Fertilizer Efficiency of Some Plant Growth Promoting Rhizobacteria for Plant Growth

Adem Gunes, Kenan Karagoz, Metin Turan, Recep Kotan, Ertan Yildirim, Ramazan Cakmakci and Fikrettin Sahin

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\textsubscript{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), N\textsubscript{2}-fixation (Cakmakci et al., 2001) and solubilization of phosphate (Sahin et al., 2004). The N\textsubscript{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 (Biswa 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\textsubscript{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 (Ataturk 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 potent 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 (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 m 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 m filters (Cutting, 1991). Supernatants were evaporated to dryness at 35°C by evaporator pumps. Dried supernatants were solved using 0.1 M KH₂PO₄ (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$_2$O$_2$. One unit of CAT activity was define as the amount of enzyme that used 1 mol H$_2$O$_2$ min$^{-1}$. The POD activity was measured by monitoring the decrease in absorbance at 470 nm in 50 mM phosphate buffer (pH 5.5) containing 1 mM guaiacol and 0.5 mM H$_2$O$_2$. One unit of POD activity was defined as the amount of enzyme that cause a 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$_2$O$_2$ 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
**Table 1: Amino acid content (pmol L⁻¹) of PGPR**

| Amino acids   | B.M (M3) | P.A (RK-92) | B.M (TV-17C) | B.M (TV-3D) | B.M (TV-87A) | B.M (TV-60D) | H.A. (KBA-10) | B.M (FDG-37) | B.M (B.M) |
|---------------|---------|-------------|--------------|-------------|--------------|--------------|---------------|--------------|-----------|
| Aspartate     | 965±7   | 868±5       | 877±7        | 128±3       | 228±5       | 91±4         | 742±4c        | 582±5       | 433±5     |
| Glutamate     | 15021±20 | 13519±17    | 16947±15     | 7501±6      | 6673±6      | 5187±9       | 10136±6       | 715±16      | 7332±6    |
| Asparagine    | 953±9   | 859±6       | 343±6        | 106±6       | 220±5       | 352±11       | 265±5         | 903±4       | 433±4     |
| Serine        | 814±11  | 735±5       | 876±8        | 151±4       | 240±4       | 81±5         | 455±6         | 532±6       | 408±3     |
| Glutamine     | 941±12  | 847±8       | 810±7        | 414±5       | 390±4       | 523±6        | 652±5         | 420±4       | 467±7     |
| Histidine     | 10585±24| 11126±12    | 12963±12     | 9186±10     | 5531±6      | 5353±7       | 9517±8        | 6897±3      | 7209±5    |
| Glycerine     | 2881±8  | 2593±9      | 594±8        | 412±8       | 813±8       | 711±5        | 255±5         | 214±6       | 114±1     |
| Threonine     | 3289±11 | 2960±8      | 677±5        | 375±6       | 733±5       | 1990±6       | 318±3         | 612±5       | 1359±8    |
| Arginine      | 8452±19 | 9007±10     | 8197±9       | 704±8       | 8290±9      | 8791±9       | 8290±9        | 7574±4      | 6551±4    |
| Alanine       | 1718±11 | 1546±5      | 974±6        | 109±5       | 563±15      | 178±3        | 2551±5        | 1217±3      | 696±6     |
| Tyrosine      | 1149±13 | 1035±6      | 1927±8       | 857±8       | 637±4       | 651±5        | 898±4         | 1089±5      | 503±5     |
| Cystine       | 4956±14 | 4490±8      | 8986±10      | 3997±6      | 2933±10     | 3203±6       | 4228±6        | 2655±3      | 2477±8    |
| Valine        | 2148±12 | 1933±9      | 3725±6       | 1711±7      | 1418±5      | 239±14       | 2069±7        | 1149±4      | 932±6     |
| Methionine    | 1292±9  | 1163±7      | 3242±8       | 1058±5      | 1028±4      | 1341±5       | 1640±6        | 824±3       | 642±4     |
| Tryptophan    | 5273±10 | 4746±9      | 945±7        | 4528±6      | 3262±3      | 3457±4       | 467±4         | 2977±5      | 2450±6    |
| Phenylalanine | 1159±8  | 1043±8      | 1094±6       | 4595±6      | 3143±5      | 786±5        | 312±5         | 223±2       | 526±7     |
| Isoleucine    | 9360±11 | 8424±6      | 9917±8       | 7282±7      | 5065±6      | 5911±6       | 8035±6        | 4952±5      | 4527±8    |
| Leucine       | 654±14  | 755±7       | 1755±8       | 812±5       | 734±5       | 661±4        | 912±5         | 411±3       | 338±6     |
| Lysine        | 11353±11| 10218±18    | 17514±8      | 14205±12    | 14093±11    | 11629±11     | 1068±4        | 10539±14    | 11024±4   |
| Hydroxyproline| 3693±5  | 2472±8      | 4432±5       | 1601±5      | 941±5       | 1205±5       | 1232±4        | 825±5       | 1247±6    |
| Sarcosine     | 4656±11 | 4191±9      | 11671±6      | 3620±3      | 2777±6      | 2962±6       | 6160±6        | 450±4       | 2024±5    |
| Proline       | 6665±8  | 1872±8      | 2080±14      | 2192±4      | 2018±4      | 1792±5       | 2705±5        | 2154±7      | 1023±6    |

