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Fertilizer Efficiency of Some Plant Growth Promoting Rhizobacteria for Plant Growth

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ABSTRACT

Use of biotechnological approaches and processes to increase of soil fertility and productivity, allow to be made sustainable agriculture with less use of chemical fertilizers. So, the aim of this study was to understand the biochemical mechanisms of action of the 10 different plant growth promoting rhizobacteria (PGPR) species (*Bacillus megaterium* (M3), *Pantoea agglomerans* (RK-92), *Bacillus megaterium* (Tv-17C), *Bacillus megaterium* (Tv-3D), *Bacillus megaterium* (Tv-87A), *Hafnia alvei* (Tv-34A), *Bacillus megaterium* (Tv-60D), *Pseudomonas fluorescens* (FDG-37), *Bacillus megaterium* (KBA-10) and *Bacillus megaterium* (Tv-91C) on their host plant. Results of this study show that the maximum amino acids etc., aspartate, asparagine, glutamine, proline, organic acid etc., malonic acid, oxalic acid and hormone etc., indol-3-acetic acid (IAA), gibberellic acid (GA) and salicylic acid (SA) super oxygen dismutase (SOD), peroxidase (POD) enzyme activity, alkaline phosphatase (ALPA) and acid phosphatase enzyme activity (APA), nutrient concentration of Ca, K, Mg, Na, P, S, N, Cu, Fe, Mn, Zn, B and Al were determined in *B. megaterium* M3, respectively. The highest cysteine, valin, methionine, tryptophan, isoleucine, leucine butyric acid, maleic acid, were determined from *B. megaterium* (Tv-17C); the highest urease (UEA) and dehydrogenase (DEA), enzymatic activities were found in *Pantoea agglomerans* (RK-92) but CAT enzyme activity was detected in *Pseudomonas fluorescens* (FDG-37). The data suggested that *B. megaterium* M3, *B. megaterium* (Tv-17C) and *Pantoea agglomerans* (RK-92) strains tested have the potential to be used as an organic fertilizer source for plant growth in sustainable and organic farming.

Key words: Amino acid, enzyme activity, hormone, organic acid, PGPR

INTRODUCTION

Plant Growth-Promoting Rhizobacteria (PGPR) are free-living microorganisms that have beneficial effects on plants by colonizing in their rhizosphere or phyllosphere (Bashan and de Bashan, 2005). In general, beneficial free-living bacteria are usually referred to as

Plant-Growth-Promoting Rhizobacteria (PGPR) which can affect plant growth directly or indirectly. One potential way to decrease negative environmental impacts resulting from continued use of chemical fertilizers is inoculation with PGPR. Apart from fixing N_2, PGPR can affect plant growth directly by the synthesis of phytohormones (auxins, cytokinins, gibberellins) and vitamins, inhibition of plant ethylene synthesis, enhanced stress resistance and improved nutrient uptake, solubilization of inorganic phosphate and mineralization of organic phosphate. Indirectly, diazotrophs are able to decrease or prevent the deleterious effects of pathogenic microorganisms (Zahir *et al*., 2004; Bashan and de Bashan, 2005; Antoun and Prevost, 2006; Podile and Kishore, 2007).

Bacteria are able to exert positive effects on plants through various mechanisms. Nitrogen fixations contributes organic nitrogen for plant growth, while the bacterial enzyme 1-Amino-Cyclopropane-1-Carboxylate (ACC) deaminase hydrolysis ACC (the immediate precursor of ethylene) and lowers the levels of ethylene produced in developing or stressed plants, promoting root elongation. Some bacteria solubilize insoluble minerals through the production of acids, increasing the availability of phosphorus and other nutrients to plants in deficient soils. Several bacteria improve plant growth through suppression of pathogens by competing for nutrients, by antibiosis, or by synthesizing siderophores which can solubilize and chelate iron from the soil and inhibit the growth of phytopathogenic microorganisms (Caballero-Mellado *et al*., 2007).

It is well known that a considerable number of bacterial species, mostly those associated with the plant rhizosphere, are able to exert a beneficial effect on plant growth. The use of those bacteria as biofertilizers in agriculture has been a focus of research for a number of years. The bacteria have been called plant growth-promoting rhizobacteria (PGPR) (Davison, 1988) and include strains in the genera *Azospirillum*, *Azotobacter*, *Bacillus*, *Enterobacter*, *Pseudomonas*, *Serratia* and *Streptomyces* (Kloepper and Beauchamp, 1992; Hoflich *et al*., 1994; Cakmakci *et al*., 2007a). The beneficial impact of PGPR are thought to be direct plant growth promotion by the production of plant growth regulators (Esitken *et al*., 2003, 2006; Orhan *et al*., 2006; Turan *et al*., 2006, 2012; Cakmakci *et al*., 2007a, b; Karakurt *et al*., 2011; Gunes *et al*., 2014), enhanced access to soil nutrient (Ogut and Er, 2006), disease control (Cuppels *et al*., 1999; Kotan *et al*., 2004, 2009; Kotan and Sahin, 2006; Erman *et al*., 2010; Fayetorbay *et al*., 2010; Karagoz and Kotan, 2010; Esitken *et al*., 2002) and associative nitrogen fixation (Zhang *et al*., 1996; Elkoca *et al*., 2007).

Bacillus species are among the most common soil bacteria groups and they are frequently isolated from the rhizosphere of plants (Bai *et al*., 2003). *Bacillus* species used as biofertilizers may have direct effects on plant growth through the synthesis of plant growth hormones (Cakmakci *et al*., 2007a, b), N2-fixation (Cakmakci *et al*., 2001) and solubilization of phosphate (Sahin *et al*., 2004). The N_2-fixing and P-solubilizing *Bacillus* spp. stimulate plant growth through enhanced N and P nutrition (Orhan *et al*., 2006), increasing the uptake of N, P, K, Ca, manganese (Mn), zinc (Zn) and Fe (Biswas *et al*., 2000; Esitken *et al*., 2003; Han and Lee, 2005; Orhan *et al*., 2006; Cakmakci *et al*., 2007a; Turan *et al*., 2012; Cakmakci *et al*., 2014). Trials with rhizosphere-associated plant growth-promoting N_2-fixing and P-solubilizing *Bacillus* species indicated yield increases in many crops such as wheat (Caceres *et al*., 1996; Ozturk *et al*., 2003) barley (Cakmakci *et al*., 2001; Ozturk *et al*., 2003) sugar beet (Cakmakci *et al*., 2001), canola (De Freitas *et al*., 1997) and maize (Pal, 1998). Because of their spore-forming ability, plant growth promoting *Bacillus* strains are readily adaptable to commercial formulation and field application (Liu and Sinclair, 1993).

