AJFAND ONLINE Scholarly, Peer Reviewed ISSN 1684 5374
Volume 12 No. 3
May 2012
African Journal of Food, Agriculture, Nutrition and Development
Published by African Scholarly Science Communications Trust

SCREENING OF PLANT GROWTH–PROMOTING RHIZOBACTERIA FROM MAIZE (*ZEA MAYS*) AND WHEAT (*TRITICUM AESTIVUM*)

Karnwal A[*1]

Arun Karnwal

[*] Corresponding Author: arunkarnwal@gmail.com

[1]Reader, Bhojia Institute of Life Sciences, Teh. Nalagarh, Budh, Baddi, Solan, H.P. 173205.

AJFAND ONLINE Scholarly, Peer Reviewed
ISSN 1684 5374
Volume 12 No. 3
May 2012
African Journal of Food, Agriculture, Nutrition and Development
Published by African Scholarly Science Communications Trust

ABSTRACT

Plant growth-promoting rhizobacteria (PGPR) are free-living soil-borne bacteria that colonize the rhizosphere and have great importance in governing the functional property of terrestrial ecosystems. In this study, rhizospheric bacteria were isolated from maize and wheat and screened for their plant growth promoting activities. These isolates were identified as *Pseudomonas*, *Bacillus*, *Azospirillum* and *Azotobacter* species. All isolates were tested for their indole acetic acid (IAA) production ability. All isolates produce the varying amounts of IAA ranging from 0.6-2.7 µg/ml. The highest concentration of IAA was produced by bacterial strain *Bacillus subtilis* AK31. A series of growth pouch and pot experiments were conducted to study the effect of bacterial inoculants on the growth of maize and wheat. It was concluded that IAA plays a key role in the growth promotion of roots in maize and wheat in growth pouch study. In maize, isolate AK1, AK21, AK31 and AK8 showed high indole acetic acid (6.86, 7.11, 7.11 and 7.36 pmol/ml, respectively) and root elongation activity (4.10, 5.00, 5.00 and 3.80 cm, respectively) after 96h of growth. In wheat, bacterial strains AK31, AK2, AK14, AK32 and AK15 showed high IAA (6.59, 5.66, 5.35, 7.53 and 5.66 pmol/ml, respectively) and root elongation (6.07, 4.00, 5.20, 6.90 and 5.20 cm, respectively) activity after 96h of growth. In pot experiments, *Bacillus* sp. AK21, *Bacillus subtilis* AK31, *Azotobacter diazotrophicus* AK14, *Microbacterium* sp. AK19 and *Pseudomonas fluorescens* AK32 showed effective results in terms of increase in root and shoot dry weight in maize (123, 130, 121, 120, 124g and 116, 126, 116, 114, 120g/pot, respectively) and wheat (130, 135, 125, 118, 140g and 105, 106, 110, 102, 110g/pot, respectively), in comparison to controls of maize and wheat crops. Thus, it might be concluded that PGPR strains AK21, AK31, AK14, AK19 and AK32 could be used as crop-enhancer and bio-fertilizer for production of maize and wheat.

Key words: Gnotobiotic assay, Maize, Wheat, Phytohormones

INTRODUCTION

Soil contains a wide diversity of microbes. Microbes are concentrated in nutrient-rich soil regions, including the topsoil layer and the region around the plant root. Roots secrete rhizodeposits that stimulate microbial life and allow fast spreading of microbes through soils. Plants can benefit from soil microbes in many ways. Certain microbes stimulate plant growth, enrich soils, degrade pollutants, or protect plants against pathogens. Analysis of the genotypic and phenotypic characteristics of indigenous rhizobacteria can help in understanding the mechanism of interactions between them and plant roots [1]. Plant growth-promoting rhizobacteria (PGPR) are free-living, soil-borne bacteria, which, when applied to seeds or crops, enhance the growth of the plant or reduce the damage from soil-borne plant pathogens [2]. The rhizosphere is the soil portion found around the root and under the influence of the root. It is the site with complex interaction between the root and associated microorganisms [3]. It has been noted by many workers that *Pseudomonas*, *Bacillus*, *Arthrobacter*, *Azospirillum*, *Klebsiella*, and *Enterobacter*, isolated from the rhizosphere of various crops, showed synergistic effects on plant growth [2, 4, 5]. Beneficial effects of micro-organisms have often been evaluated based on faster seed germination, better seedling emergence, and increased plant growth. Plant growth-promoting rhizobacteria enhance plant growth either by direct or indirect mechanisms [5]. The indirect mechanisms of plant growth promotion by PGPR include i) antibiotic production ii) depletion of iron from the rhizosphere iii) synthesis of antifungal metabolites iv) production of fungal cell wall lysing enzymes v) competition for sites on roots and vi) induced systemic resistance [2, 6, 7]. The production of phytohormones has been suggested to be one of the mechanisms by which PGPR bacteria stimulate plant growth [4]. Aslantas *et al*. [8] reported that the PGPR of apple showed the growth-promoting effect with possible involvement of the plant growth regulators indole-3-acetic acid and cytokinin. The objectives of the study were to isolate plant growth promoting rhizosphere bacteria from maize and wheat, and evaluate their beneficial effects on plant growth properties.

METHODS

Pot experiments

Soil was collected from Forest Research Institute, Dehradun. This soil had pH 6.7 and contained 7.3 g/kg organic C, 590 mg/kg N, 60 mg/kg P, and 71 mg /kg K. Kjeldahl method was used to determine the total nitrogen content in the soil. For determination of the total organic carbon content, elementary analysis was used [9], while K and P content were also determined by flame photometric method and molybdenum blue method, respectively [10, 11]. For pot experiments, maize (*Zea mays*) and wheat (*Triticum aestivum*) were used as experimental plants.