Values (n = 25) in the same row with a different letters are significantly different (p<0.05). Mean±standard deviation
Fig. 2(a-c): Antioxidant enzyme activity of (a) CAT, (b) POD and (c) SOD of some studied PGP 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 L⁻¹) of PGPR (n = 25)

| Organic acids | P.A (RR-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       | 191±3        | 692±4        | 368±3        | 110±3        | 387±5        | 403±5        | 620±3        | 413±5        | 735±5       |
| Propionic acid | 1772±6      | 1363±4       | 1143±6       | 640±4        | 783±4        | 1018±5       | 767±7        | 1495±3       | 321±4        | 904±4       |
| Tartaric acid | 893±5       | 705±3        | 1429±5       | 501±5        | 405±3        | 973±4        | 681±5        | 1676±5       | 306±3        | 1986±6      |
| Butyric acid  | 4197±5      | 1033±5       | 997±6        | 829±6        | 504±5        | 1502±6       | 4608±5       | 9256±4       | 5729±4       | 8711±5      |
| Malonic acid  | 20855±12    | 1572±4       | 6036±6       | 3418±4       | 904±3        | 4024±5       | 3895±4       | 5985±3       | 2951±6       | 7093±6      |
| Malic acid    | 3195±5      | 1193±5       | 1126±5       | 452±4        | 686±4        | 697±5        | 844±5        | 1899±5       | 862±4        | 2251±4      |
| Lactic acid   | 58787±4     | 22432±5      | 37057±4      | 20819±11     | 17667±4      | 35082±6      | 49823±7      | 66650±9      | 22544±9      | 62696±5     |
| Citric acid   | 89386±5     | 3889±6       | 7027±3       | 753±3        | 2236±5       | 3417±4       | 7542±4       | 1048±5       | 5296±6       | 2251±4      |
| Maleic acid   | 811±4       | 2016±4       | 3693±5       | 400±6        | 1159±6       | 763±5        | 407±3        | 1473±5       | 198±6        | 1746±4      |
| Fumaric acid  | 990±3       | 2213±3       | 388±3        | 110±9        | 127±5        | 138±3        | 266±3        | 835±3        | 257±3        | 247±5       |
| Succinic acid | 47914±8     | 25074±5      | 4445±4       | 21867±11     | 34417±6      | 26094±6      | 25461±7      | 40427±7      | 2945±4       | 31567±5     |

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, 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 (APEA) of some studied PGP 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 (mg kg⁻¹) | K (mg kg⁻¹) | Mg (mg kg⁻¹) | Na (mg kg⁻¹) | P (mg kg⁻¹) | S (mg kg⁻¹) | N (mg kg⁻¹) |
|--------------|--------------|-------------|--------------|--------------|-------------|-------------|-------------|
| Bacillus megaterium (M3) | 12.40<sup>a</sup> | 207<sup>a</sup> | 5.40<sup>a</sup> | 1457<sup>a</sup> | 81<sup>a</sup> | 75<sup>a</sup> | 1610<sup>a</sup> |
| Pantoea agglomerans (RK-92) | 5.25<sup>c</sup> | 147<sup>c</sup> | 3.08<sup>c</sup> | 951<sup>c</sup> | 48<sup>c</sup> | 58<sup>c</sup> | 580<sup>c</sup> |
| Bacillus megaterium (Tv-17C) | 3.11<sup>d</sup> | 162<sup>d</sup> | 4.61<sup>d</sup> | 821<sup>d</sup> | 38<sup>d</sup> | 33<sup>d</sup> | 290<sup>d</sup> |
| Bacillus megaterium (Tv-3D) | 6.35<sup>d</sup> | 126<sup>d</sup> | 2.15<sup>d</sup> | 725<sup>d</sup> | 26<sup>d</sup> | 43<sup>d</sup> | 475<sup>d</sup> |
| Bacillus megaterium (Tv-87A) | 7.12<sup>d</sup> | 146<sup>d</sup> | 1.66<sup>d</sup> | 825<sup>d</sup> | 38<sup>d</sup> | 54<sup>d</sup> | 360<sup>d</sup> |
| Hafnia alvei (Tv-34A) | 3.61<sup>e</sup> | 170<sup>e</sup> | 2.83<sup>e</sup> | 621<sup>e</sup> | 37<sup>e</sup> | 55<sup>e</sup> | 420<sup>e</sup> |
| Bacillus megaterium (Tv-60D) | 7.22<sup>e</sup> | 135<sup>e</sup> | 3.48<sup>e</sup> | 848<sup>e</sup> | 32<sup>e</sup> | 38<sup>e</sup> | 580<sup>e</sup> |
| Pseudomonas fluorescens (FDG-37) | 2.20<sup>f</sup> | 94<sup>f</sup> | 1.88<sup>f</sup> | 423<sup>f</sup> | 54<sup>f</sup> | 42<sup>f</sup> | 820<sup>f</sup> |
| Bacillus megaterium (KBA-10) | 3.12<sup>f</sup> | 110<sup>f</sup> | 0.99<sup>f</sup> | 800<sup>f</sup> | 42<sup>f</sup> | 40<sup>f</sup> | 660<sup>f</sup> |
| Bacillus megaterium (Tv-91C) | 3.52<sup>f</sup> | 199<sup>f</sup> | 1.56<sup>f</sup> | 938<sup>f</sup> | 68<sup>f</sup> | 49<sup>f</sup> | 750<sup>f</sup> |

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.
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.

