Soil Environ. 37(1): 35-44, 2018
DOI:10.25252/SE/18/872
Online ISSN: 2075-1141
Print ISSN: 2074-9546

Effect of bacterial consortia on growth and yield of maize grown in *Fusarium* infested soil

Naseem Akhtar[1], Muhammad Naveed[2], Muhammad Khalid[2], Nisar Ahmad[1], Muhammad Rizwan[3] and Saima Siddique[3]
[1] Biochemistry Section, Post-Harvest Research Center, AARI, Faisalabad
[2]Institute of Soil & Environmental Sciences, University of Agriculture, Faisalabad
[3]Applied Chemistry Research Centre, PCSIR laboratories Complex, Lahore

Abstract

Soil borne pathogens are responsible for considerable yield losses in field crops. Healthy growth and ultimate yield of the crop depends upon the efficient supply of water, nutrients and absence of biotic and abiotic stress. Under biotic stress plant growth promoting rhizobacteria (PGPR) and compost inhabiting bacteria (CIB) can help the plant to function normally by suppressing the pathogen. A pot experiment was conducted to determine the effect of PGPR and CIB on growth and yield of maize, grown in fungus infested soil. Two strains, each of PGPR (Mb4 and Mb7) and CIB (Cb4 and Cb9) were evaluated to improve the growth and yield of maize crop. Maize seeds were sterilized and inoculated with bacterial strains before sowing along with un-inoculated control for comparison. Recommended dose of fertilizers (180, 140, 90 NPK kg ha^{-1}) was applied at sowing and pots were arranged in completely randomized design. Results showed that inoculation with selected strains of bacteria, exhibited percent increase in yield of fresh cob (up to 52.69%) and dry cob (40.87%), cob length (51.42%), grain yield (up to 55.34%), 1000-grain weight (up to 37.27%), K contents in grains and straw (1.756 and 0.793, respectively), %N in grains and straw (up to 2.675 and 0.997%, respectively) and %P in grains and straw (up to 1.756 and 0.793%, respectively) compared to un-inoculated control. Keeping in view the higher yield parameters of inoculated treatments compared to un-inoculated control, it was concluded that inoculation of maize seeds with bacterial consortia suppressed the adverse effect of fungal pathogen and enhanced the growth and yield of maize crop.

Keywords: Bacterial consortia, PGPR, CIB**,** *Fusarium oxysporum,* maize

Introduction

Microbes are everywhere on the earth but the largest number of microbes reside in the rhizosphere (Carmen and Roberto, 2011). Among this microbial population, bacteria and fungi cover the highest proportion (Morgan *et al*., 2005). They compete with each other for food and space and differ from the microbes residing in the bulk soil (Chaparro *et al*., 2013). Rhizospheric bacteria are very important for proper development of plants under stressed conditions. They can be plant specific and their number can vary with age of plant, type of soil and environmental conditions (Hrynkiewicz *et al*., 2010; Bouffaud *et al*., 2012). Their effect on plants may be detrimental, beneficial or neutral (Katarzyna and Christel, 2011). Detrimental microbes may be major plant pathogens and/or minor parasites while favorable bacteria help plants to grow successfully (Bulgarelli *et al*., 2013).

Seedling blight in maize crops is due to soil born fungus *Fusarium oxysporum.* Different *Fusarium species* cause seedling blight, crown rot and scab of wheat and barley, stalk, cob and crown rot of maize (Khan *et al*., 2006). Seed infected with *Fusarium*, exhibited reduced germination, seedling emergence and caused post emergence death of wheat seedling and ear rot of maize (Charles *et al*., 2007).

Seed treatment with pesticide could be effective for control of pathogens but more severe problems may arise due to lethal residues of fungicides (Cardoso *et al*., 2010). The continuous use of pesticides is not only deteriorating the environment but also has increased the resistance in the pests (Gassmann *et al*., 2011). Extensive use of pesticide might cause soil and air pollution which might have adverse effects on the human, animals and plants health. The persistent nature of many pesticide/fungicides further aggravated the problem. Sometimes pesticides may activate into more harmful by-products that might cause more serious health problems due to their uptake by plant and ultimately by entering into the food chain (Wasim *et al*., 2009). Therefore, biocontrol may be the best alternative of chemical control for plant diseases (Killani *et al*., 2011) being economical and environment friendly (Lugtenberg and Kamilova, 2009).

*Email: nasimsajjad235@gmail.com

Beneficial bacteria (PGPR, CIB) colonize the plant root surface and increase nutrient uptake (Jing *et al*., 2007), release organic compounds (Shukla *et al*., 2011; Drogue *et al*., 2013) and suppress the diseases (Tarkka *et al*., 2008) resultantly improve the growth of plants. They produce antimicrobial metabolites and induce systemic resistance in plants (Falahian *et al*., 2007; Erdogan and Benlioglu, 2010). Plant growth promoting rhizobacteria help plants by modifying the chemical composition of root cell wall (El Zemrany *et al*., 2007) and by amplifying the lignin deposition in epidermal tissues of inoculated plants under diseased condition (Niranjan *et al*., 2012).

Inoculation of field crops with compatible strains showed better results than single inoculation (Figueiredo *et al*., 2010). Inoculation with PGPR and arbuscular mycorrhizal fungi enhanced the yield of sorghum (Kumar *et al*., 2012). Inoculation with a consortium of two PGPR and one mycorrhizal strain exhibited enhanced growth of root in maize crop (Walker *et al*., 2012). In the present study bacterial strains were characterized for siderophore and chitinase production, biofilm formation and antagonism assay and the efficient strains were used to evaluate the potential of bacterial consortia for yield and growth promotion of maize crop in fungus infested soil.

Materials and Methods

Isolation of bacteria

Bacteria were isolated using standard dilution-plating procedure as described by Pugliese *et al*. (2008). Ten gram of the sample (Compost/rhizosphere soil) was mixed with 90 mL of sterile distilled water in conical flask, shaken vigorously on a vortex mixer for 10 minutes and serially diluted up to 10^{-9}. An aliquot (0.1 mL) of these suspensions was spread on the plates of Luria-Bertani (LB) agar medium (Luria and Burrous, 1995). Inoculated plates were incubated at 26 ± 2º C for 48 h and were purified by repeated streaking on the same medium until pure colonies were established.