Intensive farming practices, that warrant high yield and quality, require extensive use of chemical fertilizers which are costly and create environmental problems. Therefore, more recently there has been a resurgence of interest in environmental friendly, sustainable and organic agricultural practices. However, yield reduction is an important problem in organic production system (Lind *et al*., 2003). Use of organic fertilizers containing sewage sludge, seaweed and lichen is known to improve plant growth and help to sustain environmental health and soil productivity (O'Connell, 1992; Turkmen *et al*., 2004; Turan and Kose, 2004). Crop, vegetable and fruits are relatively easy to produce using organic fertilizer sources as long as enough nutrients are available (Kuepper *et al*., 2003).

Objectives of the present study were to understand the action mode of the PGPR on their host plant and evaluate some chemical properties of PGPR strains as a plant nutrient source for sustainable and organic agriculture.

MATERIALS AND METHODS

Bacterial strains: All bacterial strains (*Bacillus megaterium* (M3), *Pantoea agglomerans* (RK-92), *Bacillus megaterium* (TV-17C), *Bacillus megaterium* (TV-3D), *Bacillus megaterium* (TV-87A), *Hafnia alvei* (TV-34A), *Bacillus megaterium* (TV-60D), *Pseudomonas fluorescens* (FDG-37), *Bacillus megaterium* (KBA-10) and *Bacillus megaterium* (TV-91C) tested in the present study were obtained from Dr. Recep Kotan (Atatürk University, Agriculture Faculty, Department of Plant Protection, Erzurum, Turkey). These bacteria used in this study were identified and reported as plant growth promoting bacteria and potential bio-control agents against a wide range of bacterial and fungal pathogens that cause economically important problems in agriculture (Kotan *et al*., 2005; Recep *et al*., 2009; Erman *et al*., 2010).

Bacterial growth and laboratory experiment: Bacteria were grown on Nutrient Agar (NA) for routine use and maintained in Nutrient Broth (NB) with 15% glycerol at -80°C for long-term storage. For each experiment, a single colony was transferred to 500 mL flasks containing NB and grown aerobically in flasks on a rotating shaker for 48 h at 27°C (Merck KGaA, Germany) and diluted to a final concentration of 10^8 CFU mL^{-1} (colony forming units) using sterile distilled water containing 0.025% Tween 20. Twenty-five bacteria sample of each PGPR were used in the experiment to determine organic acid, amino acid, hormone, enzyme activity and nutrient content.

Amino acid analysis: Amino acids were extracted from the samples and were analyzed as described by Aristoy and Toldra (1991), Antoine *et al*. (1999) and Henderson *et al*. (2000).

Organic acid analysis: The organic acids were analyzed by HPLC on Zorbax Eclipse-AAA 4.6×250 mm, 5 µm columns (Agilent 1200 HPLC) and absorbance of 220 nm in UV detector.

Hormone analysis: Extraction and purification processes were as described by Kuraishi *et al*. (1991), Battal and Tileklioglu (2001) and Davies (1995). The samples were filtered with Whatman No. 1 filter paper and then supernatants were filtered through 0.45 µm filters (Cutting, 1991). Supernatants were evaporated to dryness at 35°C by evaporator pumps. Dried supernatants were solved using 0.1 M KH_2PO_4 (pH 8.0). Extracts were centrifuged at 5000 rpm for 1 h at 4°C to separating fatty acids (Palni *et al*., 1983). Polyvinylpolypyrrolidone (PVPP), 1 g was prepared and added to supernatants to separate phenolic and colored matters (Qamaruddin, 1996; Chen, 1991;

Mooney and van Staden, 1984; Hernandez-Minea, 1991). Supernatants with PVPP were filtered with Whatman No. 1 filter paper to remove PVPP (Cheikh and Jones, 1994). The hormones were analyzed by HPLC on a Zorbax Eclipse-AAA C-18 column (Agilent 1200 HPLC).

Enzyme activities of PGPR: Phosphatase activity was determined using para-nitro-phenyl phosphate (pNPP) as an orthophosphate monoester analogue substrate (Tabatabai, 1982).

Antioxidant enzymes analysis of PGPR: Superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) enzyme activities in the apoplastic fractions were measured spectrophotometrically. The CAT activity was measured by monitoring the decrease in absorbance at 240 nm in 50 mM phosphate buffer (pH 7.5) containing 20 mM H_2O_2. One unit of CAT activity was define as the amount of enzyme that used 1 µmol H_2O_2 min^{-1}. The POD activity was measured by monitoring the increase in absorbance at 470 nm in 50 mM phosphate buffer (pH 5.5) containing 1 mM guaiacol and 0.5 mM H_2O_2. One unit of POD activity was defined as the amount of enzyme that cause an increase in absorbance of 0.01 min^{-1}. The SOD activity was estimated by recording the decrease in optical density of nitro-blue tetrazolium dye by the enzyme (Dhindsa *et al*., 1981; Sairam and Srivastava, 2002).

Element analysis: The Kjeldahl method and a Vapodest 10 Rapid Kjeldahl Distillation Unit (Gerhardt, Konigswinter, Germany) were used to determine total N (Bremner, 1996) Ca, Mg, Na, K, P, S, Fe, Cu, Mn, Zn, Pb, Ni and Cd contents of PGPR strains after wet digestion of dried and ground sub-samples using a HNO_3-H_2O_2 acid mixture (2:3 v/v) with three steps (first step; 145ºC, 75% RF, 5 min; second step; 180ºC, 90% RF, 10 min and third step; 100ºC, 40% RF, 10 min) in a microwave oven (Bergof Speedwave Microwave Digestion Equipment MWS-2) (Mertens, 2005a). The Ca, Mg, Na, K, P, S, Fe, Cu, Mn, Zn, Pb, Ni and Cd were determined using an Inductively Couple Plasma spectrometer (Perkin-Elmer, Optima 2100 DV, ICP/OES, Shelton, CT 06484-4794, USA) (Mertens, 2005b).