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

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

Values in the same column (n = 25) with a different letters are significantly different (p<0.05).
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\textsubscript{3}OHG OHG H\textsuperscript{+}), gaseous molecules (CO\textsubscript{2}, H\textsubscript{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\textsubscript{2}O\textsubscript{3} and MnO\textsubscript{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 have 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 (Bienelt 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 antioxidative enzyme activities, decreasing reactive oxidative oxygen species (ROS) such as H\textsubscript{2}O\textsubscript{2}, O\textsubscript{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. brasiliense* (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 N₂-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 N₂ 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 N₂-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 plant 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.

ACKNOWLEDGMENT
The authors would like to acknowledge the Atatürk University, Agriculture Faculty, Department of Plant Protection and Department of Soil Science, for giving the opportunity to set up the experiment and use of laboratory.
REFERENCES

Ae, N., J. Arihara, K. Okada, T. Yoshihara and C. Johansen, 1990. Phosphorus uptake by pigeon pea and its role in cropping systems of the Indian subcontinent. Science, 248: 477-480.

Ahemad, M. and M.S. Khan, 2010. Comparative toxicity of selected insecticides to pea plants and growth promotion in response to insecticide-tolerant and plant growth promoting Rhizobium leguminosarum. Crop Protect., 29: 325-329.

Aloni, R., E. Aloni, M. Langhans and C.I. Ulrich, 2006. Role of cytokinin and auxin in shaping root architecture: Regulating vascular differentiation, lateral root initiation, root apical dominance and root gravitropism. Ann. Bot., 97: 883-893.

Amador, J.A., A.M. Glucksman, J.B. Lyons and J.H. Gorres, 1997. Spatial distribution of soil phosphatase activity within a riparian forest. Soil Sci., 162: 808-825.

Antoine, F.R., C.I. Wei, R.C. Littell and M.R. Marshall, 1999. HPLC method for analysis of free amino acids in fish using o-phthalaldehyde precolumn derivatization. J. Agric. Food Chem., 47: 5100-5107.

Antoun, H. and D. Prevost, 2006. Ecology of Plant Growth Promoting Rhizobacteria. In: PGPR: Biocontrol and Biofertilization, Siddiqui, Z.A (Ed.). Chapter 1, Springer, Netherlands, ISBN-13: 9781402040023, pp: 1-38.

Aristoy, M.C. and F. Toldra, 1991. Deproteinization techniques for HPLC amino acid analysis in fresh pork muscle and dry-cured ham. J. Agric. Food Chem., 39: 1792-1795.

Asad, S., M. Arshad, S. Mansoor and Y. Zafar, 2009. Effect of various amino acids on shoot regeneration of sugarcane (Saccharum officinarum L.). Afr. J. Biotechnol., 8: 1214-1218.

Bai, C.E., Q. Liu, J. Lu, F. Song and J. Zhang, 2003. Corporate governance and market valuation in China. William Davidson Working Paper, 564, The William Davidson Institute at the University of Michigan Business School, Michigan, USA., May 2003.

Barlow, P.W., P. Brain and J.S. Parker, 1991. Cellular growth in roots of a gibberellin-deficient mutant of tomato (Lycopersicon esculentum Mill.) and its wild-type. J. Exp. Bot., 42: 339-351.

Bashan, Y. and L.E. de Bashan, 2005. Bacteria/Plant Growth-Promotion. In: Encyclopedia of Soils in the Environment, Hillel, D. and J.L. Hatfield (Eds.). Elsevier/Academic Press, Boston, MA., ISBN-13: 9780123485304, pp: 103-115.

Battal, P. and B. Tileklieglu, 2001. The effects of different mineral nutrients on the levels of cytokinins in Maize (Zea mays L.). Turk. J. Bot., 25: 123-130.

Biemelt, S., U. Keetman, H.P. Mock and B. Grimm, 2000. Expression and activity of isoenzymes of superoxide dismutase in wheat roots in response to hypoxia and anoxia. Plant Cell Environ., 23: 135-144.

Biswas, J.C., L.K. Ladha and F.B. Dazzo, 2000. Rhizobia inoculation improves nutrient uptake and growth of lowland rice. Soil Sci. Soc. Am. J., 64: 1644-1650.

Bolan, N.S., R. Naidu, S. Mahimairaja and S. Baskaran, 1994. Influence of low-molecular-weight organic acids on the solubilization of phosphates. Biol. Fertil. Soils, 18: 311-319.

Bottini, R., F. Cassan and P. Piccoli, 2004. Gibberellin production by bacteria and its involvement in plant growth promotion and yield increase. Applied Microbiol. Biotechnol., 65: 497-503.

