ISSN 0367-6315 (Print) / ISSN 2288-2162 (Online)
Korean J. Soil Sci. Fert. 47(3), 141-146 (2014)
http://dx.doi.org/10.7745/KJSSF.2014.47.3.141

Article

Plant Growth Promoting Activities of Some Rhizosphere Bacteria and their Effect on *Brassica rapa* Growth

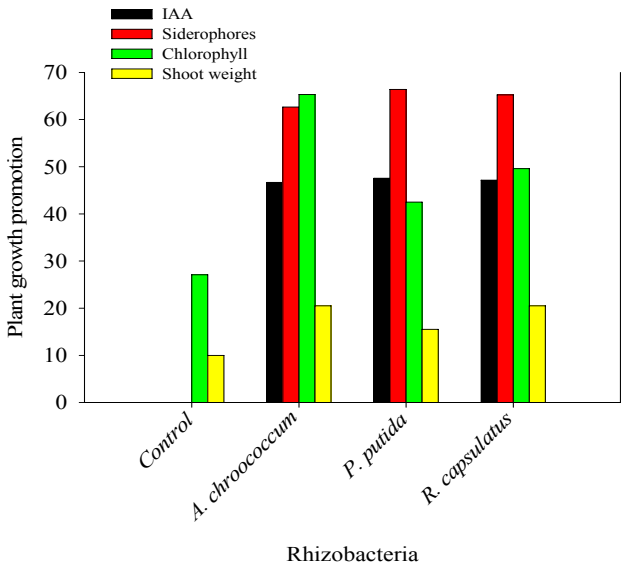
Khalid A. Hussein, Yeong Sang Jung, and Jin Ho Joo*

Department of Biological Environment, Kangwon National University, Chuncheon, Kangwon-do, Republic of Korea

(Received: November 26 2013, Accepted: May 7 2014)

The necessity to develop economical and eco-friendly technologies is steadily increasing. Plant growth promoting rhizomicrobial strains PGPR are a group of microorganisms that actively colonize plant roots and increase plant growth and yield. Pot experiments were used to investigate the potential of some rhizobacterial strains to enhance the *Brassica rapa* growth. Microbial strains were successfully isolated from the rhizosphere of *Panax ginseng* and characterized based on its morphological and plant growth promotion characters. Surface disinfected seeds of Wisconsin Fast *B. rapa* were inoculated with the selected PGPR microorganisms. The different pots treatments were inoculated by its corresponding PGPR (10^7 cfu mL^{-1}) and incubated in the growth chamber at 25°C and 65% RH, the light period was adjusted to 24 hours (day). NPK chemical fertilizer and trade product (EMRO, USA) of effective microorganisms as well as un-inoculated control were used for comparison. Plants harvested in 40 days were found to have significant increase in leaf chlorophyll units and plant height and also in dry weight of root and shoot in the inoculated seedlings. Root and shoot length and also leaf surface area significantly were increased by bacterial inoculation in sterile soil. The study suggests that *Rhodobacter capsulatus* and *Azotobacter chroococcum* are beneficial for *B. rapa* growth as they enhance growth and induced IAA production and phosphorus solubilization. This study presents some rhizomicrobial strains that significantly promoted growth of Wisconsin Fast Plant *B. rapa* in pot experiment under different soil conditions.

Key words: Pots, Rhizobacterial strains, PGPR, *Brassica rapa*



Effect of rhizobacterial inoculations for plant growth promotion traits on the chlorophyll, IAA, Siderophores, and plant weight of *Brassica rapa*.

*Corresponding author : Phone: +821053796229, E-mail: jhjoo@kangwon.ac.kr
§Acknowledgement: This study was supported by the National Research Foundation of Korea Grant funded by the Koran government (Project number: NRF-2013R1A1A2011950) and 2013 Research Grant from Kangwon National University(No. 120131336)

