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POTENTIAL OF SOME NATIVE *BACILLUS* STRAINS TO PROMOTE GROWTH OF TOMATO

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ABSTRACT

Plant growth promoting bacteria are soil inhabiting around or on the root surface and are involved in plant growth promotion and development *via* direct or indirect mechanisms. Some native strains of *Bacillus* were evaluated for their ability to promote growth of tomato. Different *in vitro* biochemical assays indicated capabilities of these bacterial agents for producing Indole acetic acid, siderophores and phosphate solubilization. Pot experiments indicated ability of *B. megaterium* FCBP520 strain to induce maximum significant increase in growth parameters like root length, shoot length and total biomass in tomato plants. *B. subtillis* FCBP170 was second best performer to induce growth promotion in tomato plants. Calorimetric assays were performed to quantify changes in plant growth related biochemicals. Both these strains induced plants for significantly higher production of total chlorophyll, anthocyanin and carotenoid contents. Overall this study presents the potential of *B.megaterium* FCBP520 and *B.subtillis* FCBP 170 to promote growth of tomato and the mechanism behind growth promotion with respect to both bacterial inducer and tomato plants. Our results may be the first to demonstrate plant growth promotion by these two bacterial strains during vegetation growth of tomato. This study will also be functionally relevant for future studies directed towards better tomato growth and ultimately better yields in agriculture system of Pakistan.

Key world: Plant Growth Promoting Bacteria (PGPB), Tomato, *Bacillus*, Indole acetic acid, Siderophores,

INTRODUCTION

The accelerated pace of increase in world population and continuously increasing environmental damage are alarming us that it will be a significant challenge to feed all of the world's people in the near future. Therefore, agricultural productivity needs to be augmented three to four times than the present situation but it needs to be in a sustainable and environmentally friendly manner. Bacteria are mainly known as pathogens but they can also exert beneficial effects on growth and development of plants. Many growth promoting bacteria have been commercialized in for use in agriculture (Bashan,1998). One important mechanism underlying growth promotion by bacteria is availability of nutrition to

plants (Bashan & Levanony, 1991). Bacteria promote growth by production of plants hormones and by facilitating nutrients uptake *via* different pathway like nitrogen fixation, phosphorus solubilization and siderophores production (Benizri *et al.*, 2001; Baudoin *et al.*, 2002; Berg *et al.*, 2002). Recent studies clues about some volatile compounds production by bacteria that promote plants growth and induce resistance in plants while chemicals fertilizers comprise low use efficacy and a small portion is up taken by plants (Bertrand *et al.*, 2000).

Bacterial organisms provide with plants with increased nutrients availability. Some previous researchers have approved non-legume nitrogen fixation ability of microbes (Bowen & Rovira, 1999;Bloemberg *et al.*, 2000; Bloemberg & Lugtenberg, 2001). Inoculations of these microbes also enhance phosphate solubilization and aid in plats to uptake phosphorous by production of organic acids (Cattelan *et al.*, 1999).

Roots are the main uptake sites of nutrients in plants (Ciccillo *et al.*, 2002). Healthy root growth ensures increased plant growth. Many rhizospheric bacteria colonize plants roots and promote root growth by producing phytohormones (Cook,2002). Bacteria with these characteristics have been identified in many genera mostly in *Bacillus* (Date,2001). Bacteria belonging to this genera form endospores to ensure enhanced life and stability in different formulations. Researchers have approved practical ability of these bacterial microbes in the field experiments. These microbes provided increased plant vigor, disease protection and high yield (**Fravel *et al.*, 1999**). This study was aimed on examining potential of native *Bacillus* strains of rhizospheric origin for growth promotion of tomato under both green house and elucidation of mechanism behind growth promotion.

MATERIALS AND METHODS

Microorganisms: *Bacillus* strains were used that were obtained from bacterial conservatories of Institute of Agricultural Sciences, University of the Punjab, Lahore Pakistan. Bacterial were grown on LB agar media and stocks kept in 80% glycerol at -20^{o}C. Bacterial inocula used in these investigations were prepared by growing them in LB broth media overnight. Bacterial cells were collected by centrifugation and re-suspended in distilled sterilized water at concentration of $1x10^{8}$*cfu*/ml by taking OD at 600 nm.