Statistical analysis: Data was sorted by PGPR species and differences among species were attained using Duncan test option in analysis of variance (SPSS., 2004). Differences were declared to be significant at $p<0.05$.

RESULTS

Amino acids and organic acid contents of PGPR: *Bacillus megaterium* M3 had the highest aspartate, glutamate, asparagine, serine, glutamine, glycine, threonine, tyrosine, proline, hydroxyproline, malonic acid, oxalic acid, propionic acid, citric acid, fumaric acid but the highest cysteine, valin, methionine, tryptophan, isoleucine, leucine butyric acid, maleic acid, were determined from *B. megaterium* (Tv-17C) (Table 1 and 2).

Hormone contents of PGPR: Similarly, when compare to hormone content of PGPR, the highest Indol-3-Acetic Acid (IAA), Gibberellic Acid (GA) and Salicylic Acid (SA) were obtained from *B. megaterium* M3 (Fig. 1).

Antioxidant enzyme and other enzyme activities: The highest SOD, POD, ALPEA and APEA were noted in *B. megaterium* M3 (Fig. 2) but the lowest was determined from *B. megaterium* Tv-3D

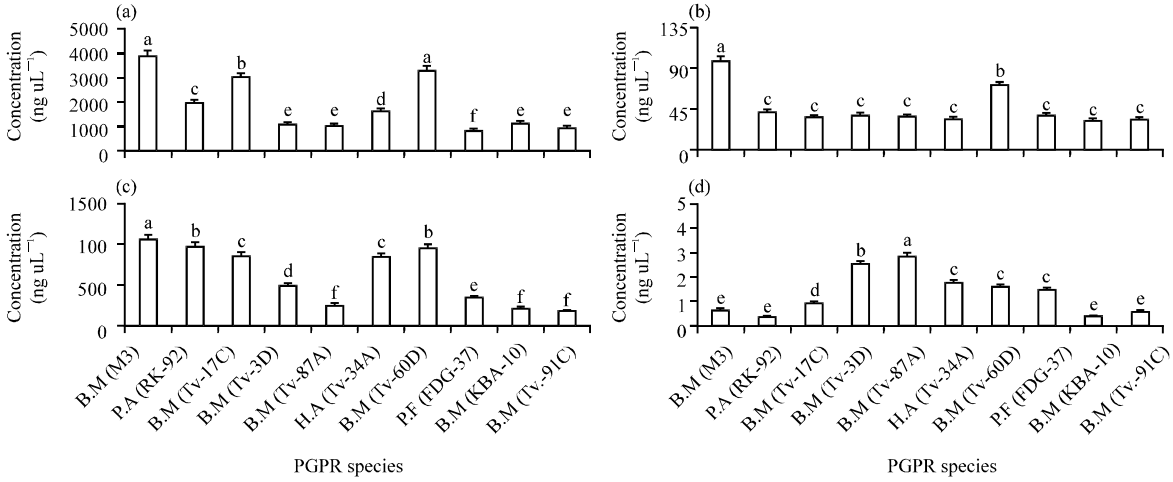

Fig. 1(a-d): (a) Gibberellic acid (GA), (b) Indole acetic acid (IAA), (c) Salicylic acid (SA) and (d) Abscisic acid (ABA) content of some studied PGPR species (Mean±Standard deviation). Different letters within a PGPR species indicate means are significantly different at p#0.05

Table 1: Amino acid content (pmol µLG^{1}) of PGPR

Amino acids	B.M (M3)	P.A (RK-92)	B.M (Tv-17C)	B.M (Tv-3D)	B.M (Tv-87A)	H.A (Tv-34A)	B.M (Tv-60D)	B.M (FDG-37)	B.M (KBA-10)	B.M (Tv-91C)
Aspartate	965±7^{a}	868±5^{b}	877±7^{b}	129±3^{g}	228±5^{f}	91±4^{g}	742±4c	582±5^{d}	433±5^{e}	730±6^{c}
Glutamate	15021±20^{a}	13519±17^{b}	16947±15^{a}	7501±6^{d}	6673±6^{e}	5187±9^{f}	10138±12^{c}	7151±6^{d}	7332±6^{d}	14122±5^{b}
Asparagine	955±9^{a}	859±6^{a}	343±6^{b}	106±6^{d}	220±5^{c}	352±11^{b}	265±5^{c}	903±4^{a}	433±4^{b}	286±8^{c}
Serine	814±11^{a}	733±5^{b}	876±8^{a}	151±4^{e}	240±4^{d}	81±5^{e}	455±6^{c}	532±6^{c}	408±3^{c}	730±6^{b}
Glutamine	941±12^{a}	847±8^{b}	810±7^{b}	414±5^{d}	390±4^{e}	523±6^{cd}	652±5^{c}	426±4^{d}	467±7^{d}	675±5^{c}
Histidine	10585±24^{c}	11126±12^{b}	12963±12^{b}	9186±10^{d}	5531±6^{f}	5353±7^{f}	9517±8^{d}	6897±3^{e}	7209±5^{e}	14969±7^{a}
Glycine	2881±8^{a}	2593±9^{a}	594±8^{d}	412±8^{e}	813±8^{c}	711±5^{c}	255±5^{f}	214±6^{f}	1141±6^{b}	495±8^{e}
Threonine	3289±11^{a}	2960±8^{b}	677±5^{c}	375±6^{e}	733±5^{c}	1999±6^{b}	318±3^{e}	612±3^{c}	1359±8^{b}	564±6^{d}
Arginine	8452±19^{b}	9007±10^{a}	8197±9^{b}	7041±8^{c}	8290±9^{b}	8791±9^{ab}	8290±6^{b}	7574±4^{c}	6551±4^{d}	8498±5^{b}
Alanine	1718±11^{b}	1546±5^{b}	974±6^{c}	109±5^{f}	563±5^{e}	178±3^{f}	2551±5^{a}	1217±3^{b}	696±6^{d}	811±4^{c}
Tyrosine	1149±13^{a}	1035±6^{a}	1927±8^{a}	857±8^{b}	637±4^{d}	651±5^{d}	898±4^{c}	1089±5^{b}	503±5^{e}	1606±6^{a}
Cystine	4956±14^{c}	4460±8^{c}	8986±10^{a}	3997±6^{cd}	2933±10^{d}	3203±6^{d}	4228±6^{c}	2655±3^{e}	2477±8^{e}	7489±8^{b}
Valin	2148±12^{b}	1933±9^{b}	3725±6^{a}	1711±7^{c}	1418±5^{c}	2391±4^{b}	2069±7^{b}	1149±4^{c}	932±6^{d}	3104±4^{a}
Methionine	1292±9^{d}	1163±7^{d}	3242±8^{a}	1058±5^{e}	1028±4^{e}	1341±5^{d}	1640±6^{c}	824±3^{e}	642±4^{f}	2701±6^{b}
Tryptophan	5273±10^{c}	4746±9^{d}	9435±7^{a}	4528±6^{d}	3262±3^{e}	3457±4^{e}	4674±5^{d}	2977±5^{f}	2450±6^{f}	7863±5^{b}
Phenylalanine	1159±8^{b}	1043±8^{b}	1094±6^{b}	4595±6^{a}	3143±5^{a}	786±5^{c}	312±3^{e}	223±3^{e}	526±7^{d}	912±6^{bc}
Isoleucine	9360±11^{a}	8424±6^{b}	9917±8^{a}	7282±7^{c}	5605±6^{d}	5911±6^{d}	8035±6^{b}	4952±5^{d}	4527±8^{d}	7431±4^{c}
Leucine	654±14^{c}	755±7^{d}	1755±6^{a}	812±5^{c}	734±5^{d}	661±4^{d}	912±5^{c}	411±3^{e}	338±6^{e}	1462±6^{b}
Lysine	11353±11^{c}	10218±18^{d}	17514±8^{a}	14205±12^{b}	14093±11^{b}	11629±11^{c}	10684±6	10539±14^{d}	11024±4^{c}	12928±6^{c}
Hydroxyproline	3693±5^{a}	2472±8^{c}	4432±5^{b}	1601±5^{d}	941±5^{f}	1205±5^{e}	1232±4^{e}	825±5^{f}	1247±6^{e}	2747±12^{c}
Sarcosine	4656±11^{c}	4191±9^{c}	11671±6^{f}	3320±3^{d}	2777±6^{e}	2962±6^{e}	6160±6^{b}	4504±6^{c}	2024±5^{e}	9725±6^{a}
Proline	6656±8^{a}	1872±8^{cd}	2080±14^{c}	2192±4^{c}	2018±4^{c}	1792±5^{c}	2705±5^{c}	2154±7^{c}	1023±6^{d}	5547±5^{b}