Introduction

The environmental-unfriendly nature and the high production cost of the chemical fertilizers, have led to microbial biofertilizers and the plant growth promoting rhizobacteria in order to improve the crop yield and also maintain the environment. The microbial inoculants which are prepared for enhancing plant health and growth are generally referred to effective microorganisms or biofertilizers that are mostly isolated from rhizosphere. The rhizosphere is the part of soil surrounding the root where complex interactions (are active) between the root and the present microorganisms (Sylvia et al., 1998). Plant growth promoting rhizobacteria (PGPR) bacteria are those which colonize plant roots and enable to increase plant growth and yield (Wu et al., 2005). Active motility using flagella and chemotactic responses facilitate PGPR to reach root surfaces (Steenhoudt and Vanderleyden 2000). Root exudates such as organic acids, amino acids, and certain sugars may be helpful in chemotaxis as attractants (Welbaum et al., 2004). High concentration of easily degradable substrates in root exudates lead to a proliferation of microorganisms in rhizosphere and thus plant select and maintain its specific microflora that may have neutral, deleterious or beneficial effects on the plant (Bais et al., 2006). It is not fully understood how PGPRs promote plant growth, however, there are supposed mechanisms, such as-production of phytohormons (Egamberdiyeva, 2007), asymbiotic N_2 fixation (Mrkovack and Milic 2001), biosynthesis of siderophores (Salantur et al., 2006), solubilisation of inorganic phosphates (Cattelan et al., 1999), secretion of antibiotics, enzymes and/or fungicidal compounds (Ahmad et al., 2006). Due to their ability to fix the atmospheric nitrogen and nodulation in the root, rhizobia were the first classified species as biofertilizers and have been packaged as commercial inoculants (Kannaiyan, 2002). *Azotobacter* and *Azospirillum* strains application increased canola yield (21.17%), pod per plant (16.05%) and number of branches (11.78%) (Yasari and Patwardhan, 2007). Shaharoona et al., (2006) stated that seed inoculation with rhizobacteria enhanced shoot dry weight and yield of maize. Promotion in plant growth and yields of different crops in response to inoculation with rhizomicroorganisms was demonstrated by other researchers (Gravel et al., 2007; Kozdroja et al., 2004; Shaharoona et al., 2006). The aim of the study was to investigate the potential of some rhizobacterial strains like *Pseudomonas putida*, and *Azotobacter chroococcum* that possess plant growth promotion characteristics to enhance the *Brassica rapa* growth in pots experiment.

Materials and Methods

Microorganisms and growth conditions Bacterial strains were cultivated on different Agar media. Various species were isolated using dilution plate method on Nutrient Agar medium according to their different colonies shapes. Pseudomonas Agar selective media was used to obtain the *Pseudomonas* species. The media was prepared by adding pancreatic digest of casein 10 g; proteose peptone 10 g; K_2HPO_4 1.5 g; $MgSO_4.7H_2O$ 1.5 g; and agar 15 g to 1,000 mL of distilled water. Stock cultures of bacteria were maintained on nutrient agar grown at 31°C. *Azotobacter* sp was selected on *Azotobacter* Agar Modified Ⅰ media.

Identification and characterization of the rhizosphere bacterial isolates Bacterial colonies with different characters were tested biochemically and identified using the Bergy's manual of systematic Bacteriology. Moreover, universal primer 16F27N (5-CCAGAGTTTGATCMTGGCT CAG-3) according to Brosius et al. (1978) was used for the amplification of 16S r-RNA of the bacterial strains. A representative sequence was queried against NCBI GenBank BLAST search. Salkowski indicator was used to measure the isolates' ability to produce IAA auxin (Benizri et al., 1998), and phosphate solubilization (Pikovskaya, 1948), and siderophore production was analyzed on chrome azurol S agar plates (Schywn and Nielands, 1987).

Phosphate solubilizing activity Tricalcium phosphate (Ca_3 $(PO_4)_2$) was considered as a model compound for measuring the potential or relative rates of microbial solubilization. Solubilization of precipitated Ca_3 $(PO_4)_2$ in solid agar medium (contained g L^{-1}: yeast extract, 0.5; dextrose, 10; calcium phosphate, 5; ammonium sulphate, 0.5; potassium chloride, 0.2; magnisium sulphate, 0.1; manganese sulphate, 0.0001; ferrous sulphate, 0.0001, agar; 15) has been used for the isolation of phosphate solubilizing microorganisms (Pikovskaya 1948). Rhizosphere microorganisms on precipitated calcium phosphate agar produces clear zones around their colonies if they are capable of solubilizing calcium phosphate. Phosphate solubilization was estimated quantitatively by inoculating loop full of spore's suspensions of the isolates in 10 mL Pikovskaya's broth at initial pH adjusted to 7. Bacteria were incubated at 30°C for 3 days and fungi at 27°C for 7 days. The soluble inorganic P concentration was analyzed by IC (Ion Chromatography).