Cross check of pathogen

The pathogen (*Fusarium oxysporum*) was collected from Department of Plant Pathology, Ayub Agricultural Research Institute, Faisalabad, isolated by tissue segment method and identified by using colony morphology and microscopic features. Pathogenicity was tested on live plant by cut stem method (Singh *et al*., 1991). The mycelial suspension of the isolates was produced in broth medium of 250 mL in conical flasks. The mycelium of the isolate was filtered through the cheesecloth by gentle pressing to remove excess liquid and blending for 30 seconds in blender at the rate of 5 g of mycelium per liter of sterile deionized water. The resulting suspension was used as inoculum. The inoculum was freshly prepared before the applications. Three weeks old seedlings of maize, grown in sterilized potted soil were inoculated with the mycelial suspension of the fungal isolate. The pathogen was re-isolated from the infected plants and identified using colony morphology and microscopic features (Singh *et al*., 1991) and compared the characteristics with the initial isolates.

Characterizations of bacterial isolates

Seventy five strains of each PGPR and CIB were isolated from maize rhizosphere and compost, respectively. Twenty five strains were selected for preliminary tests (*in vitro* disease suppression assay), and two each of PGPR (Mb1 to Mb25) and CIB (Cb1 to Cb25) were selected for further evaluation under pot condition. The selected strains were further characterized for beneficial plant growth promoting traits following standard methods as mentioned in Bergey's Manual of Systematic Bacteriology (Krieg and Holt, 1984). Bacterial strains (Mb4, Mb7, Cb4 and Cb9) were characterized for siderophore production and chitinase activities, by following the protocols described by Clark and Bavoil (1994) and Chernin *et al*. (1998) respectively. Biofilm formation was carried out by following the procedure adopted by Peeters *et al*. (2008).

Antagonism assay

The target pathogen was inoculated on PDA at four equidistant peripheral points while compost inhabiting bacteria were inoculated at the center of Petri plates. In another set, the target pathogens were inoculated at the center and CIB at four equidistant peripheral points of Petri plates. Same was repeated for the PGPR in place of compost inhabiting bacteria. The experiment was replicated thrice and incubated at 28± 1 ºC. Inhibition of pathogens was observed after every 12 h. The means of the three measurements were recorded for each pathogen. Inhibition of pathogen (%) over control was calculated by using the following formula (Vincent, 1947),

$$\text{Percent Inhibition of mycelium (I)} = \frac{C-T}{C} \times 100$$

Where 'I' is percent inhibition of mycelium, 'C' is growth of mycelium in control and 'T' is growth of mycelium in inoculated treatment.

Preparation of inoculum and seed inoculation

Inocula of selected strains of PGPR (Mb4 and Mb7) and CIB (Cb4 and Cb9) were prepared separately in 250 mL conical flask having 100 mL LB broth. The inoculated culture was incubated at 28±1º C in the orbital shaking incubator at 100 rpm for 48 hours. An optical density of

0.5, recorded with an optical density meter at wavelength of 535 nm was achieved by dilution to maintain uniform cell density (10^8–10^9 CFU mL^{-1}) prior to seed inoculation. A consortium was prepared by mixing bacterial strains cultures in 1:1 ratio. The seeds of maize were inoculated by coating with peat-based slurry containing 2-days old inocula of respective strains and sugar solution (10%). Whereas the seeds for control were treated with peat containing sterilized broth and solution of sugar (Yadav *et al*., 2010). Inoculated seeds were air dried under shade before sowing. *Fusarium oxysporum* culture was prepared in 250 mL flak contain potato dextrose broth (PDB)

Pot experiment

Pot experiment was carried out in sandy clay loam soil having pH, 7.9; EC, 1.4 dS m^{-1}; nitrogen, 0.030%; available phosphorus, 7.2 mg kg^{-1} and extractable K 120 mg kg^{-1} at wire house of Soil Bacteriology Section, Agriculture Biotechnology Research Institute, AARI, Faisalabad. Each pot contained 12 kg soil. Recommended dose of NPK fertilizers (180, 140, 90 NPK kg ha^{-1}) was applied as urea, single super phosphate, and sulphate of potash). The whole P and K were supplemented as basal dose whereas N was added in two splits (Once after germination and then at tasseling stage). Equal volume of fungus (*Fusarium oxysporum*) inoculum (10^6 spores mL^{-1}) was added at the time of pot filling by mixing manually into soil. Surface disinfected maize seeds were inoculated by peat based inocula/slurry (consist of peat, sugar and bacterial culture, multiplied on LB broth media) while control was treated with slurry prepared with peat, sugar and LB broth without bacteria cells (un-inoculated). Seed of each treatment was sown at the depth of 3.81 cm. Each treatment was replicated thrice and arranged according to completely randomized design. Good quality canal water [EC = 0.7 dS m^{-1}, SAR = 0.5 (mmol L^{-1})1/2 and RSC = 0.05 mmolc L^{-1}] meeting the irrigation quality criteria for crops (Ayers and Westcot 1985) was used for irrigation. Data regarding disease suppression and growth promotion was collected during the growth period while yield data was collected at harvest.

Grains and straw samples were digested using tri-acid mixture (nitric acid, sulfuric acid and perchloric acid (9:2:1 (v/v)) and analyzed phosphorus and potassium following protocol given by Ryan *et al*. (2001). Soil and plant samples were digested with sulfuric acid and catalyst mixtures following Gunning and Hibbard's method and for the distillation of ammonia into nitrogen, 4% boric acid was utilized using macro Kjeldhal's apparatus (Jackson, 1967) while available phosphorus was determined by Olsen method (Olsen and Sommer, 1982). Extractable potassium was determined using flame photometer Jenway PFP-7.

Statistical analysis

Standard errors of means of the data were computed following Steel *et al*. (1997) using the statistical software STATISTIX v8.1 whilst means were compared by means of Duncan's Multiple Range Test (Duncan, 1955).