Values (n = 25) in the same row with a different letters are significantly different (p#0.05). Mean±standard deviation

(Fig. 2 and 3). The highest urease (UEA) and dehydrogenase (DEA) enzymatic activities were found in *Pantoea agglomerans* (RK-92) but CAT enzyme activity was detected in *Pseudomonas fluorescens* (FDG-37) (Fig. 2 and 3).

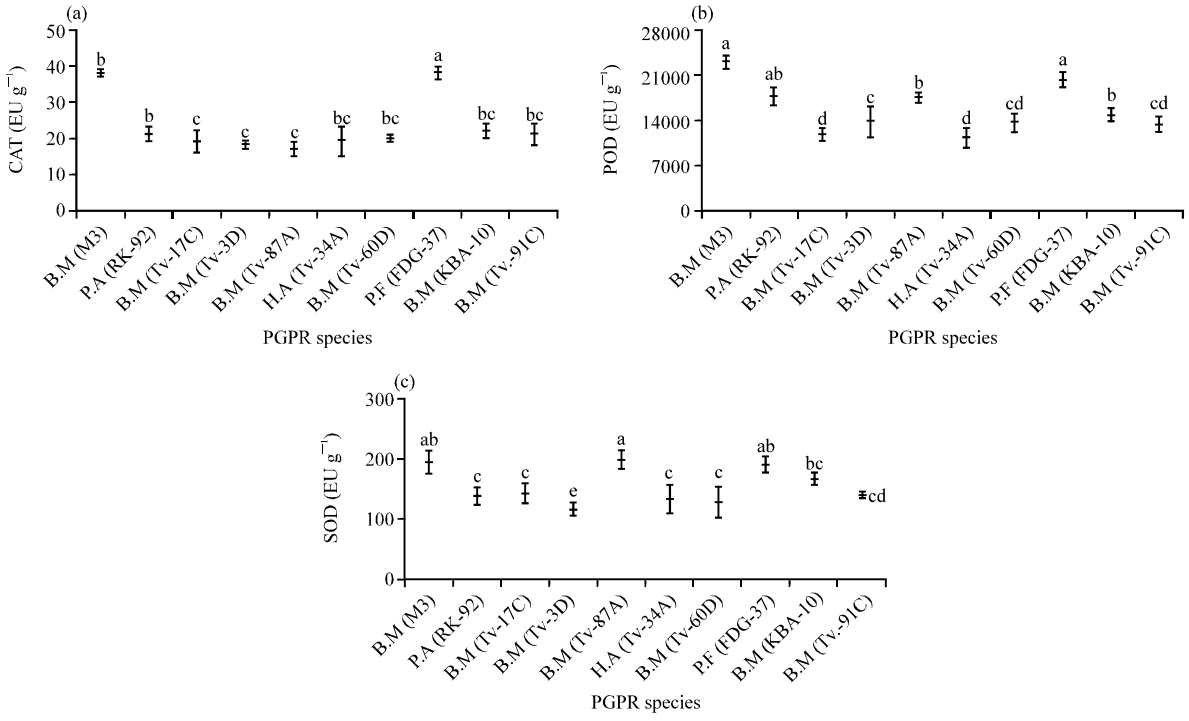

Fig. 2(a-c): Antioxidant enzyme activity of (a) CAT, (b) POD and (c) SOD of some studied PGPR species (Mean±Standard Deviation). Different letters within a PGPR species indicate means are significantly different at p#0.05

Table 2: Organic acid content (ng µLG^{1}) of PGPR (n = 25)