Characterization of *Bacillus* strains for production of plant growth promoting substances: Strains were first characterized for production of plant growth promoting substances like siderophores production, IAA production and phosphorus solubilization. Briefly, Siderophore production was tested qualitatively by Chrome Azural S (CAS) assay as described by Garland (1996). IAA production was noticed by adopting Paulitz and Belanger reagent method (Paulitz & Belanger, 2001). Phosphate solubilization was assessed by observing decolorization of bromophenol blue

according to method of Jetiyano & Kloepper (2002).

Potential of Bacillus strains to promote growth of tomato under Greenhouse conditions: A pot experiment was conducted to assess beneficial effects of our bacterial strains on plant growth and development. Plastic pots of 10 inch diameter were filled with sterilized sandy loamy soil as growth media. Three different tomato varieties were used for experimentation. Ten seeds of a single tomato variety were sown in each pot. One hundred ml of bacterial inoculum was provided in each pot. Control treatment was provided with 100 ml of distilled sterilized water. Al the treatments were arranged in complete randomized block design with five replications and performed twice. After forty days of incubation under greenhouse environment, harvesting of plants was done and different growth parameters were recorded.

Quantification of growth related biochemicals in tomato plants: Growth related biochemicals were quantified in tomato plants by calorimetric assays under influence of bacterial strains. For that purpose leaf contents were extracted with 80% acetone. Total chlorophyll and carotenoids were quantified by adopting method of Mathre *et al.,* (1999). Anthocyanins were quantified by extracting leaf contents in methanol/ HCL/water (90:1:1) solution by adopting methodology of Lubeck *et al.,* (2000).

Statistical analysis: Data were subjected to analysis of variance (ANOVA) and Duncan's new multiple range test (DNMRT) using DSASTAT statistical package (Onofri Italy).

RESULTS

Production of plant growth related substances: *In vitro* tests such as siderophores production, phosphate solubilization and IAA production for detection of plant growth promotion activities were carried out. Among all the *Bacillus* strains BT002, BS170, BM520 and BM521 were found positive for IAA production (Table 1). Only two strains were got positive for siderophores production (Table 1). Three strains viz: BS170, BM520 and BM522 were capable of phosphate solubilization (Table 1).

Potential of *Bacillus* strains to promote growth of tomato under greenhouse conditions: Bacterization of tomato plants showed significant increase in all variables measured in this study when plants were inoculated with *Bacillus* strains as compared to non-inoculated plants (Table 2). However, the magnitude of growth promotion varied among the both strains. To denote changes in growth parameters, average values were used obtained from data of all three tomato varieties. When tomato plants were grown with the strain BM520, maximum significant increases in shoot and a root length were observed (Table 2). This strain induced tomato plants for 72.3% and 87.4% increase over control (IOC) in shoot length and root length respectively. In the same way, BS170 (63.1% IOC) and BM520 (54.2% IOC) significantly increased shoot length.

Table -1: Characterization of *Bacillus* strains for production of growth promotion related substances.

Code	*Bacillus* strains		IAA Production	Siderophores production	Phosphorus solubilization
	Bacillus species	#			
BF324	*B. fortis*	FCBP 324	-	-	-
BF223	*B. fortis*	FCBP 223	-	-	-
BT199	*B. theruingenesis*	FCBP 199	-	-	-
BT002	*B. theruingenesis*	FCBP 002	+	-	-
BS525	*B. subtillis*	FCBP 525	-	+	-
BS170	*B. subtillis*	FCBP 170	+	-	+
BS530	*B. subtillis*	FCBP 530	-	-	-
BM520	*B. megaterium*	FCBP 520	+	+	+
BM521	*B. megaterium*	FCBP 521	+	-	-
BM522	*B. megaterium*	FCBP 522	-	-	+
BM523	*B. megaterium*	FCBP 523	-	-	-
BM524	*B. megaterium*	FCBP 524	-	-	-

(+) = Activity Present. (-) = No activity seen.

Likewise, inoculation of plants with bacteria showed significant increases in root lengths. Highest significant increase of 70.6% IOC in root volume was provided by BM520 inoculation, and was followed by BS170 (36.8% IOC) (Table 2). The results of total biomass followed the same trends as those of plant height and root length. In comparison with the control plants tomato plants co-cultivated with bacteria provided significant increase in total fresh biomass. Highest increase in total fresh biomass was observed for BM520 (96.7% IOC) and BS170 (73% IOC). Similar results were also recorded in case of total dry biomass. BS174 treated plants provided highest increase in total dry biomass (72.2% IOC) compared to control.

Table -2: Effect of *Bacillus* strains on growth parameters of tomato.