Table1: Characterization of PGPR and CIB isolates

Treatment	Siderophore production	Chitinase	Antagonism (I %)	Biofilm formation Absorbance	
				600 nm	595 nm
Mb4	++	++	84	4.095	2.971
Mb5	-	-	84	2.018	1.775
Mb6	++	-	82	2.508	1.556
Mb7	++	++	81	3.314	2.860
Cb1	-	++	86	2.341	1.29
Cb 4	++	++	86	3.210	2.78
Cb 8	-	++	88	2.117	1.56
Cb 9	++	++	86	4.081	2.54

Mb4= *Bacillus megaterium*, Mb7= *Pseudomonas aeruginosa*, Cb4= *Serratia* spp., Cb9= *Pseudomonas fluorescens*

Results

Characterization of PGPR and CIB

All the isolates (Mb4, Mb7, Cb4, and Cb9) were characterized for siderophore production, chitinase production, antagonistic effect against *Fusarium oxysporum* and biofilm formation (Table 1). Four strains i.e. Mb4, Mb7, Cb4, Cb9 were proficient siderophore producers and showed efficient chitinase activity. Percent antagonism by Mb4 and Mb7 ranged from 82 to 84% and it varied from 86 to 88% by Cb4 and Cb9. Biofilm formation at absorbance of 595 and 600 nm was observed and results showed that Mb4 (4.095, 2.971 nm), Mb7 (3.314, 2.860 nm), Cb4 (3.210, 2.78 nm) and Cb9 (4.081, 2.54 nm) were efficient for biofilm formation at both the wavelengths while Cb9 was the most effective strain for biofilm formation.

Effect of bacterial consortia on growth parameters of maize

Leaf size is related to photosynthesis of the plant, so health of the crop is affected by leaf area. Leaf length was positively affected by bacterial inoculation. Increase in leaf area was higher by single inoculation but combination of two and three strains of Mb and Cb gave better result while maximum leaf length was recorded with consortium of all the four strains (88.40 cm) compared to control (62.2 cm). All the inoculated treatments showed leaf width, significantly higher than control. Maximum leaf width was recorded with consortium of four (7.9 cm) followed by Mb4 + Cb4 + Cb9 (7.87 cm), Mb7 + Cb4 + Cb9 (7.7 cm) and Cb9 + Mb4 + Mb7 (7.5 cm) which were significantly higher than control (3.9 cm). Inoculated treatments gave

Table 2: Effect of consortium of bacterial strains on leaf length, leaf width and intermodal distance of maize grown in fungus infested soil

Treatment	Leaf length (cm)	Leaf width (cm)	Leaf area (cm^2)	Inter-nodal distance (cm)
Control	62.20 h	3.90 k	242.18 h	10.20 c
Mb4	78.50 efg	5.70 j	447.15 g	13.50 ab
Mb7	75.30 g	6.00 ij	452.00 g	13.40 abc
Cb4	78.03 fg	6.6 fgh	514.8 ef	12.20 bc
Cb9	79.90 ef	6.27 hi	529 def	12.90 abc
Mb4 +Cb4	80.90 def	7.13 cde	576.89 bc	12.90 abc
Mb4 +Cb9	82.00 cde	6.73 efg	552.27 cde	13.00 abc
Mb7 + Cb4	81.90 cde	6.63 fgh	543.67 c-f	12.50 abc
Mb7 + Cb9	81.7cdef	6.6 f gh	536 c-f	13.70 ab
Mb4 +Cb4 + Cb9	86.00 ab	7.87 ab	663.00 a	14.60 ab
Mb7 + Cb4 + Cb9	85.30 abc	7.70 ab	656.81 a	15.0 ab
Cb4 + Mb4 +Mb7	84.4b cd	7.43 bcd	594.26 b	13.70 ab
Cb9 + Mb4 +Mb7	85.50 bc	7.50 abc	675.45 a	14.40 ab
Consortium of all the four strains	88.40 a	7.90 a	676.82 a	15.50 a
LSD	3.8250	0.4347	41.736	3.2027

Means sharing the same letter are not significantly different from each other (data is the mean of three repeats)
Mb4= *Bacillus megaterium*, Mb7= *Pseudomonas aeruginosa*, Cb4= *Serratia* spp., Cb9= *Pseudomonas fluorescens*

Table 3: Effect of consortium of bacterial strains on stem diameter, plant height, root length and shoot dry weight of maize grown in fungus infested soil

Treatment	Stem diameter (mm)	Plant height (cm)	Root length (cm)	Shoot dry Weight (g pl^{-1})
Control	8.40 i	131.2 hi	65.00f	41.50 ef
Mb4	9.30 h	137.7 efg	69.00def	44.44 de
Mb7	9.50 gh	135.9 gh	70.33def	44.17de
Cb4	9.70 fg	136.7 fg	67.00 ef	45.51de
Cb9	9.90 f	137.4 fg	70.67 de	45.61de
Mb4 +Cb4	10.47 d	140.0 defg	71.34cbe	47.45cd
Mb4 +Cb9	10.50 d	142.5 cde	73.67 bcd	48.70 bcd
Mb7 + Cb4	10.30 de	137.3 fg	73.65bcd	48.23 bcd
Mb7 + Cb9	9.93 ef	140.7 def	76.67bc	49.00 bcd
Mb4 +Cb4 + Cb9	11.70 bc	143.7 cd	77.67b	53.22 ab
Mb7 + Cb4 + Cb9	12.40 b	147.4 abc	86.65a	54.74 a
Cb4 + Mb4 +Mb7	12.50 b	145.7 bc	85.33 a	54.80a
Cb9 + Mb4 +Mb7	12.27 b	150.7 a	83.55 a	54.83 a
Consortium of all the four strains	13.30 a	149.4ab	87.00 a	55.55a
LSD	0.3964	4.8463	5.6166	5.2626

Means sharing the same letter are not significantly different from each other (data is the mean of three repeats)
Mb4= *Bacillus megaterium*, Mb7= *Pseudomonas aeruginosa*, Cb4= *Serratia* spp., Cb9= *Pseudomonas fluorescens*

higher leaf surface area than un-inoculated control (242.18 cm^2). Inter-nodal distance, stem diameter and plant height was higher by inoculated treatments, however effect of single inoculation was less than the consortium of two and three strains while maximum increase was observed with consortium of all the four strains.