Pots experiment The study was conducted in growth chamber (DS 54 GLP, DASOL Scientific Co., Ltd., Korea). Clean pots (7.5 x 7.5 x 9 cm) containing approximately 500 g soil were filled with the prepared collected soil. Agriculture soil was collected from Korean ginseng field near Kangwon National University, Chuncheon, Kangwon-do, Korea, air-dried, ground, sieved (2 mm mesh) and analyzed for physico-chemical characteristics. The experimental soil were analyzed for pH (McLean, 1982), texture (Gee and Bauder, 1986), total mineral N, NH_4-N and NO_3-N (Bremner

Table 1. Physicochemical properties of the experimental soil.

pH	EC	NH_4-N	NO_3-N	Exchangeable cations				Soil texture		
				Ca^{2+}	Mg^{2+}	K^+	Na^+	Sand	Silt	Clay
(1:5)	dS m^{-1}	mg kg^{-1}		---------- $cmol_{(+)}kg^{-1}$ ----------				---------- % ----------		
6.7	0.24	5.98	9.97	10.94	1.23	0.44	0.34	45.10	31.90	23.00

Table 2. Characteristics of rhizobacterial strains.

Strains	IAA production (mg kg^{-1})	% Siderophores units	Phosphate solubilization (mg L^{-1})
Azotobacter chroococcum	46.68	62.65	863.42
Psuedomonas aeruginosa	31.03	73.60	382.88
P. fluorescens	35.91	69.86	885.40
P. putida	47.54	66.40	533.80
Rhodobacter capsulatus	47.14	65.23	525.11
Lactobacillus sp.	23.48	11.06	804.96

1982), and exchangeable cations (Ca^{2+}, Mg^{2+}, K^+, and Na^+) (Thomas, 1982) (Table 1).

Five hundred grams of soil was put in each pot for different treatments and three replicates were done for each treatment. Fifty mL of distilled water was added to each jar before autoclaving three times at 121°C for 30 min. The soil was fertilized with 120: 60: 50 kg/ha of nitrogen, phosphorus and potassium (NPK). Seeds of Wisconsin Fast Plants were surface-sterilized with sodium hypochlorite NaOCl (0.02%) for 1 min, and rinsed thoroughly 3 times in sterile distilled water. Six bacterial strains were *Azotobzcter chroococcum*, *Pseudomonas putida*, *P. aeruginosa*, *P. fluorescens*, *Rhodobacter capsulatus*, and *Lactobacillus* sp. The bacterial strains were selected for this study on the basis of their high performance in previous physiological experiments, such as IAA production and phosphorus solubilization. Pure cultures were grown in NB at 30°C and diluted in sterile distilled water containing 0.025% Tween 80. Seeds were dipped into individual culture broth (cell density about 10^7 cells / mL^{-1}) and agitated for one hour with spore's suspension. Wisconsin Fast seeds were sown three to a pot at surface. Pots were placed in a germination chamber. This provided a 24 h photoperiod, temperatures of 25°C, and relative humidity of 65%. Pots were watered by distilled water to 60% water holding capacity twice daily. Inoculation was achieved by applying 1 mL of bacterial suspension at 10^7 CFU mL^{-1} on each seed (Yolcu et al., 2011). Moreover, there is a treatment of Effective Microorganisms (EMRO, USA), that was used as a trade product of biofertilizer containing (*Lactobacillus kefir*, *Candida versatilis*, and *Rhodopseudomonas palustris*) and diluted by SD H_2O according to the manufacturer instructions. The uninoculated treatment received sterile water as a control. After 40 days of sowing, the data in terms of the chlorophyll amount shoot and root height (cm), leaf length and width (cm), and dry weight (mg) of shoot and root were recorded.