Organic acids	B.M (M3)	P.A (RK-92)	B.M (Tv-17C)	B.M (Tv-3D)	B.M (Tv-87A)	H.A (Tv-34A)	B.M (Tv-60D)	B.M (FDG-37)	B.M (KBA-10)	B.M (Tv-91C)
Oxalic acid	806±6^{a}	191±3^{e}	692±4^{b}	368±3^{d}	110±3^{e}	387±5^{d}	403±3^{c}	620±3^{b}	413±5^{c}	735±5^{a}
Propionic acid	1772±6^{a}	1363±4^{b}	1143±6^{c}	640±4^{e}	783±4^{e}	1018±5^{c}	767±7^{e}	1495±3^{b}	321±4^{f}	904±4^{d}
Tartaric acid	893±5^{d}	705±3^{d}	1429±5^{c}	501±5^{e}	405±3^{e}	973±4^{cd}	681±5^{e}	1676±5^{b}	306±3^{f}	1986±6^{a}
Butyric acid	4197±5^{d}	1033±5^{e}	9972±6^{a}	829±6^{g}	594±5^{g}	1502±6^{f}	4608±5^{d}	9256±4^{a}	5729±4^{c}	8711±5^{b}
Malonic acid	20855±12^{a}	1572±4^{h}	6036±6^{c}	3418±5^{f}	904±3i	4024±5^{e}	3895±7^{f}	5985±3^{d}	2951±6^{g}	7093±6^{b}
Malic acid	3195±5^{a}	1193±5^{c}	1126±5^{c}	452±4^{e}	686±4de	697±5de	844±5 ^{d}	1899±5^{b}	862±4^{d}	2251±4^{b}
Lactic acid	58787±4^{b}	22432±5^{e}	37057±4^{d}	20819±11^{e}	17667±4^{f}	35982±6^{d}	49823±7^{c}	66650±9^{a}	22544±9^{e}	62696±5^{a}
Citric acid	8938±5^{a}	3889±6^{d}	7027±3^{b}	753±3^{h}	2236±5^{e}	1597±4^{f}	3417±7^{d}	7542±4^{b}	1048±5^{f}	5296±6^{c}
Maleic acid	811±4^{f}	2016±4^{b}	3693±5^{a}	400±6^{g}	1159±6^{e}	763±5^{f}	407±3^{g}	1473±5^{d}	198±6^{h}	1746±4^{c}
Fumaric acid	990±3^{a}	221±3^{e}	388±3^{d}	110±9	127±5	584±5^{c}	266±3^{e}	835±3^{b}	257±3^{e}	247±5^{e}
Succinic acid	47914±8^{a}	25074±5^{cc}	44434±4^{ab}	21867±11	34417±6^{b}	26994±6	25461±7^{c}	40427±7^{b}	29454±4^{bc}	31567±5^{b}

Values in the same row with a different letters are significantly different (p#0.05). Data is taken as Mean±SD

Macro-micro and heavy metal content of PGPR: The levels of minerals important for plant nutrition are presented in Table 3 and 4. There were statistical significant differences between the PGPR species in respect of total microelement concentration. The highest concentration of Ca, K, Mg, Na, P, S, N, Cu, Fe, Mn, Zn, B and Al were obtained from *B. megaterium* M3. With regard to Cd, Ni, Cr and Pb, *Hafnia alvei* (Tv-34A) had the highest content.

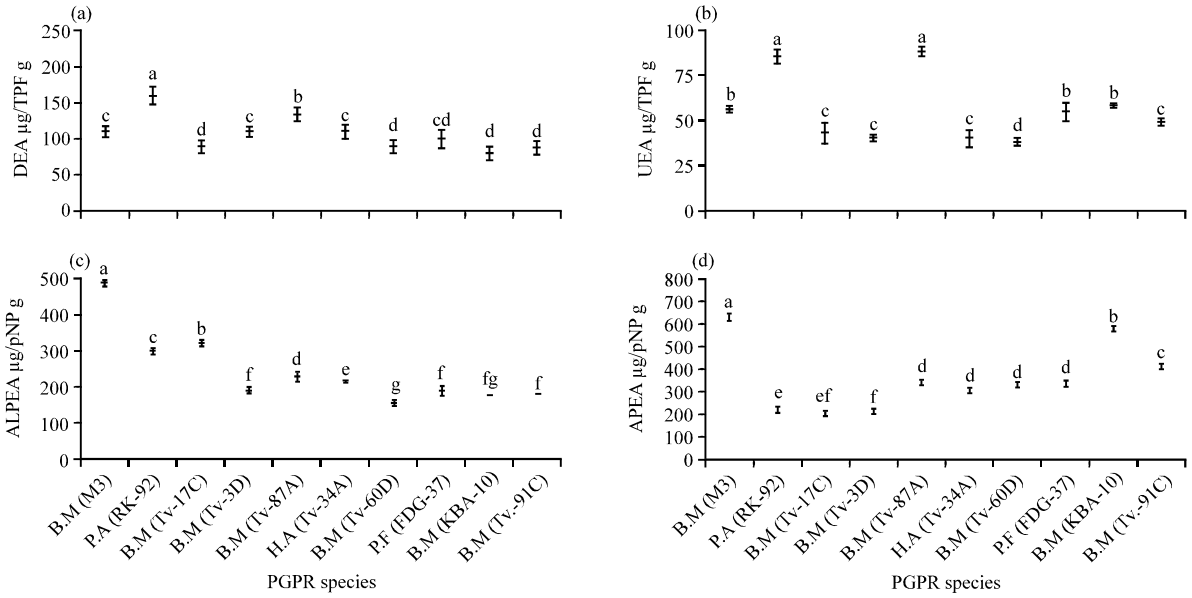

Fig. 3(a-d): (a) Dehydrogenase activity (DEA), (b) Urease activity (UEA), (c) Alkaline phosphatase activity (ALPEA) and (d) Acid phosphatase activity of (APEA) some studied PGPR species (Mean±Standard Deviation). Different letters within a PGPR species indicate means are significantly different at p#0.05