Root length is related to healthy growth of the plant because it anchors the plant and enables it to uptake required amount of water and nutrients. Root length was increased by inoculation with bacterial consortia compared to un-inoculated control. Longest root was observed by inoculation with consortium of all the four strains (87 cm), medium length was with consortium of Mb7 with Cb4 and Cb9 and smaller with single inoculation however all the inoculated treatments showed longer root than control (65 cm). Shoot dry weight was also higher by inoculation with bacterial consortium of all the four strains (55.55 g $plant^{-1}$) which was statistically at par with consortium of Cb9 with Mb4 and Mb7 and significantly better than single strain inoculation.

Effect of bacterial consortia on yield parameters of maize

Cob weight was affected by inoculation with bacterial consortia. Fresh cob weight was higher by inoculation with consortium of four strains (107.45 g cob^{-1}) which were statistically at par with consortium of Cb4 and Cb9 with Mb4 and Mb7 showing 52.69, 49.4 and 47.98% increase respectively over control. Eminent increase in cob weight was also observed by inoculation with consortium of two and three strains which were significantly higher than single treatments. Consortium of all the four strains showed 40.87% increase in cob weight followed by consortium of Cb9 with Mb4 and Mb7 which showed 39.78% increase over control. Percent increase in dry cob weight ranged from 39.78% by consortium of triple strains to 28.29% by consortium of two strains while single strain inoculation showed increase up to 22.06% (Cb9). Highest cob length was observed with consortium of four which showed 51.42% increase over control. Cob length ranged from 15 to 17.33 cm by consortium of three strains while cob length with consortium of two strains ranged from 13.34 to 14.67 cm and was significantly higher than control (12.33 cm).

Grain yield is the ultimate capitulate and of actual interest. Grain yield was higher by all the inoculated treatments than control. Significantly higher grain yield 55.77 g cob^{-1} was perceived by inoculation with consortium of all the four strains which showed 55.34% increase in grain yield while 37.27% increase in thousand grain weight over control. Other prominent values for thousand grain weight were 156, 153.5, and 153.34 g, with consortium of different combination of three strains which was significantly higher than control (120.44 g).

Effect of bacterial consortia on NPK contents of grain and straw of maize

Inoculation positively affected the chemical composition of maize, grown in fungus infested soil. Percent nitrogen in grain (2.675%), percent nitrogen of straw (0.997%), phosphorus percentage in grain and straw

Table 4: Effect of consortium of bacterial strains on yield parameters of maize

Treatment	Fresh cob yield (g cob^{-1})	Air dried cob yield (g cob^{-1})	Cob length (cm)	Grain yield (g cob^{-1})	1000 Grain weight (g)
Control	70.37 i	45.60 h	12.33b	35.90f	120.44g
Mb4	91.5 fg	53.45 fg	14.33ab	40.43ef	132.1de
Mb7	89.76fg	54.53efg	13.45b	42.33def	126.45ef
Cb4	81.00 h	50.31gh	13.33b	42.01def	126.6ef
Cb9	90.98 fg	55.66 d-g	13.77ab	40.37ef	126.33ef
Mb4 +Cb4	97.77cde	58.50 b-f	14.44ab	45.43cde	135.23d
Mb4 +Cb9	97.98 cde	58. 99 a-e	14.51ab	44.55def	132.34d
Mb7 + Cb4	94.79 ef	57.55 c-f	14.67ab	44.14def	141.1c
Mb7 + Cb9	97.28 de	55.34 d-g	13.34b	47.31 bcd	137.0 cd
Mb4 +Cb4 + Cb9	100.44 bcd	60.54a-d	15.00 ab	52.44 ab	150.7 b
Mb7 +Cb4 + Cb9	102.85abc	62.64abc	17.33ab	50.05bc	156.0 b
Cb4 + Mb4 +Mb7	105.13ab	62.54abc	16.54ab	50.44 bc	153.34 b
Cb9 + Mb4 +Mb7	104.14 ab	63.74 ab	16.67ab	51.41 ab	153.5 b
Consortium of all the four strains	107.45a	64.24 a	18.67a	55.77a	165.34a
LSD	5.2163	5.4738	5.1064	5.3289	5.5473

Means sharing the same letter are not significantly different from each other (data is the mean of three repeats)
Mb4= *Bacillus megaterium*, Mb7= *Pseudomonas aeruginosa*, Cb4= *Serratia* spp., Cb9= *Pseudomonas fluorescens*

(1.411 and 0.793% respectively) and maximum potassium in grain (1.756%) and straw (1.064%) (Table 6) was recorded with the treatments, inoculated with consortium of all the four strains (Mb4, Mb7, Cb4 and Cb9). Medium concentration of N, P and K was observed in treatments, inoculated with consortium of three and lower with single inoculation. All the inoculated treatments showed higher phosphorus and potassium by grain and straw of maize was also higher in treatments, inoculated with consortium of four strains of bacteria (Table 5).

Discussion

Seedling blight suppresses the growth by blocking the vascular tissues of the plant. Plant growth promoting

Table 5 Effect of consortium of bacterial strains on chemical composition of grain and straw of maize

Treatment	% N in grains	% N in straw	% P in grains	% P in straw	% K in grains	% K in straw
Control	1.69 j	0.677 k	0.937 k	0.483 m	1.27 l	0.822 m
Mb4	1.945 g	0.753 i	1.097 i	0.607 j	1.4497 j	0.932 k
Mb7	1.92 h	0.747 j	1.117 h	0.553 l	1.43 k	0.933 k
Cb4	1.887 i	0.767 h	1.067 j	0.58 k	1.44 jk	0.893 l
Cb9	1.897 i	0.753 i	1.127 gh	0.62 i	1.51 i	0.95 j
Mb4 + Cb4	1.997 f	0.797 f	1.203 e	0.673 f	1.63 e	1.007 h
Mb4 + Cb9	2.007 f	0.776 g	1.177 f	0.654 h	1.532 h	0.96 i
Mb7 + Cb4	2.11 d	0.77 h	1.134 g	0.687 e	1.563 g	1.017 g
Mb7 + Cb9	2.08 e	0.827 e	1.239 d	0.667 g	1.607 f	1.027 f
Mb4 + Cb4 + Cb9	2.12 cd	0.843 d	1.297 c	0.788 b	1.67 d	1.059 b
Mb7 + Cb4 + Cb9	2.565 b	0.987 b	1.354 b	0.777 c	1.734 b	1.034 e
Cb4 + Mb4 + Mb7	2.107 d	0.847 d	1.23 d	0.766 d	1.673 d	1.043 d
Cb9 + Mb4 + Mb7	2.147 c	0.877 c	1.287c	0.767 d	1.72 c	1.054 c
consortium of all the four strains	2.675 a	0.997 a	1.411a	0.793 a	1.756 a	1.064 a
LSD	0.0204	4.856E 03	0.0141	4.580E 03	0.014	3.948 E03