Bradley, D.B. and D.H. Sieling, 1953. Effect of organic anions and sugars on phosphate precipitation by iron and aluminum as influenced by pH. Soil Sci., 76: 175-179.

Bremner, J.M., 1996. Nitrogen-Total. In: Methods of Soils Analysis: Chemical Methods, Sparks, D.L. (Ed.). American Society of Agronomy (ASA) and Soil Science Society of America (SSSA), Madison, WI., USA., pp: 1085-1121.
Caballero-Mellado, J., J. Onofre-Lemus, P. E.L. Santos and L. Martinez-Aguilar, 2007. The tomato rhizosphere, an environment rich in nitrogen-fixing Burkholderia species with capabilities of interest for agriculture and bioremediation. Applied Environ. Microbiol., 73: 5308-5319.

Cacciari, I., D. Lippi, T. Pietrosanti and W. Pietrosanti, 1989. Phytohormone-like substances produced by single and mixed diazotrophic cultures of Azospirillum and Arthrobacter. Plant Soil, 115: 151-153.

Caceres, E.A.R., G.G. Anta, J.R. Lopez, C.A. di Ciocco, J.C.P. Basurco and J.L. Parada, 1996. Response of field-grown wheat to inoculation with Azospirillum brasilense and Bacillus polymyxa in the semiarid region of Argentina. Soils Fertil., 10: 13-20.

Cakmakci, R., F. Kantar, O.F. Algur, 1999. Sugar beet and barley yields in relation to Bacillus polymyxa and Bacillus megaterium var. iphosphaticum inoculation. J. Plant Nutr. Soil Sci., 162: 437-442.

Cakmakci, R., F. Kantar and F. Sahin, 2001. Effect of N2-fixing bacterial inoculations on yield of sugar beet and barley. J. Plant Nutr. Soil Sci., 164: 527-531.

Cakmakci, R., F. Donmez, a. Aydin and F. Sahin, 2006. Growth promotion of plants by plant growth-promoting rhizobacteria under greenhouse and two different field soil conditions. Soil Biol. Biochem., 38: 1482-1487.

Cakmakci, R., M. Erat, U. Erdogan and M.F. Donmez, 2007a. The influence of plant growth-promoting rhizobacteria on growth and enzyme activities in wheat and spinach plants. J. Plant Nutr. Soil Sci., 170: 288-295.

Cakmakci, R., M.F. Donmez and U. Erdogan, 2007b. The effect of plant growth promoting rhizobacteria on barley seedling growth, nutrient uptake, some soil properties and bacterial counts. Turk. J. Agric. For., 31: 189-199.

Cakmakci, R., M. Turan, M. Gulluce and F. Sahin, 2014. Rhizobacteria for reduced fertilizer inputs in wheat (Triticum aestivum spp. vulgare) and barley (Hordeum vulgare) on Aridisols in Turkey. Int. J. Plant Prod., 8: 1735-8043.

Cheik, N. and R.J. Jones, 1994. Disruption of maize kernel growth and development by heat stress (role of cytokinin/abscisic acid balance). Plant Physiol., 106: 45-51.

Chen, W.S., 1991. Changes in cytokinins before and during early flower bud differentiation in Lychee (Litchi chinensis Sonn.). Plant Physiol., 96: 1203-1206.

Cuppels, D., F. Sahin and S.A. Miller, 1999. Management of bacterial spot of tomato and pepper using a plant resistance activator in combination with microbial biocontrol agents. Phytopathology, 89: 19-25.

Cutting, J.G.M., 1991. Determination of the cytokinin complement in healthy and witchesbroom malformed protease. J. Plant Growth Regul., 10: 85-89.

Davies, P.J., 1995. The Plant Hormones: Their Nature, Occurrence and Functions. In: Plant Hormones; Physiology, Biochemistry and Molecular Biology, Davies, P.J. (Ed.). Kluwer Academic Publishers, Dordrecht, Boston, pp: 1-38.

Davison, J., 1988. Plant beneficial bacteria. Nature Biotechnol., 6: 282-286.

De Freitas, J.R., M.R. Banerjee and J.J. Germida, 1997. Phosphate-solubilizing rhizobacteria enhance the growth and yield but not phosphorus uptake of canola (Brassica napus L.). Biol. Fertil. Soils, 24: 358-364.

De Salamone, I.E.G., R.K. Hynes and L.N. Nelson, 2001. Cytokinin production by plant growth promoting rhizobacteria and selected mutants. Can. J. Microbiol., 47: 404-411.
Dhindsa, R.S., P. Plumb-Dhindsa and T.A. Thorpe, 1981. Leaf senescence: Correlated with increased levels of membrane permeability and lipid peroxidation and decreased levels of superoxide dismutase and catalase. J. Exp. Bot., 32: 93-101.

Elkoca, E., F. Kantar and F. Sahin, 2007. Influence of nitrogen fixing and phosphorus solubilizing bacteria on the nodulation, plant growth and yield of chickpea. J. Plant Nutr., 31: 157-171.