Table 3: Macro element content of some studied PGPR species

PGPR species	Ca	K	Mg	Na	P	S	N
	(mg kgG^{1})						
Bacillus megaterium (M3)	12.40^{a}	207^{a}	5.40^{a}	1457^{a}	81^{a}	75^{a}	1610^{a}
Pantoea agglomerans (RK-92)	5.25^{d}	147^{d}	3.08^{c}	951^{b}	48^{c}	58^{b}	580^{d}
Bacillus megaterium (Tv-17C)	3.11^{e}	162^{c}	4.61^{b}	821^{b}	38^{d}	33^{d}	290^{g}
Bacillus megaterium (Tv-3D)	6.35^{c}	126^{e}	2.15^{d}	725^{b}	26^{e}	43^{c}	475^{e}
Bacillus megaterium (Tv-87A)	7.12^{b}	146^{d}	1.66^{e}	825^{b}	38^{d}	54^{b}	360^{f}
Hafnia alvei (Tv-34A)	3.61^{e}	170^{c}	2.83^{d}	621^{bc}	37^{d}	55^{b}	420^{e}
Bacillus m^{e}gaterium (Tv-60D)	7.22^{b}	135^{f}	3.48^{c}	848^{b}	32^{d}	38^{cd}	580^{d}
Pseudomonas fluorescens (FDG-37)	2.20^{f}	94^{h}	1.88^{e}	423^{c}	54^{bc}	42^{c}	820^{b}
Bacillus megaterium (KBA-10)	3.12^{f}	110^{f}	0.99^{f}	800^{b}	42^{c}	40^{c}	660^{c}
Bacillus megaterium (Tv-91C)	3.52^{f}	199^{b}	1.56^{e}	938^{b}	68^{b}	49^{c}	750^{c}

Values (n = 25) in the same column with different letters are significantly different (p#0.05)

DISCUSSION

Effects on amino acids and organic acids produced from PGPR on plant growth: a considerable number of fertilizer sources, mostly those associated with hormone, organic or amino acid contents promote plant growth. These ingredients render insoluble forms of plant nutrients into soluble forms through the process of acidification, chelation and exchange reactions. This process not only compensates for the higher cost of manufacturing fertilizers in industry but also mobilizes the fertilizers added to soil. Organic fertilizer source of PGPR, especially low grade and its use in agriculture has received great attention. Foliar feeding, using bio based, natural organic foliar fertilizer, is an effective method for correcting soil deficiencies and overcoming the soil's sustainability to transfer nutrients to the plant.

Table 4: Micro element and heavy metal content of some studied PGPR species

	Micro elements and heavy metals (mg kgG¹)									
PGPR species	Cu	Fe	Mn	Zn	B	Al	Cd	Cr	Ni	Pb
Bacillus megaterium (M3)	0.31[a]§	1.64[a]	0.55[a]	2.41[a]	0.66[a]	0.94[a]	0.013[c]	0.011[c]	0.011[d]	0.05[b]
Pantoea agglomerans (RK-92)	0.10[d]	0.86[b]	0.10[d]	1.69[b]	0.44[c]	0.30[d]	0.013[c]	0.018[b]	0.014[c]	0.04[b]
Bacillus megaterium (Tv-17C)	0.21[b]	0.82[b]	0.24[c]	1.80[b]	0.63[a]	0.62[b]	0.013[c]	0.013[c]	0.010[d]	0.11[a]
Bacillus megaterium (Tv-3D)	0.28[b]	0.25[c]	0.13[d]	0.89[d]	0.35[d]	0.40[c]	0.020[a]	0.012[c]	0.016[f]	0.11[a]
Bacillus megaterium (Tv-87A)	0.27[b]	0.77[b]	0.50[a]	1.66[c]	0.40[c]	0.30[d]	0.020[a]	0.016[b]	0.012[d]	0.08[a]
Hafnia alvei (Tv-34A)	0.38[a]	0.76[b]	0.33[b]	1.18[c]	0.52[b]	0.23[e]	0.023[a]	0.019[a]	0.024[a]	0.10[a]
Bacillus megaterium (Tv-60D)	0.30[a]	0.91[b]	0.32[b]	1.30[c]	0.30[d]	0.29[e]	0.018[b]	0.010[c]	0.019[b]	0.09[a]
Pseudomonas fluorescens (FDG-37)	0.19[c]	0.84[b]	0.23[c]	1.14[c]	0.13[f]	0.31[d]	0.023[a]	0.014[c]	0.018[b]	0.12[a]
Bacillus megaterium (KBA-10)	0.14[d]	0.99[b]	0.27[c]	0.83[d]	0.24[e]	0.29[d]	0.018[b]	0.011[c]	0.011[d]	0.12[a]
Bacillus megaterium (Tv-91C)	0.01[d]	0.18[d]	0.32[b]	1.39[c]	0.32[d]	0.24[e]	0.022[a]	0.011[c]	0.015[c]	0.11[a]

Values in the same column (n = 25) with a different letters are significantly different (p#0.05)

Amino acids presence in the medium may promote shoot production-through the differentiation of dividing cells that is the reason that it possess comparatively low growth potential because the majority of dividing cells become differentiated rather undergoing faster cell proliferation (Asad *et al*., 2009). Organic acids have a potential role as metabolically active solutes for the osmotic adjustment and the balance of cation excess in the plant. Organic acids also participate as key components in the mechanisms that some plants use to cope with nutrient deficiencies, metal tolerance and plant-microbe interactions operating at the root-soil interface. Because of its high affinity for di-and tri-valent cations, citrate and other organic acids can displace P from insoluble complexes, making it more soluble and thus available for plant uptake and stimulate nitrate uptake of plant (Struthers and Sieling, 1950; Bradley and Sieling, 1953). Exogenous amino acids can modulate membrane permeability and ion uptake and probably this is the major component by which amino acids help in mitigating drought or salt stress effects.

In this study, *B. megaterium* M3, *B. megaterium* (Tv-91C) and *B. megaterium* (Tv-17C) species/strains may have beneficial effect on plant growth under unfavorable plant growth condition due to their high level of amino acid and organic acid contents. This suggests that amino acid and organic acid production in the PGPR, or a change in the rhizosphere's chemical properties could benefit to plant growth. Similar findings were reported in previous studies showing that application of PGPR may stimulate yield, growth and nutrient element uptake from soil in different plant species under stress plant growth conditions for different crops. Proline, alanine, serine and asparagine also delayed wilting of maize under stress conditions, proline, glycine, alanine, leucine, threonine, lysine, arginine, tryptophan and phenylalanine inhibited stomatal opening while histidine, methionine, aspartic acid, glutamic acid, asparagine and glutamine promoted stomatal opening of *Vicia faba*, histidine, proline, glutamine, methionine and glycine promoted calcium uptake in *Phaseolus* seedlings, proline relieved salt toxicity in barley plant lets by changing salt transport from root to shoot and increasing proline content increased K^+ content and alleviated salt stress effects on growth of *Vigna radiate* cultures (Thakur and Rai, 1985; Rai and Sharma, 1991; Rai and Rana, 1996; Lone *et al*., 1987; Kumar and Sharma, 1989).