Means sharing the same letter are not significantly different from each other (data is the mean of three repeats)
Mb4= *Bacillus megaterium*, Mb7= *Pseudomonas aeruginosa*, Cb4= *Serratia* spp., Cb9= *Pseudomonas fluorescens*

Table 6: Effect of consortium of bacterial strains on uptake of NPK by grains and straw of maize grown in pots

Treatment	N uptake grain (mg pot^{-1})	N uptake straw (mg pot^{-1})	P uptake grain (mg pot^{-1})	P uptake straw (mg pot^{-1})	K uptake grain (mg pot^{-1})	K uptake straw (mg pot^{-1})
Control	606.71	280.955	336.383	200.445	455.93	341.13
Mb4	786.3635	334.6332	443.5171	269.7508	586.11371	414.1808
Mb7	812.736	329.9499	472.8261	244.2601	605.319	412.1061
Cb4	792.7287	349.0617	448.2467	263.958	604.944	406.4043
Cb9	765.8189	343.4433	454.9699	282.782	609.587	435.5755
Mb4+Cb4	907.2371	378.1765	546.5229	319.3385	740.509	477.8215
Mb4+Cb9	894.1185	377.912	524.3535	318.498	682.506	467.52
Mb7+Cb4	931.354	371.371	500.5476	331.3401	689.9082	490.4991
Mb7+Cb9	984.048	405.23	586.1709	326.83	760.2717	503.23
Mb4+Cb4+Cb9	1111.728	448.6446	680.1468	419.3736	875.748	563.5998
Mb7+Cb4+Cb9	1283.783	540.2838	677.677	425.3298	867.867	566.0116
Cb4+Mb4+Mb7	1062.771	464.156	620.412	419.768	843.8612	571.564
Cb9+Mb4+Mb7	1103.773	480.8591	661.6467	420.5461	884.252	577.9082
Consortium of all the four strains	1491.848	553.8335	786.9147	440.5115	979.3212	591.052

Mb4= *Bacillus megaterium*, Mb7= *Pseudomonas aeruginosa*, Cb4= *Serratia* spp., Cb9= *Pseudomonas fluorescen*

percentage of NPK than control. Uptake of nitrogen, rhizobacteria and CIB compete with pathogen to colonize

the root, thus restrain the pathogen and help plant to grow successfully in fungus infested soil. Fungal pathogen stunted the growth and sometime fully destroyed the developmental activities of plant. Inoculation with selected strains of PGPR and CIB help plant to grow, survive and positively influence the ultimate yield of maize in infested soil. Healthy growth of the crop is responsible for better yield. Biotic stress stunted the growth which leads to the poor yield of the crop.

Under biotic stress, different mechanisms were adopted by bacteria to promote growth of the plant. They provide relief to the plant by improving nutrition (Peralta *et al*., 2013), producing requisite hormone to suppress the pathogens (Saharan and Nehra, 2011) and by releasing siderophore (Arruda *et al.,* 2013). Siderophore production by bacteria is very important with respect to pathogen control. It chelates the micronutrients, necessary for growth and pathogenicity of the pathogens and creates famine of these nutrients for pathogens to kill them. It was also endorsed by other scientists who observed that siderophore producers support the plant against disease attack (Wani *et al*., 2007) and enable to bear biotic and abiotic stress (Usha *et al*., 2011).

Another way to control pathogen is the ability to produce antifungal enzymes. Therefore chitinase producing bacterial strains have better antifungal activities. Our selected strains were good chitinase producers and it was observed that better chitinase and siderophore producing strains helped plants to survive and produced higher biomass in fungus infested soil. These strains antagonized the pathogens up to 86% which was due to production of antifungal metabolites. It was observed previously that PGPR act as defensive mediators against soil-borne plant pathogens (Lowe *et al*., 2012) due to the production of antifungal enzymes (Laslo *et al*., 2012). Compost inhabiting bacteria, particularly fluorescent pseudomonads and certain *Bacillus* spp effectively controlled the root diseases (fungal and bacterial) of agricultural crops (Ahmadzadeh *et al*., 2004).

In fungus infested soil, consortium of bacterial strains helps plant to function normally by contending the pathogen. They compete with pathogen for food and space by root colonization and by producing various antibiotic compounds. They have multiple functions in the rhizosphere and approaching towards plant growth promotion. Suppression of pathogen through competition for food and space enables the bacteria to support the better growth and yield of the crop in fungus infested soil. Root colonization is important criteria to suppress the pathogen and biofilm is the measure of root colonizing ability of microbes. It is the biological response to compete for root niches and nutrients and inducing systemic resistance in host plants (Haas and Défago, 2005). In the current study, selected bacterial strains showed higher values for biofilm formation. Biofilm formation is important for plant microbe interaction to control the pathogen (Timmusk, 2005). It is a bio barrier on the roots against pathogens (Morikawa *et al*., 2006). Root length significantly increased by inoculation with consortium of beneficial bacteria. Our study exhibited that seed inoculation with consortia resulted in developed and improved root architecture due to which uptake of nutrients (N, P, and K) is enhanced. The increased uptake of nitrogen, phosphorus and potassium was due to solubilization of nutrients (Ranjan *et al*., 2013), production of siderophore (Sayyed *et al*., 2010) and well developed root system (Karnwal, 2009) due to inoculation with consortium of bacteria strains (Figueiredo *et al*., 2010).