Treatments	Shoot Length (cm)	Root Length (cm)	Total biomass (g)	
			Fresh	Dry
BF324	21.13 $^{E-G}$	10.29 $^{E-H}$	44.14 ^{E}	05.52 ^{B}
BF223	24.32 ^{DE}	13.67 ^{CD}	33.29 ^{GH}	04.69 $^{C-E}$
BT199	18.91^{GH}	08.61 ^{H}	34.28 ^{GH}	04.46 ^{DE}
BT002	23.63 $^{D-F}$	12.83 $^{C-F}$	47.06 ^{CD}	05.71 ^{AB}
BS525	21.07 $^{E-G}$	13.03 ^{CD}	39.68 ^{F}	05.01 ^{C}
BS170	34.51 ^{B}	23.55 ^{B}	54.47 ^{A}	05.89 ^{AB}
BS530	17.26 ^{H}	11.37 $^{D-G}$	38.04 ^{F}	04.61 $^{C-E}$
BM520	38.44 ^{A}	26.10 ^{A}	56.57 ^{A}	06.02 ^{A}
BM521	19.60^{F-H}	10.71 $^{E-H}$	35.36 ^{G}	04.72 ^{CD}
BM522	22.37 $^{E-G}$	12.94 $^{C-E}$	45.29 ^{DE}	05.67 ^{AB}
BM523	26.16 ^{CD}	10.86 $^{D-H}$	48.05 ^{C}	05.75 ^{AB}
BM524	19.54 $^{F-H}$	09.87 $^{F-H}$	34.70 ^{GH}	04.77 ^{CD}
Control	17.57 ^{H}	09.17^{GH}	31.17 ^{H}	04.15 ^{E}

Values are the mean within the column sharing the same letters do not differ significantly according to DNMRT at p=0.05.

Quantification of Plant growth related bio-chemicals: In this study we have found that bacterial treatments significantly increased growth related biochemicals in tomato plants under influence of bacterial strains as compared with the control (Table 3). In particular, root inoculation of BM520 promoted production of total chlorophyll, carotenoids and anthocyanin in tomato plants (Table 3). The highest significant increases in total chlorophyll (62.9% IOC), Anthocyanin (37.2% IOC) and carotenoids (68.95 IOC) contents were obtained from BM520 applications (Table 3). Here BS170 performed second best and induced 62.9%, 37.5% and 68.3% more production of total chlorophyll, anthocyanin and carotenoids content in tomato plants as compared to untreated control (Table 3).

Table -3: Changes in growth related bio-chemicals in tomato plants under influence of *Bacillus* strains.

Treatments	Anthocyanin	Carotenoids	Total Chlorophyll contents
BF324	29.87 $^{G-I}$	24.29 $^{C-E}$	48.28 ^{E}
BF223	32.53 ^{FG}	22.51 $^{E-G}$	57.29 ^{BC}
BT199	30.21 $^{F-H}$	24.75 $^{C-E}$	46.89 ^{F}
BT002	35.12 $^{A-C}$	23.39 $^{D-F}$	59.83 ^{B}
BS525	30.97 $^{E-G}$	21.64 $^{F-H}$	58.55 ^{BC}
BS170	36.88 ^{AB}	27.81 ^{AB}	59.68 ^{B}
BS530	28.34 ^{HI}	22.51 $^{E-G}$	51.41 ^{DE}
BM520	37.05 ^{A}	29.20 ^{A}	63.42 ^{A}
BM521	34.91 $^{A-D}$	21.84 $^{F-H}$	56.21 ^{C}
BM522	33.76 $^{B-E}$	26.73 ^{BC}	51.26 ^{DE}
BM523	31.54 $^{E-G}$	25.25 $^{B-D}$	52.70 ^{D}
BM524	31.72 $^{D-G}$	24.56 $^{C-E}$	46.37 ^{F}
Control	26.55 ^{I}	19.11 ^{H}	38.61 ^{H}

Values are the mean within the column sharing the same letters do not differ significantly according to DNMRT at p=0.05.

DISCUSSIONS

Numerous microbes are thought to be plant growth promoting with diverse mechanisms. The exact mechanism by which these microbes encourage plant growth is yet not clear. Several authors reported that production of phytohormones, phosphate solubilization and promotion of the nutrient uptake are involved in plant growth promotion. Current investigation summarizes effect of bacillus inoculation on growth promotion of tomato under pot experiment. Hypothesis of growth promotion came true with positive effects of inoculations on different growth parameters of tomato plants like increase in shoot and root length and biomass. *Bacillus* strains provided comparatively better performance as compared to other strains as proved by data analysis. Co-cultivation of tomato with *B.megaterium* FCBP520 (BM520) stimulated its growth nearly two folds whereas addition of *B.subtillis* FCBP 170 (BS170) into pot mix provided

with second best growth promotion potentials.