Erman, M., R. Kotan, R. Cakmakci, F. Cig, F. Karagoz and M. Sezen, 2010. Effect of nitrogen fixing and phosphate-solubilizing Rhizobacteria isolated from Van Lake Basin on the growth and quality properties in wheat and sugar beet. Proceedings of the Turkey IV Organic Farming Symposium, June 28-July 1, 2010, Erzurum, Turkey, pp: 325-329.

Esitken, A., H. Karlidag, S. Ercisli and F. Sahin, 2002. Effect of foliar application of Bacillus subtilis Osu-142 on the yield growth and control of shot-hole disease (Corneum blight) of apricot. Gartenbauwissenschaft, 67: 139-142.

Esitken, A., H. Karlidag, S. Ercisli, M. Turan and F. Sahin, 2003. The effect of spraying a growth promoting bacterium on the yield, growth and nutrient element composition of leaves of apricot (Prunus armeniaca L. cv. Hacihaliloglu). Aust. J. Agric. Res., 54: 377-380.

Esitken, A., L. Pirlak, M. Turan and F. Sahin, 2006. Effects of floral and foliar application of plant growth promoting rhizobacteria (PGPR) on yield, growth and nutrition of sweet cherry. Scientia Horticulturae, 110: 324-327.

Evans, A. and T.J. Anderson, 1990. Aliphatic acids: Influence on sulfate mobility in a forested cecil soil. Soil Sci. Soc. Am. J., 54: 1136-1139.

Farzana, Y. and O. Radizah, 2005. Influence of rhizobacterial inoculation on growth of the sweetpotato cultivar. Am. J. Biochem. Biotechnol., 1: 176-179.

Fayetorbay, D., K. Karagoz, F. Dadasoglu, B. Comakli, R. Cakmakci and R. Kotan, 2010. Common vetch (Vicia sativa) growth and yield in relation to single and mixed cultures of plant growth promoting bacteria, mineral and organic fertilizers. Proceedings of the Turkey IV Organic Farming Symposium, June 28-July 1, 2010, Erzurum, Turkey, pp: 696-701.

Felici, C., L. Vettori, E. Giraldi, L.M.C. Forino, A. Toffanin, A.M. Tagliasacchi and M. Nuti, 2008. Single and co-inoculation of Bacillus subtilis and Azospirillum brasilense on Lycopersicon esculentum: Effects on plant growth and rhizosphere microbial community. Applied Soil Ecol., 40: 260-270.

Fox, T.R. and N.B. Comerford, 1990. Low-molecular-weight organic acids in selected forest soils of the southeastern USA. Soil Sci. Soc. Am. J., 54: 1139-1144.

Fukaki, H., Y. Okushima and M. Tasaka, 2007. Auxin-mediated lateral root formation in higher plants. Int. Rev. Cytol., 256: 111-137.

Glick, B.R., 1995. The enhancement of plant growth by free-living bacteria. Can. J. Microbiol., 41: 109-117.

Gunes, A., M. Turan, M. Gulluce and F. Sahin, 2014. Nutritional content analysis of plant growth-promoting rhizobacteria species. Eur. J. Soil Biol., 60: 88-97.

Gutierrez-Manero, F.J., B. Ramos-Solano, a. Probanza, J. Mehouachi, F.R. Tadeo and M. Talon, 2001. The plant-growth-promoting rhizobacteria Bacillus pumilus and Bacillus licheniformis produce high amounts of physiologically active gibberellins. Physiol. Plant., 111: 206-211.

Gyaneshwar, P., L.J. Parekh, G. Archana, P.S. Poole, M.D. Collins, R.A. Hutson and G.N. Kumar, 1999. Involvement of a phosphate starvation inducible glucose dehydrogenase in soil phosphate solubilization by Enterobacter asburiae. FEMS Microbiol. Lett., 171: 223-229.
Han, H.S. and K.D. Lee, 2005. Physiological responses of soybean-Inoculation of Bradyrhizobium japonicum with PGPR in saline soil conditions. Res. J. Agric. Biol. Sci., 1: 216-221.

Henderson, J.W., R.D. Ricker, B.A. Bidlingmeyer and C. Woodward, 2000. Rapid, accurate and reproducible HPLC analysis of amino acids. Amino acid analysis using Zorbax Eclipse AAA columns and the Agilent 1200 HPLC. Agilent Technologies 2000, Part No5980-1193E:10.

Hernandez-Minea, F.M., 1991. Identification of cytokinins and the changes in their endogenous levels in developing Citrus sinensis leaves. J. Horticult. Sci., 66: 505-511.

Hoflich, G., W. Wiehe and G. Kuhn, 1994. Plant growth stimulation by inoculation with symbiotic and associative rhizosphere microorganisms. Experientia, 50: 897-905.

Jones, D.L. and P.R. Darrah, 1994. Role of root derived organic acids in the mobilization of nutrients from the rhizosphere. Plant Soil, 66: 247-257.

Jones, D.L., 1998. Organic acids in the rhizosphere: a critical review. Plant Soil, 205: 25-44.

Kang, H.M. and M.E. Saltveit, 2001. Activity of enzymatic antioxidant defense systems in chilled and heat shocked cucumber seedling radicles. Physiol. Plant., 113: 548-556.