Results also showed the increase in root and shoot biomass by inoculation with bacterial consortia. PGPR has the capability to promote plant growth and productivity through synthesizing phytohormone, increasing the nutrient availability, antagonizing phytopathogens and decreasing metal toxicity (Burd *et al*., 2000). The improving effect of seed inoculation with bacterial consortia on shoot dry weight and yield of maize was reported by Shaharoona *et al*. (2006). Such an enhancement might be due to the ability of bacteria to fix nitrogen, solubilize the phosphate and production of growth regulators (Salantur at el., 2006). Consortium inoculation increased the leaf area responsible for efficient photosynthesis and finally the yield of the crop. It is due to well-developed root system, efficient root colonization and resultantly the uptake of water for photosynthesis. Elkoca *et al*. (2008) determined that dry matter, grain yield and phosphorus uptake was significantly higher by co-inoculation than single inoculated treatments in legumes. Other scientists also observed the increase in plant height and leaf area by inoculation with bacterial consortia (Govindappa *et al.,* 2011). Increased growth was also observed in wheat (Salantur *et al*., 2006), tomato (Gravel *et al*., 2007), chickpea (Verma *et al*., 2010), fodder galega (Egamberdieva *et al*., 2010) and maize (Pacôme *et al*., 2013) due to co-inoculation. Under diseased condition it may be due to the production of siderophore, antifungal compounds, auxin biosynthesis and efficient root colonization. Biocontrol strategy depends on the nutrient management, better root colonization (biofilm formation) and production of siderophore and chitinase. Present study exhibited that selected strains of bacteria (Mb4, Mb7, Cb4 and Cb9) possessed these characteristics, therefore had suppressive effect on the pathogen which cause seedling blight in maize. Their combined activity showed higher efficiency for growth promotion in fungus infested soil than

the single inoculation but consortium of all the four strains showed the best in results this contest.

Conclusion

Beneficial bacterial population played important role in growth and yield promotion through different mechanisms. They suppressed the pathogen through competition for food and space on root, production of antifungal metabolites and by inducing systemic resistance in plant. They promoted growth through solubilizing the nutrients and making it available to plant. However their efficiency may vary under different conditions. The selected bacterial strains suppressed the pathogen because they were screened against the *Fusarium oxysporum* which caused seedling blight in maize and their efficiency may vary with change in causal organism or disease. Their effectiveness in other crops may also be different. To avoid the harmful effect of pesticide the bacterial consortia may be extended in other crops under stressful condition. The selected strains may be tested under abiotic stresses because the characteristics described above, enabled them to promote growth under stressful condition. Nevertheless the given consortium of bacterial strains can effectively be used to increase the growth of maize in *Fusarium* infested soil.

References

Ahmadzadeh, M., A.S. Tehrani and K.T. Jahromi. 2004. Study on production of some antimicrobial metabolites by fluorescent pseudomonads. *Iranian Journal of Agricultural Sciences* 35(3): 731-739.

Arruda, L., A. Beneduzi, A. Martins, B. Lisboa, C. Lopes, F. Bertolo, L. Maria, P. Passaglia, L.K. Vargas. 2013. Screening of rhizobacteria isolated from maize (*Zea mays L.*) in Rio Grande do Sul State (South Brazil) and analysis of their potential to improve plant growth. *Applied Soil Ecology* 63: 15– 22.

Ayers, R.S. and D.W. Westcot. 1985. Water quality for agriculture. FAO irrigation and drainage papers 29 (rev. 1). FAO, Rome.

Bouffaud, M.L., M. Kyselkova, B. Gouesnard, G. Grundmann, D. Muller and Y. Moënne Loccoz. 2012. Is diversification history of maize influencing selection of soil bacteria by roots. *Molecular Ecology* 21: 195–206.

Bulgarelli, D., K. Schlaeppi, S. Spaepen, E. VerLorenvan Themaat and P. Schulze-Lefert. 2013. Structure and functions of the bacterial microbiota of plants. *Annual Review of Plant Biology* 64: 807–838.

Burd, G.I., D. G. Dixon and B. R. Glick. 2000. Plant growth promoting rhizobacteria that decrease heavy metal toxicity in plants. *Canadian Journal of Microbiology* 33(3): 237-245.

Cardoso, R.A., L.T.A. Pires, T.D. Zucchi, F.D. Zucchi and T.M.A.D. Zucchi. 2010. Mitotic crossing-over induced by two commercial herbicides in diploid strains of the fungus *Aspergillus nidulans*. *Genetic and Molecular Research* 9: 231-238.

Carmen, B. and D. Roberto. 2011. Soil Bacteria support and protect plants against abiotic stresses, abiotic stress in plants - Mechanisms and Adaptations, Shanker (Ed.), ISBN: 978-953-307-394-1.

Chaparro, J.M., D. V. Badri, M.G. Bakker, A. Sugiyama, D.K. Manter and J.M. Vivanco. 2013. Root exudation of phytochemicals in *Arabidopsis* follows specific patterns that are developmentally programmed and correlate with soil microbial functions. PLoS ONE 8: e55731.

Charles, W. Bacon and dorothy M. Hinton. 2007. Potential for control of seedling blight of wheat caused by *Fusarium graminearum* and related species using the bacterial endophyte *Bacillus mojavensis*. *Biocontrol Science and Technology* 17(1/2): 81-94.

Chernin, L.S., M.K. Winson, J.M. Thompson, S. Haran, B.W. Bycroft, I. Chet, P. Williams and G. S. A. B. Stewart. 1998. Chitinolytic activity in *Chromobacterium violaceum*: substrate analysis and regulation by Quorum sensing. *Journal of Bacteriology* 180: 4435-4441.

Clark, V.L. and P.M. Bavoil. 1994. Methods in Enzymology 235(A), Academic Press, London, pp. 315-372.

Drogue, B., E. Combes-Meynet, Y. Moënne-Loccoz, F. Wisniewski-Dyé and C. Prigent-Combaret. 2013. Control of the cooperation between plant growth-promoting rhizobacteria and crops by rhizosphere signals in Vol.1and 2, Molecul. *Microbial Ecology and Rhizosphere*. F.J.de Bruijn (ed) & Sons, Inc.). 281–294.