Numerous functional aspects of bacterial microbes have been studied extensively to uncover mechanism behind plant growth promotion. Some direct mechanisms are nitrogen fixation, phosphate solubilization, production of siderophores, phytohormones and some volatile compounds (Giacomodonato *et al.,* 2001). Direct enhancement of mineral uptake due to increases in specific ion fluxes at the root surface in the presence of PGPR has also been reported (Bashan & Levanony,1991; Bertrand *et al.,* 2000). These bacterial microbes provide higher production of plants under natural ecosystem (Rosas *et al.,* 2009). Colonization of plant roots at early stages is preferred for sustainable higher yield in plants (Cakmakci *et al.,* 2006). Some beneficial influences of these growth promoting bacterial microbes include stimulation of root growth, induction of resistance against biotic and abiotic stresses (Lugtenberg & Kamilova, 2009). Higher production of plant growth related biochemicals like chlorophyll, carotenoids and anthocyanin contents under influence of bacterial inoculation has also been described extensively (Elkoca *et al.,* 2008). Based on the findings of current study, it was clear that can be applied as plant growth promoting microbes for field application. Both of these bacterial strains can be applied in field conditions to get higher production of tomato crop. These microbes can be raised and stored in different carrier materials for large scale use because of their ability to form resting spores to ensure viability for long terms (Trivedi *et al,* 2005; Trivedi & Pandey, 2008a). In case of commercially developed plant growth promoting bacterial strains, mostly belong to genus *Bacillus*. These play dual role by inducing resistance against plant diseases along with growth promotion (Ghosh *et al.,* 2003; Liu *et al,* 2006; Trivedi *et al,* 2008; Trivedi & Pandey, 2008b; Pandey *et al.,* 2011; Zhao *et al.,* 2011). Normal growth of the tomato plants was observed when control plants were not provided with bacterial inducers.

Various aspects of functional efficiency of PGPR have been studied extensively. The PGPR influence plant growth through direct or indirect mechanisms. The examples of direct mechanism(s) may include: growth promotion by providing fixed nitrogen to the host plant, phosphate solubilization, production of phytohormones, and sequestration of iron by siderophores, and lowering of plant ethylene levels. The indirect mechanisms mainly involve biocontrol of plant pathogens that may be associated with antibiosis and production of antimicrobial substances, such as lytic enzymes and biocidal volatiles (Chaurasia *et al.,* 2005; Compant *et al,* 2005). Natural agriculture ecosystems depend upon such beneficial microorganisms to sustain higher crop productivity (Rosas *et al.,* 2009). Selection of early root colonizing PGPR, which directly or indirectly influence plant growth and

productivity, is preferred (Cakmakci *et al*, 2006). The beneficial influence has been reported in terms of biofertilization, stimulation of root growth, rhizoremediation, plant stress management and biocontrol (Lugtenberg & Kamilova, 2009). Increase in plant growth related parameters including biochemicals, such as the chlorophyll content, due to bacterial inoculation in crop plants, under cold on highland areas of Turkey, has also been reported (Elkoca *et al*., 2008). Based on the results of laboratory as well as field based studies, it was established that these two species of *Bacillus* are suitable PGPRs for field application. These can be raised in suitable formulations and stored without the loss of the beneficial traits over storage (Trivedi *et al.*, 2005; Trivedi & Pandey, 2008a). However, for field application, it is desirable to select a PGPR that benefits large number of plant species, under a particular environment. Most of the commercially developed PGPR belong to the genus *Bacillus* and among the genus, strains of *B.subtilis* have been recognized as PGPR, mainly because of their disease reducing ability and resistance to environmental stresses due to spore forming nature. The importance and preference for the *Bacillus* species in field applications is of late getting attention (Ghosh *et al.*, 2003; Liu *et al*, 2006; Trivedi *et al*, 2008; Trivedi & Pandey, 2008b; Pandey *et al*, 2011; Zhao *et al*, 2011).

For advancement in the area of PGPR research, deeper understanding of the rhizosphere ecology, with focus on the microbial communication during plant-microbe and microbe-microbe interactions, needs attention. Knowledge on these lines is likely to provide clues to many observations noted in respect of the 'microbial shift' in the rhizosphere microflora due to PGPR application.

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