Results

Plant growth promotion activities *Pseudomonas putida*, *Rhodobacter capsulatus*, and *Azotobacter chroococcum* showed high productivity of indole acetic acid IAA by 47.54 mg kg^{-1}, 47.14 mg kg^{-1}, and 46.68 mg kg^{-1}, respectively. *P. fluorescens* and *P. aeruginosa* produced 35.91 mg kg^{-1} and 31.03 mg kg^{-1} of IAA, respectively, while Lactobacillus sp. produced only 23.48 mg kg^{-1} of IAA. The highest bacterial siderophores production was shown by *P. aeruginosa*, *P. fluorescens*, and *P. putida*. They demonstrated 73.60% units, 69.86% units, and 66.40% units, respectively. However, *Lactobacillus* sp. showed the lowest siderophores production by only 11% units. Relatively high siderophores production was shown by *A. chroococcum* 62.65% units. For phosphate solubilization, *P. fluorescens*, *A. chroococcum*, and *Lactobacillus* sp. demonstrated 885.4 mg L^{-1}, 863.4 mg L^{-1}, and 804.9 mg L^{-1}, respectively. However, *P. aeruginosa* showed low level of phosphate solubilization. Also, *P. putida* and *R. capsulatus* showed little higher solubility of phosphate (Table 2).

Pots experiment For leaf chlorophyll density, the most condensed chlorophyll level was demonstrated in *Azotobacter chroococcum* leaves 65.3 SPAD units followed by the NPK fertilizer 55.5 SPAD units. The bacteria mixture of *Pseudomonas aeruginosa* and *P. fluorescens* showed low value of chlorophyll 28.7 SPAD units. *Rhodobacter capsulatus* also showed high level of chlorophyll density of 49.6 SPAD

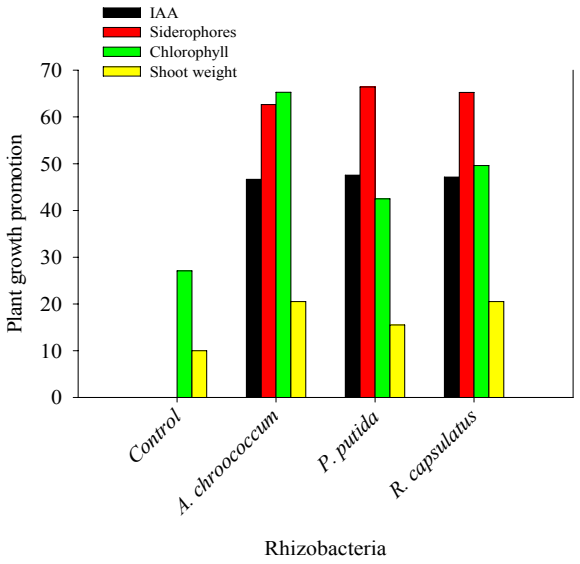


Fig. 1. Effect of rhizobacterial inoculations for plant growth promotion traits on the chlorophyll, IAA, Siderophores, and plant weight of *Brassica rapa*.

units (Fig. 1). Molasses treatment without inoculation showed the lowest result of chlorophyll density in the leaf of 27.1 SPAD units. For leaf length, the longest leaves were shown by NPK fertilizer 4.2 cm followed by *A. chroococcum* and *R. capsulatus* showed 4 cm, while the commercial effective microorganisms (EM) showed leaf length of 3.9 cm (Fig. 1). The lowest leaf length was demonstrated by bacteria mixture (*Pseudomonas aeruginosa* and *P. fluorescens*) of 3 cm while molasses control demonstrated leaf length of only 1.9 cm. For leaf width, it was quite similar to leaf length, the NPK chemical fertilizer showed the widest leaf diameter of 2.1 cm as well as *A. chroococcum. R. capsulatus* came in the next inferior of 2 cm leaf width. The trade effective microorganisms (EM) solution demonstrated 1.8 cm leaf width followed by bacteria mixture of 1 cm. The molasses showed only 0.8 cm. For the shoot length, NPK chemical fertilizer was superior by 16.9 cm length. *R. capsulatus* showed 15.1 cm followed by *A. chroococcum* 14.6 cm shoot length then *P. putida* and the trade EM (Table 3).