Duncan, D.B. 1955. Multiple range and multiple F-test. Biometrics. 11: 1-42.

Egamberdieva, D., G. Berg, K. Lindstrom and L.A. Rasanen. 2010. Co-inoculation of *Pseudomonas spp*. with Rhizobium improves growth and symbiotic performance of fodder galega (*Galega orientalis* L.). *European Journal of Soil Biology* 46: 269-272.

El Zemrany, H., S. Czarnes, P.D. Hallett, S. Alamercery, R. Bally and L. Jocteur Monrozier. 2007. Early changes in root characteristics of maize (*Zea mays*) following seed inoculation with the PGPR *Azospirillum lipoferum* CRT1. *Plant Soil* 291: 109–118.

Elkoca, E., F. Kantar and F. Sahin. 2008. Influence of nitrogen fixing and phosphate solubilizing bacteria on nodulation, plant growth and yield of chickpea. *Journal of Plant Nutrition* 33: 157-171.

Erdogan, O. and K. Benlioglu. 2010. Biological control of *Verticillium* wilts on cotton by the use of fluorescent *Pseudomonas* spp. under field conditions. *Biological Control* 53: 39–45.

Falahian, F., Z. Oraghi Ardebili and H. Fahimi. 2007. Effect of mycorrhizal fungi on some defense enzymes against *Gaeumannomyces graminis* in Wheat. *Pakistan Journal of Biological Sciences* 10(14): 2257-2266.

Figueiredo, M.V.B., L. Seldin, F.F. de Araujo and R. L. R. Mariano. 2010. Plant growth promoting rhizobacteria: fundamentals and applications. Plant Growth and Health Promoting Bacteria, D.K. Maheshwari (ed.). DOI 10.1007/978-3-642-13612-2_2.

Gassmann, A.J., J. L. Petzold-Maxwell, R. S. Keweshan, M. W. Dunbar. 2011. Field evolved resistance to Bt maize by western corn rootworm. PLoS ONE 6: e22629.

Govindappa, M., V. Ravishankar Rai and S. Lokesh. 2011. Screening of *Pseudomonas fluorescens* isolates for biological control of *Macrophomina phaseolina* root rot of safflower. *African Journal of Agriculture Research* 6(29): 6256-6266.

Gravel, V., H. Antoun and R. J. Tweddell. 2007. Growth stimulation and fruit yield improvement of greenhouse tomato plants by inoculation with *Pseudomonas putida* or *Trichoderma atroviride*: Possible role of indole acetic acid (IAA). *Soil Biology and Biochemistry* 39(8): 1968-1977.

Haas, D. and G. Defago. 2005. Biological control of soil borne pathogens by fluorescent pseudomonads. *Nature Reviews Microbiology* 3: 307-319.

Hrynkiewicz, K., C. Baum and P. Leinweber. 2010. Density, metabolic activity and identity of cultivable rhizosphere bacteria on *Salix viminalis* in disturbed, arable and landfill soils. *Journal of Plant Nutrition and Soil Science* 173:747–756.

Jackson, M.L. 1967. Soil Chemical Analysis. Prentice-Hall, New Delhi.

Jing, Y.D., Z.L. He and X.E. Yang. 2007. Role of soil rhizobacteria in phytoremediation of heavy metal contaminated soils. *Journal of Zhejiang University Science* 8(3): 192–207.

Karnwal, A. 2009. Production of indol acetic acid by fluorescent pseudomonas in the presence of L-tryptophan and rice root exudates. *Journal of Plant Pathology* 91 (1): 61-63.

Katarzyna, H. and B. Christel. 2011. The Potential of Rhizosphere Microorganisms to Promote the Plant Growth in Disturbed Soils. Environmental Protection Strategies for Sustainable Development, pp.35-64.

Khan, M. R., S. Fischer, D. Egan and F. M. Doohan. 2006. Biological control of *Fusarium* seedling blight disease of wheat and barley. *Phytopathology* 96: 386-394.

Killani, A. S., R. C. Abaidoo, A. K. Akintokun and M. A. Abiala. 2011. Antagonistic effect of indigenous *Bacillus subtilis* on root/soil-borne fungal pathogens of cowpea. *Science publications* 3(3): 11-18.

Krieg, N.R. and J.G. Holt. 1984. Bergey's Manual of Systematic Bacteriology. Vol. 1. Williams and Wilkins, Baltimore, MD, USA.Pp. 694.

Kumar, V., M.V. Sarma, K. Saharan, R. Srivastava, L. Kumar and V. Sahai *et al*., 2012. Effect of formulated root endophytic fungus *Piriformospora indica* and plant growth promoting rhizobacteria fluorescent pseudomonads R62 and R81 on *Vigna mungo*. *World Journal of Microbiology and Biotechnology* 28: 595–603.

Laslo, E., E. Gyorgy, G. Mara, E. Tamas, B. Abraham and S. Lanyi. 2012. Screening of plant growth promoting rhizobacteria as potential microbial inoculants. *Crop Protection* 40: 43-48.

Lowe, A., S. Raffery-McArdle and A.C. Cassells. 2012. Effects of AMF and PGPR root inoculation and a foliar chitosan spray in single and combined treatments on powdery mildew disease in strawberry. *Agriculture and Food Science* 21: 28–38.

Lugtenberg, B. and F. Kamilova. 2009. Plant-Growth-Promoting Rhizobacteria. *Annual Review of Microbiology* 63:541–56

Luria, S.E. and J.W. Burrous. 1995. Hybridization between Escherichia coli and Shigella. *Journal of Bacteriology* 74: 461-476.

Morgan, J.A.W., G.D. Bending and P.J. White. 2005. Biological costs and benefits to plant–microbe interactions in the rhizosphere. *Journal of Experimental Botany* 56: 1729–1739.

Morikawa, M., S. Kagihiro, M. Haruki, K. Takano, S. Branda, R. Kolter and S. Kanaya. 2006. Biofilm formation by a *Bacillus subtilis* strain that produces g-polyglutamate. *Microbiology* 152: 2801-2807.

