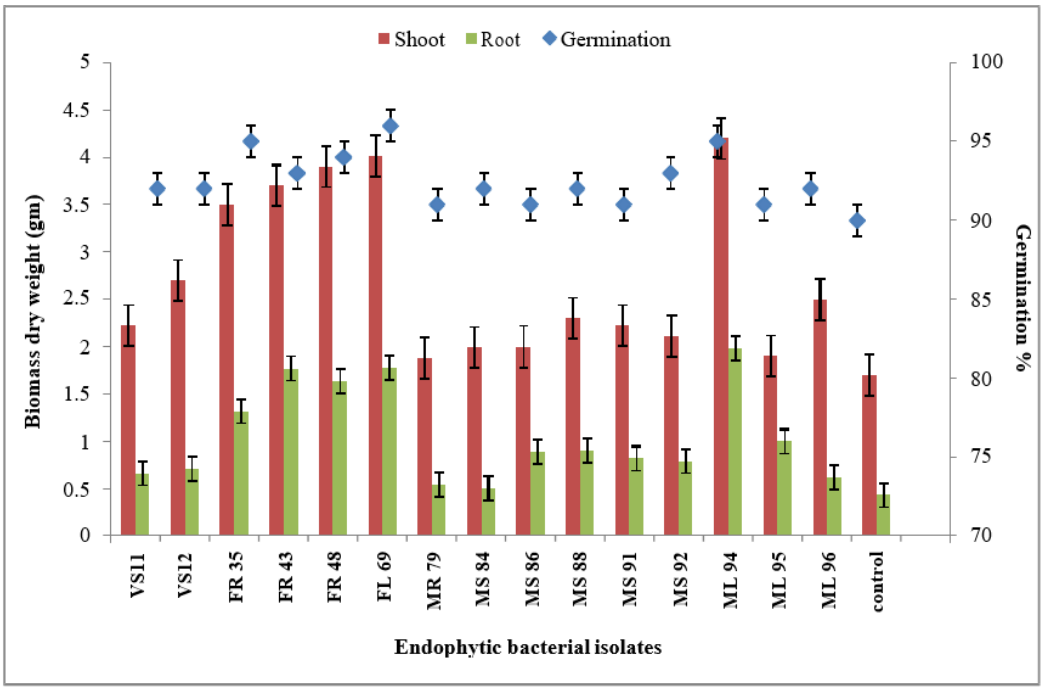

Fig 1: Effect of endophytic bacterial isolates on maize germination and plant biomass Principal Component Analysis

Principal Component Analysis was done to correlate the PGP traits of endophytic bacterial isolates with plant biometric parameters. PCA analysis extracted seven factors, of which based on eigen value five factors explained near 99% variability. Factor F1 explaining 65% variability was significantly correlated with five variables (IAA, ARA, Germination %, shoot and root biomass), whereas factor F2 explained phosphorus solubiliation and siderophore production. Twelve out of fifteen isolates explained the variability covered under factor F1, whereas factor F2 was explained by rest three isolates. Overall the isolates have been clustered in three groups, with most proficient isolates ML94, FL69 and FR43 in cluster I (Fig. 2).

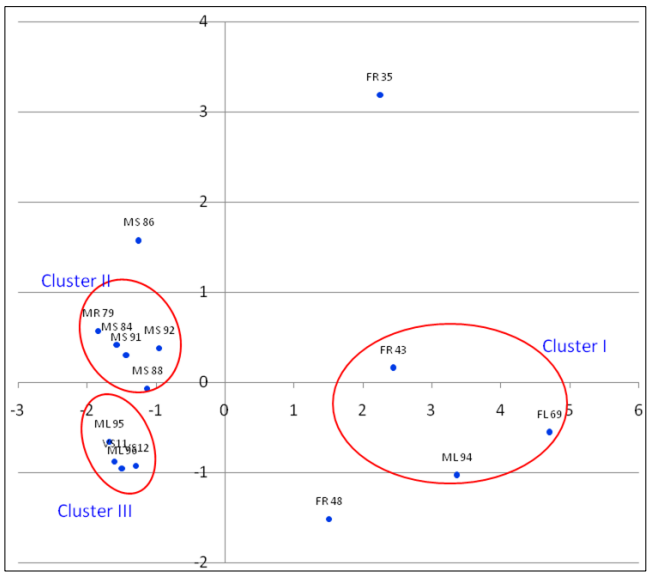

Fig 2: Principal Component analysis of maize endophytic bacterial isolates

Conclusion

Overall the present study provides information about variable load of diverse endophytic bacteria in maize plant with respect to crop duration and plant tissues. Flowering growth stage and root tissue of maize favored more colonization of bacterial endophytes. The beneficial traits possessed by these bacteria strengthen their probable role in seed germination and plant growth.

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