The lowest shoot length was demonstrated by bacteria inoculation which showed 9.3 cm, while un-inoculated control gave only 10.5 cm. For the root length, the longest plant root was demonstrated by the NPK chemical fertilizer 6.7 cm, followed by *A. chroococcum* and *P. putida* showed 6.5 cm. However, *R. capsulatus* demonstrated 6.3 cm root length. The lowest root length was shown by the commercial EM product of 4.3 cm, while the un-inoculated control demonstrated 6.1 cm of root length. For the shoot dry weight, the highest dry weight by was shown by the NPK fertilizer which demonstrated 21.5 mg. These dry weights of shoot were followed by *A. chroococcum* and *R. capsulatus* demonstrating 20.5 mg (Fig. 1). The commercial EM solution yielded 19 mg of shoot dry weight. *P. putida* and the bacteria mixture showed 15.5 mg of the shoot dry weight. The lowest shoot dry weight was 11 mg and shown by the control of the un-inoculated molasses. For the root dry weight, almost it was similar to shoot. The highest results were demonstrated by *A. chroococcum* and NPK showing 0.93 mg. Then *R. capsulatus* came in the next by 0.91 mg. The commercial biofertilizer (EM) showed 0.79 mg followed by *P. putida* giving 0.75 mg, while the molasses control showed only 0.72 mg root dry weight (Table 3). The microbial counting of soils showed that the highest bacterial count was found in the soil inoculated with bacterial mixture 4.11 x 10^7 CFU g $soil^{-1}$. Soils treated with *P. putida* and the commercial EM solution showed 3.87 x 10^7 CFU g $soil^{-1}$ and 3.15 x 10^7 CFU g $soil^{-1}$, respectively. This was followed by 2.24 x 10^7 CFU g $soil^{-1}$ counted in *R. capsulatus* soil. *A. chroococcum* showed 2.20 x 10^7 CFU g $soil^{-1}$. The NPK fertilizer demonstrated the lowest bacterial count 7.55 x 10^6 CFU g $soil^{-1}$. However, the soil treated by the un-inoculated molasses (control) showed 1.79 x 10^7 CFU g $soil^{-1}$. (Table 4).

Table 3. Effects of different EM inoculations on the *Brassica rapa* growth.

Treatment	Chlorophyll (SPAD unit)	Leaf		Length		Dry weight	
		---------- per plant (cm) ----------				-- per plant (mg) --	
		Length	Width	Shoot	Root	Shoot	Root
Control	27.1g	1.8d	0.7c	10.5d	6.0bc	10.0d	0.72b
Chemical fertilizer	55.5b	4.2a	2.1a	16.9a	6.7b	21.5a	0.93a
Azotobacter chroococcum	65.3a	4.0ab	2.1a	14.6bc	6.5bc	20.5ab	0.93a
P. aeruginosa, P. fluorescens, and *Lactobacillus* sp. *co-inoculation*	28.7f	3.0c	1.0bc	9.3e	6.0bc	15.5c	0.75b
P. putida	42.5d	3.5bc	1.1b	13.8c	6.5bc	15.5c	0.75b
Commercial EM	39.6e	3.9ab	1.8a	13.8c	4.9c	19.0b	0.79b
Rhodobacter capsulatus	49.6c	4.0ab	2.0a	15.1b	6.3bc	20.5ab	0.91a

Table 4. Effect of EM inoculation on the bacterial density of the soil.

Treatment	Bacterial density CFU/g soil x 10^4
Control	1788
Chemical fertilizer	755
Azotobacter chroococcum	2195
P. aeruginosa, *P. fluorescens*, and Lactobacillussp. Co-inoculation	4105
P. putida	2865
Commercial EM	3150
Rhodobacter capsulatus	2235