Niranjan, R. S., S.N. Lavanya, K.N. Amruthesh, S.R. Niranjana, M.S. Reddy and H.S. Shetty. 2012. Histochemical changes induced by PGPR during induction of resistance in pearl millet against downy mildew disease. *Biological Control* 60: 90–102.

Olsen, S.R. and L.E. Sommers. 1982. Phosphorus. In: Method of Soil Analysis, Chemical and Microbiological Properties. *American Society of Agronomy* Madison. WI, USA. Part 2. (2): 403-430.

Pacôme, A., Noumavo1, Eméric Kochoni, O. Yédéou, Didagbé, Adolphe Adjanohoun, Marcellin Allagbé, Rachidatou Sikirou, W. Emma, Gachomo, Simeon O. Kotchoni, Lamine Baba-Moussa. 2013. Effect of different plant growth promoting rhizobacteria on maize seed germination and seedling development. *American Journal of Plant Sciences* 4: 1013-1021.

Peeters, E., H.J. Nelis and T. Coenye. 2008. Comparison of multiple methods for quantification of microbial biofilms grown in microtiter plates. *Journal of Microbiological Methods* 72: 157–165.

Peralta, K.D., T. Araya, S. Valenzuela, K. Sossa, M. Martinez, H. Pena-Cortes and E. Sanfuentes. 2013. Production of phytohormones, siderophores and population fluctuation of two root-promoting rhizobacteria in Eucalyptus globulus cuttings. *World Journal of Microbiology and Biotechnology* 28: 2003-2014.

Pugliese, M., B.P.Liu, M.L. Gullino and A. Garibaldi. 2008. Selection of antagonists from compost to control soil borne pathogens. *Journal of Plant Diseases and Plant Protection* 115: 220-228.

Ranjan, A., M. Rajan, Mahalakshmi and M. Sridevi. 2013. Isolation and characterization of phosphate-solubilizing bacterial species from different crop fields of Salem, Tamil Nadu, India. *International Journal of Nutrition, Pharmacology, Neurological Diseases* 3(1): 29-33.

Ryan, J., G. Estefan and A. Rashid. 2001. Soil and Plant Analysis Laboratory Manual. Jointly published by the *International Centre of Agricultural Research in Dry Areas* (ICARDA), Aleppo, Syria and National Agricultural Research Centre (NARC), Islamabad.

Saharan, B.S. and V. Nehra. 2011. Plant growth promoting rhizobacteria: a critical review. *Life Science and Medical Research* 21:1–30.

Salantur, A., A. Ozturk and S. Akten. 2006. Growth and yield response of spring wheat (*Triticum aestivum* L.) to inoculation with rhizobacteria. *Plant and Soil Environment* 52(3): 111–118.

Sayyed, R.Z., N.S. Gangurde, P.R. Patel, S.A. Joshi and S.B. Chincholkar. 2010. Siderophore production by Alcaligenes faecalis and its application for growth promotion in *Arachis hypogaea*. *Indian Journal of Biotechnology* 9: 302-307.

Shaharoona, B., M. Arshad., Z.A. Zahir and A. Khalid. 2006. Performance of *Pseudomonas spp.* containing ACC-deaminase for improving growth and yield of maize (*Zea mays* L.) in the presence of nitrogenous fertilizer. *Soil Biology and Biochemistry* 38: 2971–2975.

Shukla, K.P., S. Sharma, N.K. Singh, V. Singh, K. Tiwari and S. Singh. 2011. Nature and role of root exudates: efficacy in bioremediation. *African Journal of Biotechnology* 10: 9717–9724.

Singh, K., J. C. Frisvad, U. Thrane and S.B. Mathur. 1991. An illustrated manual on identification of some seed-borne *Aspergilli, Fusaria, Penicillia* and their mycotoxins. Danish Government Institute of Seed Pathology, Copenhagen, Denmark.

Steel, R.G.D., J.H. Torrie and D.A. Dicky. 1997. Principles and Procedures of Statistics-A Biometrical Approach (3rd Ed.) McGraw-Hill Book International Co., Singapore.

Tarkka, M., S. Schrey and R. Hampp. 2008. Plant associated micro-organisms. In: Nautiyal CS, Dion P (eds) Molecular mechanisms of plant and microbe coexistence. Springer, New York, pp. 3–51.

Timmusk, S., N. Grantcharova and E.G.H. Wagner. 2005. *Paenibacillus polymyxa* invades plant roots and forms biofilm. *Applied and Environmental Microbiology* 71: 7292–7300.

Usha, R.M., Arundhathi and G. Reddy. 2011. *Bacillus cereus* and *Enterobacter cancerogenus* screened for their efficient plant growth promoting traits rhizobacteria (PGPR) and antagonistic traits among sixteen bacterial isolates from rhizospheric soils of pigeon pea. *African Journal of Microbiology Research* 5(15): 2090-2094.

Verma, J., J. Yadav and K.N. Tiwari. 2010. Impact of plant growth promoting rhizobacteria on crop production. *International Journal of Agriculture Research* 11: 954-983.

Vincent, J.M. 1947. Distortion of fungal hyphae in the presence of certain inhibitors.*Nature*150:850.

Walker, V., O. Couillerot, A.Von Felten, F. Bellvert, J. Jansa, M. Maurhofer, *et al*., 2012. Variation of secondary metabolite levels in maize seedling roots induced by inoculation with *Azospirillum*, *Pseudomonas* and *Glomus* consortium under field conditions. *Plant Soil* 356: 151–163.

Wani, P.A., M.S. Khan and A. Zaidi. 2007. Effect of metal tolerant plant growth promoting *Bradyrhizobium* sp. (vigna) on growth, symbiosis, seed yield and metal uptake by green gram plants. *Chemosphere* 70: 36-45.

Wasim, A., D. Sengupta and A. Chowdhury. 2009. Impact of pesticides use in agriculture: Their benefits and hazards. *The Journal of Interdisciplinary Toxicology* 2(1): 1–12.

Yadav, J., J.P. Verma and K.N. Tiwari. 2010. Effect of plant growth promoting rhizobacteria on seed germination and plant growth chickpea (*Cicer arietinum* L.) under *in Vitro* Conditions, *Biological Forum-Annual of International Journal*. 2(2):15-18.