Karagoz, K. and R. Kotan, 2010. Effects of some plant growth promoting bacteria on growth of lettuce and bacterial leaf spot disease. Turkey Biyoloji Mucadele Dergisi, 1: 165-179.

Karakurt, H., R. Kotan, F. Dadasoglu, R. Aslantas and F. Sahin, 2011. Effects of plant growth promoting rhizobacteria on fruit set, pomological and chemical characteristics, color values and vegetative growth of sour cherry (Prunus cerasus cv. Kutahya). Turk. J. Biol., 35: 283-291.

Karakurt, H., R. Kotan, R. Aslantas, F. Dadasoglu and K. Karagoz, 2010. Inoculation effects of Pantoea agglomerans strains on growth and chemical composition of plum. J. Plant Nutr., 33: 1998-2009.

Khalid, A., M. Arshad and Z.A. Zahir, 2004. Screening plant growth-promoting Rhizobacteria for improving growth and yield of wheat. J. Applied Microbio., 96: 473-480.

Khan, M.S. and A. Zaidi, 2007. Synergistic effects of the inoculation with plant growth-promoting rhizobacteria and an arbuscular mycorrhizal fungus on the performance of wheat. Turk. J. Agric. For., 31: 355-362.

Kloepfer, J.W. and C.J. Beauchamp, 1992. a review of issues related to measuring colonization of plant roots by bacteria. Can. J. Microbiol., 38: 1219-1232.

Kotan, R. and F. Sahin, 2006. Biological control of Pseudomonas syringae pv. syringae and nutritional similarity in carbon source utilization of pathogen and its potential biocontrol agents. J. Turk. Phytopathol., 35: 1-13.

Kotan, R., F. Sahin and A. Ala, 2004. Nutritional similarity in carbon source utilization of Erwinia amylovora and its potential biocontrol agents. J. Turk. Phytopathol., 33: 25-38.

Kotan, R., F. Sahin and A. Ala, 2005. Identification and pathogenicity of bacteria isolated from pome fruits trees in eastern Anatolia region of Turkey. J. Plant Dis. Protect., 113: 8-13.

Kotan, R., N. Dikbas and H. Bostan, 2009. Biological control of post harvest disease caused by Aspergillus flavus on stored lemon fruits. Afr. J. Biotechnol., 8: 209-214.

Kuepper, G.L., H. Born and J. Bachmann, 2003. Organic culture of bramble fruits. Horticulture Production Guides. http://www.agmrc.org/media/cms/bramble_b301192e65279.pdf

Kumar, V. and D.R. Sharma, 1989. Effect of exogenous proline on growth and ion content in NaCl stressed and nonstressed cells of mungbean, Vigna radiata var. radiate. Ind. J. Exp. Biol., 27: 813-815.

Kuraishi, S., K. Tasaki, N. Sakurai and K. Sadatoku, 1991. Changes in levels of cytokinins in etiolated squash seedlings after illumination. Plant Cell Physiol., 32: 585-591.
Lind, K., G. Lafer, K. Schloffer, G. Innerhoffer and H. Meister, 2003. Organic Fruit Growing. CABI Publishing, Wallingford, UK., ISBN-13: 9781845933401, Pages: 281.

Liu, Z.L. and J.B. Sinclair, 1993. Colonization of soybean roots by Bacillus megaterium B 153-2-2. Soil Biol. Biochem., 25: 849-855.

Lone, M.I., J.S.H. Kueh, R.G.W. Jones and S.W.J. Bright, 1987. Influence of proline and glycinebetaine on salt tolerance of cultured barley embryos. J. Exp. Bot., 38: 479-490.

Madhaiyan, M., S. Poonguzhali, B.G. Kang, Y.J. Lee, J.B. Chung and T.M. Sa, 2010. Effect of co-inoculation of methylotrophic Methylobacterium oryzae with Azospirillum brasilense and Burkholderia pyrocinia on the growth and nutrient uptake of tomato, red pepper and rice. Plant Soil, 328: 71-82.

Malhotra, M. and S. Srivastava, 2009. Stress-responsive indole-3-acetic acid biosynthesis by Azospirillum brasilense SM and its ability to modulate plant growth. Eur. J. Soil Biol., 45: 73-80.

Marschner, H., 1995. Mineral Nutrition of Higher Plants. 2nd Edn., Academic Press Ltd., London, UK., ISBN-13: 978-0124735439, Pages: 889.

Mertens, D., 2005a. AOAC Official Method 922.02. In: Plants Preparation of Laboratory Sample: Official Methods of Analysis, Horwitz, W. and G.W. Latimer (Eds.). 18th Edn., Chapter 3, AOAC-International, Gaitherburg, Maryland, USA., pp: 1-2.

Mertens, D., 2005b. AOAC Official Method 975.03. In: Metal in Plants and Pet Foods: Official Methods of Analysis, Horwitz, W. and G.W. Latimer (Eds.). 18th Edn., Chapter 3, AOAC-International Suite 500, 481. North Frederick Avenue, Gaitherburg, Maryland, USA., pp: 3-4.