Discussion

This pot experiments were conducted to evaluate the effectiveness of inoculation with rhizomicrobes containing phosphate solubilizing and IAA activity for improving growth of Wisconsin Fast Plants *Brassica rapa*. Plant growth promoting rhizobacteria (PGPR) are able to colonize very efficiently the roots or the rhizosphere soil of crop plants. They fulfill vital functions for plant growth and health by mechanisms. They mediate the nutrient uptake and increase the hormonal biosynthesis (Nihorimbere, 2011). According to our results, the highest shoot length was shown by NPK chemical fertilizer of 16.9 cm length, *Rhodobacter capsulatus*, and *Azotobacter chroococcum* showed high productivity of indole acetic acid IAA (Fig. 1). Consequently, in the pots experiment *R. capsulatus* showed high level of chlorophyll density of 49.6 SPAD units. Also, *R. capsulatus* showed 15.1 cm followed by *A. chroococcum* 14.6 cm shoot length then *P. putida* and the trade EM. IAA and other plant hormones were hypothesized to be responsible for enhanced growth of canola, tomato, and wheat in non-sterile soil inoculated with *Azotobacter paspali* (Abbass and Okon 1993). *P. fluorescens, A. chroococcum, and Lactobacillus sp.* demonstrated the highest phosphate solubilization by 885.4 mg L^{-1}, 863.4 mg L^{-1}, and 804.9 mg L^{-1}, respectively. The lowest dry weight was shown by the control of the SD water. However, the highest results of root and shoot dry weights were demonstrated by *A. chroococcum* and NPK. Phosphate solubilizing bacteria and *Azospirillum* are the most effective components of bio-fertilizers significantly contributing N, P and K to plants and also providing resistance to drought conditions (Smith and Read 1997). Phosphorus in soil is often insoluble thereby unavailable to plants (Singh and Kapoor, 1994). The rhizobacteria can solubilize phosphates in vitro and most of them act as PGPR (Joo et al., 2004). Although, many researches has been done on utilization of phosphorus solubilising micro-organisms in order to contribute soluble phosphate to plants, its large real application has not been found. Gaur et al. (1980) demonstrated the beneficial effect of phosphate solubilizers on grain yield of rice. Many researches are concerning in developing bio-inoculant for degradation of organic wastes and preparation of decomposing cultures (Poonguzhali et al., 2008). All bacterial isolates showed high production of siderophores except *Lactobacillus* sp. The highest bacterial siderophores production was shown by *P. aeruginosa*, *P. fluorescens*. Our strain of *Azotobacter chroococcum* showed remarkable high activity for IAA production, phosphate solubilization, and siderophores synthesis, as well as, remarkable enhancing to the *B. rapa* growth. Strains of *Pseudomonas putida* and *Pseudomonas fluorescens* could increase root and shoot elongation in canola (Glick et al., 1997) as well as wheat and potato (de Freitas and Germida, 1992), (Frommel et al., 1993). The understanding of rhizosphere ecology, including the function and diversity of micro fauna that dwell there, is necessary before soil microbial application in the rhizosphere. Das, et al. (2008) showed that the amount of available nitrogen (N), phosphorus (P) and potassium (K) contents in soil have been increased significantly up to third month in the inoculated soil. *Azotobacter* and *Azospirillum* strains application increased canola yield (21.17%), pod per plant (16.05%) and number of branches (11.78%) (Yasari and Patwardhan, 2007).

References

Abbass Z., and Okon Y. 1993. Plant growth promotion by Azotobacter paspali in the rhizosphere. Soil. Biol. Biochem. 25(8):1075-1083.

Ahmad, F., Ahmad, I. and Khan M.S. 2006. Screening of free-living rhizospheric bacteria for their multiple plant growth promoting activities. Microbial. Res. 36:1-9.

Bais P, Weir TL, Perry LG, Gilroy S, and Vivanco JM. 2006. The role of root exudates in rhizosphere interactions with plants and other organisms. Annu. Rev. Plant Biol. 57:233-266.

Bremner JM, and Mulvaney CS. 1982. Nitrogen-Total. In: Page AL (ed) Methods of soil analysis, part 2 chemical and microbiological properties. ASA, Madison, WI, pp 595-624.

Brosius, J., Palmer, M. L., Kennedy, P. J. and Noller, H. F. 1978. Complete nucleotide sequence of a 16S ribosomal RNA gene from Escherichia coli. Proc Natl Acad Sci USA 75, 4801-4805.

Cattelan, A. J., Hartel, P. G. and Fuhrmann, J. J. 1999. Screening for plant growth-promoting rhizobacteria to promote early soybean growth. Soil Sci.Soc.Am. J., 63:1670-1680.

Das, K., Dang, R., and Shivananda, T.N. 2008. Influence of bio-fertilizers on the availability of nutrients (N, P and K) in soil in relation to growth and yield of Stevia rebaudiana grown in South India International Journal of Applied Research in Natural Products 1(1):20-24.

de Freitas, J. R. and Germida, J. J. 1992. Growth promotion of

winter wheat by fluorescent pseudomonads under growth chamber conditions. Soil. Biol. Biochem. 24:1127-1135.

Egamberdiyeva D. 2007. The effect of plant growth promoting bacteria on growth and nutrient uptake of maize in two different soils. Appl. Soil. Eco. 36:184-189.

Frommel., M. I. Nowak J. and Lazarovits, G. 1993. Treatment of potato tubers with a growth promoting Pseudomonas sp.: Plant growth responses and bacterium distribution in the rhizosphere. Plant .Soil. 150(1):51- 60.