The most useful PGPR application to stimulate yield of some fruit such as mulberry (*Morus alba* L.), apricot (*Prunus armenia* L.), sweet cherry (*Prunus avium* L.) and raspberry (*Rubus ideaus* L.) (Esitken *et al*., 2003, 2006; Orhan *et al*., 2006; Turan *et al*., 2006, 2007) and cereal crop species such as wheat (Cakmakci *et al*., 2007a; Turan *et al*., 2012) and other cereal crops such as maize and barley (Malhotra and Srivastava, 2009) have been subjected to seed inoculation.

Effects on hormone and nutrient content of PGPR on plant nutrition: The present results showed that the highest gibberellic acid, salicylic acid and IAA were found from *B. megaterium* M3, followed by *B. megaterium* (Tv-17C) and *B. megaterium* (Tv-60D) but ABA from *B. megaterium* (Tv-87A) (Fig. 1). Direct mechanisms of PGPR facilitates plant growth is including the production of plant growth regulators or phytohormones (Glick, 1995). The production of phytohormones such as, auxins (IAA), cytokinins and gibberellins by natural soil microbial communities have been reported by various workers over the last 20 years (Poonguzhali *et al*., 2008; Ahemad and Khan, 2010). Indol-3-acetic acid, a main auxin in plants, is known to control many important physiological processes of plants, such as, cell enlargement, cell division, root initiation, growth rate, phototropism, geotropisms and apical dominance etc. (Zaidi *et al*., 2009). In plant cells, IAA is largely formed by de novo synthesis from tryptophan that undergoes either oxidative deamination or decarboxylation with indole-3-acetic aldehyde as an intermediate. Indole-3-acetic acid (IAA) controls a wide variety of processes in plant development, control many important physiological processes of plants, such as cell enlargement, cell division, root initiation, growth rate, phototropism, geotropisms and apical dominance etc. and plays a key role in shaping plant root architecture such as regulation of lateral root initiation, root vascular tissue differentiation, polar root hair positioning, root meristem maintenance and root gravitropism (Aloni *et al*., 2006; Fukaki *et al*., 2007). Production of IAA is widespread among rhizobacteria (Khalid *et al*., 2004; Patten and Glick, 1996; Spaepen *et al*., 2007), with increasing numbers of endophytic IAA-producing PGPR being reported (Tan and Zou, 2001). Cytokinins stimulate plant cell division, control root meristem differentiation, inhibit primary root elongation and lateral root formation but can promote root hair development (Riefler *et al*., 2006; Silverman *et al*., 1998). Cytokinin production has been reported in various PGPR including, *Arthrobacter* spp., *Azospirillum* spp., *Pseudomonas fluorescens* and *Paenibacillus polymyxa* (Cacciari *et al*., 1989; De Salamone *et al*., 2001; Perrig *et al*., 2007; Timmusk *et al*., 1999). The cytokinin receptors play a complimentary role in plant growth promotion by *B. megaterium* (Ortiz-Castro *et al*., 2008). Gibberellins enhance the development of plant tissues particularly stem tissue and promote root elongation and lateral root extension (Barlow *et al*., 1991; Yaxley *et al*., 2001). Production of gibberellins have been documented in several PGPR such as *Azospirillum* spp., *Azotobacter* spp., *Bacillus pumilus*, *B. licheniformis*, *Herbaspirillum seropedicae*, *Gluconobacter diazotrophicus* and rhizobia (Bottini *et al*., 2004; Gutierrez-Manero *et al*., 2001).

When the crop is inoculated with PGPR strains which are capable of IAA production significantly increased the plant growth by enhancing N, P, K, Ca and Mg uptake of sweetpotato cultivars (Farzana and Radizah, 2005). Most of the PGPR strains analyzed in the present study were found to contain significant quantities of variety of essential nutrients. Results in this study demonstrated that the highest Ca, K, Mg, Na, P, S, N, Cu, Fe, Mn, Zn, B and Al in B were obtained from *B. megaterium* M3. With regard to Cd, Ni, Cr and Pb, *Hafnia alvei* (Tv-34A) had the highest content. The data suggested that some PGPR strains tested had a very high nutritional potential and their mineral content was even greater than that of some organic fertilizer sources. Plant developmental processes are controlled by internal signals that depend on the adequate supply of mineral nutrients by soil to roots. Thus, the availability of nutrient elements can be a major constraint to plant growth in many environments of the world, especially the tropics where soils are extremely low in nutrients. Plants take up most mineral nutrients through the rhizosphere where microorganisms interact with plant products in root exudates. Plant root exudates consist of a complex mixture of organic acid anions, phytosiderophores, sugars, vitamins, amino acids, purines,

nucleosides, inorganic ions (e.g., HCO_3^-, OH^-, H^+), gaseous molecules (CO_2, H_2), enzymes and root border cells which have major direct or indirect effects on the acquisition of mineral nutrients required for plant growth.

Acetic acid, glycolic, malonic, oxalic, formic and abscisic acid play a crucial role in nutrient acquisition (P, Fe and Mn) by plants growing in low nutrient soils and their release in response to nutrient starvation differs between plant species (Ae *et al*., 1990; Fox and Comerford, 1990; Smith, 1969, 1976; Vancura and Hovadik, 1965). The concentrations of fumaric, malic and citric acids can also chelate Fe and Mn in iron and manganese oxides (i.e., Fe_2O_3 and MnO_2), thus making them available for uptake by the plant (Ohwaki and Hirata, 1992; Marschner, 1995). Similarly, these acid anions form complexes with Ca, Al and Fe present in soil as insoluble phosphates of calcium, iron and aluminium and liberate P for uptake by roots (Marschner, 1995). Additionally, these acids can desorb P from sesquioxide surfaces by anion exchange (Bolan *et al*., 1994; Jones, 1998; Jones and Darrah, 1994; Parfitt, 1979) and also maintain sulphate mobility in rhizosphere soil through competitive displacement from adsorption sites (Evans and Anderson, 1990).