Mooney, P.A. and J. van Staden, 1984. Seasonal changes in the levels of endogenous cytokinins in Sargassum heterophyllum (Phaeophyceae). Bot. Mar., 27: 437-442.

Mullen, M.D., 2005. Phosphorus in Soils: Biological Interactions. In: Encyclopedia of Soils in the Environment, Hillel, D., J.L. Hatfield, D.S. Powlson, C. Rosenzweig and K.M. Scow (Eds.). Vol. 3. Elsevier, London, UK., ISBN-13: 9780123485304, pp: 210-215.

O'Connell, P.F., 1992. Sustainable agriculture: a valid alternative. Outlook Agric., 21: 5-12.

Ogut, M. and F. Er, 2006. Micronutrient composition of field-grown dry bean and wheat inoculated with Azospirillum and Trichoderma. J. Plant Nutr. Soil Sci., 169: 699-703.

Ohwaki, Y. and H. Hirata, 1992. Differences in carboxylic acid exudation among P-starved leguminous crops in relation to carboxylic acid contents in plant tissues and phospholipid level in roots. Soil Sci. Plant Nutr., 38: 235-243.

Orhan, E., A. Esitken, S. Ercisli, M. Turan and F. Sahin, 2006. Effects of Plant Growth Promoting Rhizobacteria (PGPR) on yield, growth and nutrient contents in organically growing raspberry. Sci. Horticul., 111: 38-43.

Ortiz-Castro, R., E. Valencia-Cantero and J. Lopez-Bucio, 2008. Plant growth promotion by Bacillus megaterium involves cytokinin signaling. Plant Signal. Behav., 3: 263-265.

Ozturk, A., O. Caglar and F. Sahin, 2003. Yield response of wheat and barley to inoculation of Plant growth promoting rhizobacteria at various levels of nitrogen fertilization. J. Plant Nutr. Soil Sci., 166: 262-266.

Pal, S.S., 1998. Interactions of an acid tolerant strain of phosphate solubilizing bacteria with a few acid tolerant crops. Plant Soil, 198: 169-177.

Palni, L.M., R.E. Summons and D.S. Letham, 1983. Mass spectrometric analysis of cytokinins in plant tissues: V. Identification of the cytokinin complex of datura innoxia crown gall tissue. Plant Physiol., 72: 858-863.
Parfitt, R.L., 1979. The availability of P from phosphate-goethite bridging complexes. Desorption and uptake by ryegrass. Plant Soil, 53: 55-65.
Patten, C.L. and B.R. Glick, 1996. Bacterial biosynthesis of indole-3-acetic acid. Can. J. Microbiol., 42: 207-220.
Patten, C.L. and B.R. Glick, 2002. Role of Pseudomonas putida indole-acetic acid in development of the host plant root system. Applied Environ. Microbiol., 68: 3795-3801.
Penrose, D.M., B.A. Moffat and B.R. Glick, 2001. Determination of 1-aminocycopropane-1-carboxylic acid (ACC) to assess the effects of ACC deaminase-containing bacteria on roots of canola seedlings. Can. J. Microbiol., 47: 77-80.
Perrig, D., M.L. Boiero, O.A. Masciarelli, C. Penna, O.A. Ruiz, F.D. Cassan and M.V. Luna, 2007. Plant-growth-promoting compounds produced by two agronomically important strains of Azospirillum brasilense and implications for inoculant formulation. Applied Microbiol. Biotech., 75: 1143-1150.
Podile, A.R. and K.G. Kishore, 2007. Plant Growth-Promoting Rhizobacteria. In: Plant-Associated Bacteria, Gnanamanickam, S.S. (Ed.). Springer, New York, USA., ISBN-13: 9781402045370, pp: 195-230.
Poonguzhali, S., M. Madhaiyan, M. Thangaraju, J. Ryu, K. Chung and T. Sa, 2005. Effects of co-cultures, containing N-fixer and P-solubilizer, on the growth and yield of pearl millet (Pennisetum glaucum (L.) R. Br.) and blackgram (Vigna mungo L.). J. Microbiol. Biotech., 15: 903-908.
Poonguzhali, S., M. Madhaiyan and T. Sa, 2008. Isolation and identification of phosphate solubilizing bacteria from Chinese cabbage and their effect on growth and phosphorus utilization of plants. J. Microbiol. Biotechnol., 18: 773-777.
Qamaruddin, M., 1996. Appearance of the zeatin riboside type of cytokinin in Pinus sylvestris seeds after red light treatment. Scand. J. For. Res., 6: 41-46.
Rai, V.K. and U. Rana, 1996. Modulation of calcium uptake by exogenous amino acids, in Phaseolus vulgaris seedlings. Acta Physiol. Plant., 18: 117-120.
Rai, V.K. and U.D. Sharma, 1991. Amino acids can modulate ABA induced stomatal closure, stomatal resistance and K fluxes by exogenous amino acids in Vicia faba leaves. Beitr Biol. Plant, 66: 393-405.
Recep, K., S. Fikrettin, D. Erkol and E. Cafer, 2009. Biological control of the potato dry rot caused by Fusarium species using PGPR strains. Biol. Control, 50: 194-198.
Riefler, M., O. Novak, M. Strnad and T. Schmulling, 2006. Arabidopsis cytokinin receptor mutants reveal functions in shoot growth, leaf senescence, seed size, germination, root development and cytokinin metabolism. Plant Cell, 18: 40-54.
Rodriguez, H. and R. Fraga, 1999. Phosphate solubilizing bacteria and their role in plant growth promotion. Biotechnol. Adv., 17: 319-339.
SPSS., 2004. SPSS® 13.0 Base User's Guide. SPSS Inc., Chicago, IL., USA.
Sahin, F., R. Cakmakci and F. Kantar, 2004. Sugar beet and barley yields in relation to inoculation with N2-fixing and phosphate solubilizing bacteria. Plant Soil, 265: 123-129.
Sairam, R.K. and G.C. Srivastava, 2002. Changes in antioxidant activity in sub-cellular fractions of tolerant and susceptible wheat genotypes in response to long-term salt stress. Plant Sci., 162: 897-904.
Silverman, E.P., A.A. Assiamah and D.S. Bush, 1998. Membrane transport and cytokinin action in root hairs of Medicago sativa. Planta, 205: 23-31.
Smith, W.H., 1969. Release of organic materials from the roots of tree seedlings. For. Sci., 15: 138-143.