Gaur, A.C., Oswal, K.P. and Mathur, R.S. 1980. Save super phosphate by P-solubilizing microorganisms. Kheti. 32:23-25.

Gee GE, and Bauder JW. 1986. Particle-size analysis. In: A Klute (ed) Methods of soil analysis, Part 1 physical and mineralogical methods. ASA, Madison WI, pp 383-411.

Glick, B.R., Changping, L. Sibdas G. and Dumbroff, E. B. 1997. Early development of canola seedlings in the presence of the plant growth promoting rhizobacterium Pseudomonas putida GR12-2. Soil Biol. Biochem. 29:1233-1239.

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

Joo GJ, Kim YM, Lee IJ, Song KS, and Rhee IK. 2004. Growth promotion of red pepper plug seedlings and the production of gibberellins by Bacillus cereus, Bacillus macroides and Bacillus pumilus. Biotechnol. Lett. 26:487-491.

Kannaiyan, S. 2002. Biofertilizers for sustainable crop production. In: Kannaiyan, S (ed) Biotechnology and biofertilizers, Kluwer Academic Publishers, Dordrecht, The Netherlands, pp. 9-49.

Kozdroja J., Trevorsb J.T. and van Elsasc J.D. 2004. Influence of introduced potential biocontrol agents on maize seedling growth and bacterial community structure in the rhizosphere. Soil. Biol. Biochem. 36:1775-1784.

McLean EO. 1982. Soil pH and lime requirement. In: Page AL (ed) Methods of soil analysis, part 2 chemical and micro-biological properties. ASA, Madison, WI, pp 199-224.

Mrkovacki, N., and Milic V. 2001. Use of Azotobacter chroococcum as potentially useful in agricultural application. Ann. Microbiol. 51:145-158.

Nihorimbere, V., Ongena, M., Smargiassi, M., Thonart, P. 2011. Beneficial effect of the rhizosphere microbial community for plant growth and health. Biotechnol. Agron. Soc. Environ. 15(2):327-337.

Pikovskaya R.I. 1948. Mobilization of phosphorus in soil connection with the vital activity of some microbial species. Microbiologiya 17:362-370.

Poonguzhali S, Madhaiyan M, Sa T. 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.

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

Schwyn B, Neilands JB. 1987. Universal chemical assay for the detection and determination of siderophores. Anal Biochem. 160:47-56.

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

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

Singh S., Kapoor K. 1994. Solubilization of insoluble phosphates by bacteria isolated from different sources. Environ. Ecol. 12:51-55.

Smith S.E., and Read D.J. 1997. Mycorrhizal Symbiosis, 2nd Edn. Academic Press, San Diego. p.126.

Steenhoudt, O., and J. Vanderleyden. 2000. *Azospirillum*, a free-living nitrogen-fixing bacterium closely associated with grasses: genetic, biochemical and ecological aspects. FEMS Microbiol. Rev. 24:487-506.

Sylvia, D.M., Fuhrmann, J.J., Hartel, P.G. and Zuberer, D.A. 1998. Principles and applications of soil microbiology. Prentice-Hall, Inc. Upper Saddle River, NJ. 550 pp.

Thomas G.W. 1982. Exchangeable cations. In: Page AL (ed) Methods of soil analysis part 2 chemical and microbiological properties. American Society of Agronomy, Madison,WI, pp 159-166.

Welbaum, G., A. V. Sturz, Z. Dong, and J. Nowak. 2004. Fertilizing soil microorganisms to improve productivity of agroecosystems. Crit. Rev. Plant Sci. 23:175-193.

Wu S.C., Cao Z.H.., Li Z.G.., Cheung K.C. and Wong, M.H. 2005. ffects of biofertilizer containing N-fixer, P and K solubilizers and AM fungi on maize growth: a greenhouse trial. Geoderma. 125:155-166.

Yasari, E., Patwardhan, A.M. 2007. Effects of Aztobacter and Azospirillium inoculations and chemical fertilizers on growth and productivity of Canola. Asi. J. Plant. Sci., 6(1):77-82.

Yolcu H., Metin T., Anastasios, L., Ramazan, Ç., Ali, K. 2011. Effects of plant growth promoting rhizobacteria and manure on yield and quality characteristics of Italian ryegrass under semi arid conditions. Autralian Journal of Crop Science. 5(13): 1730-1736.