Effects on enzyme activity of PGPR on plant growth under stress condition: Our data showed that SOD, POD and CAT contents of *B. megaterium* (M3), *P. fluorescens* (FDG-37) and *B. megaterium* (Tv-87A) were higher than the other PGPR species tested in this study (Fig. 2). The antioxidant enzyme activities has been reported to increase under cold, saline, high light and soil pollution conditions in the case of cucumber seedlings (Kang and Saltveit, 2001), olive (*Olea europea* L.), wheat (Biemelt *et al*., 2000). Our data supported the evidence that PGPR application may also assist growth by alleviating negative effects of cold stress via promoting accumulation of antioxidant enzyme activities, decreasing reactive oxidative oxygen species (ROS) such as H_2O_2, O_2 and OH in response to cold stress. The P-solubilising PGPR strains application also altered ALPEA and APEA of soil. The highest ALPEA and APEA activity of soil was obtained from *B. megaterium* M3 (Fig. 3). ALPEA and APEA are involved in the transformation of organic and inorganic compounds in soil (Amador *et al*., 1997). An increase of phosphatase activities can improve the P nutrient status of the soil. Mineralization of soil organic P (Po) plays an imperative role in phosphorus cycling of a farming system. Alkaline and APE use organic phosphate as a substrate to convert it into inorganic form. Principal mechanism for mineralization of soil organic P is the production of acid phosphatases (Rodriguez and Fraga, 1999). Release of organic anions and production of siderophores and acid phosphatase by plant roots/microbes or alkaline phosphatase (Tarafdar *et al*., 1988) enzymes hydrolyze the soil organic P or split P from organic residues.

The findings showed that PGPR strains consistently increase plant growth and yield and alleviate some deleterious stress of plant with having organic acid, amino acid, hormone and nutrient content quality of crops. In agreement with other reports (Sahin *et al*., 2004; Khan and Zaidi, 2007), the data suggested that bio-inoculation of PGPR strains can improve growth, nutrient uptake and the nutritional quality as shown for barley (Cakmakci *et al*., 1999; Sahin *et al*., 2004) and in pearl millet and blackgram (Poonguzhali *et al*., 2005), potentials for improving plant yields by combining PGPR by co-inoculation have also been a subject of several researchers for more than a decade (Cakmakci *et al*., 1999; Felici *et al*., 2008). Seed inoculation of the *A. brasilense* (Madhaiyan *et al*., 2010), B. OSU-142 and B. M-3 (Sahin *et al*., 2004) strains alone or under dual inoculation increased the plant growth in terms of shoot or root length and increased the nutrient

uptake in plants. In general, microbial inoculation of seeds with effective B. OSU-142 and *A. brasilense* sp. 245, alone or in mixed inoculation with B. M-3, may substitute costly mineral fertilizers and be used in organic and sustainable agriculture in crop production. Bacteria like *Azospirillum* and *Bacillus* are widely used in organic production systems and they are also important N_2-fixing, P-solubilizing and phytohormone-producing microorganisms, resulting in improved growth and yield of crops (Spaepen *et al*., 2008). One of the most often reported PGPR is M-3 in Turkey which have range of reported properties, including N_2-fixation, P-solubilization, IAA and cytokinin production and increased root and shoot growth and yield (Sahin *et al*., 2004; Cakmakci *et al*., 2006, 2007b; Karakurt *et al*., 2011).

It is well known that PGPR strains that produce plant hormones such as auxins and cytokinins can stimulate plant cell elongation or cell division and/or change bacterial 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity (Cakmakci *et al*., 2007a) which prevents the production of the plant growth-inhibiting hormone, ethylene (Patten and Glick, 2002; Penrose *et al*., 2001). Rhizosphere bacteria's ability of solubilize insoluble P minerals has been attributed to their capacity to reduce pH by excretion organic acid (Gyaneshwar *et al*., 1999; Mullen, 2005). In previous studies, it was reported that application of *Bacillus megaterium* Tv-17C, *Bacillus megaterium* Tv-3D, *Bacillus megaterium* Tv-87A, *Hafnia alvei* Tv-34A, *Bacillus megaterium* Tv-60D, *Pseudomonas fluorescens* FDG-37, *Bacillus megaterium* KBA-10 and *Bacillus megaterium* Tv-91C strains used in the present study may stimulate yield and quality parameters in some plants such as sugar beet, common vetch and wheat (Erman *et al*., 2010; Fayetorbay *et al*., 2010; Karagoz and Kotan, 2010; Karakurt *et al*., 2011).

In the other hand Karagoz and Kotan (2010) reported that *Pantoea agglomerans* RK-92 and *Bacillus megaterium* KBA-10 strains not only have N_2-fixation, P-solubilization properties and a positive effect on lettuce growth but also ability to suppress bacterial leaf spot of lettuce caused by *Xanthomonas axonopodis* pv. *vitiens*. In addition, Karakurt *et al*. (2010) reported that *Pantoea agglomerans* RK-92 strain caused a statistically significant increase on plant growth parameters of one-year-old saplings at 'sekerpare' apricot cultivar. It's reported that this strains have an antibacterial and/or antifungal activity; can be used as a bacterial biocontrol agents against plat pathogens (Kotan *et al*., 2004, 2009; Kotan and Sahin, 2006).

The present study reveals that PGPR species tested in this study were rich in hormone (gibberellic acid, salicylic acid, indole acetic acid), organic acid (oxalic acid, lactic acid, tartaric acid, malic acid), amino acid (proline, methionine, cystine, asparagine, alanine, proline), minerals (N, P, K Ca, Mg, S, Fe, Cu, Mn, Zn and B), antioxidant enzyme, enzyme activity and nutritional potential for plant growth and their nutritional value was greater than that of some organic fertilizer. These would be more beneficial under environmental or nutrient stress condition. Moreover, PGPR species are the least expensive sources for number of hormone and nutrients and provide macro and micro minerals sustainable or organic farming. Further studies are required to determine the efficiency of PGPR application some cultivated plant under field conditions with multiple soil types and to better understand the additional benefits of these PGPR beyond their chemical capacity, as well as economic feasibility of PGPR addition for varies crops.

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