Smith, W.H., 1976. Character and significance of forest tree root exudates. Ecology, 57: 324-331.

Spaepen, S., J. Vanderleyden and R. Remans, 2007. Indole-3-acetic acid in microbial and microorganism-plant signaling. FEMS Microbiol. Rev., 31: 425-448.

Spaepen, S., S. Dobbelaeere, A. Croonenborghs and J. Vanderleyden, 2008. Effects of Azospirillum brasilense indole-3-acetic acid production on inoculated wheat plants. Plant Soil, 312: 15-23.

Struthers, P.H. and D.H. Sieling, 1950. Effect of organic anions on phosphate precipitation by iron and aluminum as influenced by pH. Soil Sci., 69: 205-213.

Tabatabai, M.A., 1982. Soil Enzymes. In: Methods of Soil Analysis, Part 2, Agronomy 9, American Society of Agronomy, Page, A.L., R.H. Miller and D.R. Keeney (Eds.). Madison, Wis., USA., pp: 903-947.

Tan, R.X. and W.X. Zou, 2001. Endophytes: a rich source of functional metabolites. Nat. Prod. Rep., 18: 448-459.

Taraftdar, J.C., A.V. Rao and K. Bala, 1988. Production of phosphatases by fungi isolated from desert soils. Folia Microbiol., 33: 453-457.

Thakur, P.S. and V.K. Rai, 1985. Exogenously supplied amino acids and water deficits in Zea mays cultivars. Biol. Plant, 27: 485-461.

Timmusk, S., B. Nicander, U. Granhall and E. Tillberg, 1999. Cytokinin production by Paenibacillus polymyxa. Soil Biol. Biochem., 31: 1847-1852.

Turan, M. and C. Kose, 2004. Seaweed extracts improve copper uptake of grapevine. Acta Agric. Scand. Sec. B: Soil Plant Sci., 54: 213-220.

Turan, M., N. Ataoglu and F. Sahin, 2006. Evaluation of the capacity of phosphate solubilizing bacteria and fungi on different forms of phosphorus in liquid culture. J. Sustainable Agric., 28: 99-108.

Turan, M., N. Ataoglu and F. Sahin, 2007. Effects of Bacillus FS-3 on growth of tomato (Lycopersicon esculentum L.) plants and availability of phosphorus in soil. Plant Soil Environ., 53: 58-64.

Turan, M., M. Gulluce and F. Sahin, 2012. Effects of plant-growth-promoting rhizobacteria on yield, growth and some physiological characteristics of wheat and barley plants. Commun. Soil Sci. Plant Analy., 43: 1658-1673.

Turkmen, O., S. Sensoy, A. Dursun and M. Turan, 2004. Sewage sludge as a substitute for mineral fertilization of Spinach (Spinacia oleracea L.) at two growing periods. Acta Agric. Scand. Sec. B: Soil Plant Sci., 54: 102-107.

Vancura, V. and A. Hovadik, 1965. Root exudates of plants: II. Composition of root exudates of some vegetables. Plant Soil, 22: 21-32.

Yaxley, J.R., J.J. Ross, L.J. Sherriff and J.B. Reid, 2001. Gibberellin biosynthesis mutations and root development in pea. Plant Physiol., 125: 627-633.

Zahir, Z.A., M. Arshad and W.T. Frankenberger Jr., 2004. Plant growth promoting rhizobacteria: Applications and perspectives in agriculture. Adv. Agron., 81: 97-168.

Zaidi, A., M.S. Khan, M. Ahemad and M. Oves, 2009. Plant growth promotion by phosphate solubilizing bacteria. Acta Microbiologica Immunologica Hungarica, 56: 263-284.

Zhang, F., N. Dashti, H. Hynes and D.L. Smith, 1996. Plant growth promoting rhizobacteria and soybean [Glycine max (L.) Merr.] nodulation and nitrogen fixation at suboptimal root zone temperatures. Ann. Bot., 77: 453-460.