Isolation and characterization of soil microorganisms

For the isolation of bacterial population, both maize and wheat plants were grown in the field. Plants were harvested 30 days after seed emergence were harvested. After harvesting, the roots and shoots were separated. To isolate the rhizospheric bacteria, loosely held soil to fresh roots were gently shaken with 9 ml of sterile distilled water

containing 1 ml of 40% tetramethylthiuramdisulphide solution for 30 min. From the suspension, a serial dilution (1:100) was prepared, and the resulting suspensions were spread over the surface of a succinate agar medium (sodium glutamate 1.00 g, sodium succinate 5.00 g, ammonium sulphate 0.40 g, magnesium sulphate 0.20 g, dipotassium hydrogen sulphate 0.50 g, agar agar 15.0 g). Six inoculated plates per dilution were incubated at 28°C and the bacterial colony forming units (CFUs) were counted after 72 hours of incubation. After getting the pure culture of isolated bacterial population, identification and characterization was done by using morphological, physiological, Biochemical and bacterial detection kit methods (BIOCON INC., INDIA).

IAA production

After the confirmation of IAA production activity by isolated bacterial isolates, bacterial strains were grown in succinate broth with and without tryptophan (500 mg/ml) and incubated at 28°C for 72 hours. A 2ml culture was taken from each tube and centrifuged at 10,000 rpm for 15 min (REMI RM-12C DX). One milliliter of the supernatant was taken in a tube to which 100 ml of 10 mM orthophosphoric acid and 2 ml of reagent (1 ml of 0.5 M $FeCl_3$ in 50 ml of 35% $HClO_4$) were added. After 25 minutes (after color density reached its maximum), the mixture was read in UV-spectrophotometer at 530nm absorbance. The quantity of IAA was determined by comparison with a standard curve using pure IAA [12].

Gnotobiotic assay of Growth Pouch Study (GPS)

The gnotobiotic assay was performed as described by Lifshitz *et al.* [13]. Seeds were surface-sterilized by treatment with 95% ethanol (v/v) for 10 to 20 s, followed by soaking in 20% bleach (v/v) for 10 min. Seeds were washed with sterile distilled water 5-7 times in order to remove excess bleach. The seeds were then air dried by placing them in the laminar flow hood for 24 h. Surface-sterilized seeds were picked at random and placed onto half- strength tryptic soy agar (TSA) plates and incubated at 27ºC for 24 h to further check for any contamination. Seeds were considered to be free from contamination if there were neither bacterial nor fungal growth occurring on plates incubated with surface-sterilized seeds.

A 100-fold dilution of the bacterial cells grown in half strength tryptic soy broth (TSB) was performed using 0.1 M $MgSO_4$. Surface-sterilized seeds were soaked in 10 ml of bacterial suspension for 10-15 min with gentle agitation.

Growth pouch experiments were conducted in order to determine the ability of PGPR strains to produce the phytohormone, indole-3-acetic acid (IAA) in the presence of maize and wheat roots. The effect of PGPRs (plant growth promoting rhizobacteria) inoculation on maize and wheat (individually) and the concentration of IAA in the rhizosphere were measured at different time intervals (24h, 48h, 72h and 96h). The growth pouches were filled with 25 ml of Hoagland's nutrient solution and seeds treated with bacterial strains (maize and wheat, individually) were transferred aseptically to growth pouches (3 seeds per pouch and 3 pouches per treatment) [14]. Seeds treated with 0.1 M $MgSO_4$ were considered as controls. The growth pouches were incubated in a growth cabinet with gentle shaking at 100 rpm to create aerobic

conditions for growth of PGPR strains. Supernatants from growth pouches (10 ml) were obtained by centrifugation at 4000 rpm for 20 min at 4°C (REMI RM-12C DX) and filtration using 0.22 µm membrane filters. The phytohormone IAA was assayed using ELISA kits (Phytodetek, Agdia Inc, Elkhart, IN, USA). Stock solutions of the IAA (10 µmole/ml) were prepared in absolute methanol. Standard concentrations of 78-2500 pmoles/ml (IAA) were used. One hundred microlitres of standard or the sample were used for each assay.

The intensity of the color was read at 405nm using an ELISA plate reader and related to phytohormone concentrations by means of a standard curve.

Plant growth promotion

The effect of isolated strains on plant growth was studied in pot experiments. The inoculation treatments were setup in a randomized design with six replicates.

The day before sowing, pots were filled with 350 g soil. The soil was moistened with water and maintained at 60% of its moisture holding capacity. Four seeds of maize and 4 of wheat were sown per pot. After germination, plants were thinned to two per pot. The bacteria were cultured in glycerin-peptone-medium. Tubes were secured on a rotary shaker on 120rpm for 72 hours at 23ºC. Seedlings of these plants were inoculated with 1 ml of the bacterial suspension, which resulted in an inoculum's density of 10^6cfu/ml. The control was considered as un-inoculated plants. Plants were placed in a temperature-regulated growth chamber at a light intensity 20 kLux for 16 hours at 16°C during the day and 12°C at night.

Data analysis

The data were analysed for significant differences ($P < 0.05$) of main effects using 2-way ANOVA and Student-Newman-Keuls tests.

RESULTS

All sixty isolates were screened for their PGPR activities and fifteen of them were selected for further studies due to their higher IAA production ability than the rest of isolates. Among the isolates, the representative bacterial strains in the rhizosphere were identified as *Bacillus lentus* AK1, Bacillus sp. AK21, *Azospirillum brasilense* AK11, *Bacillus subtilis* AK31, *Bacillus lentus* AK8, Bacillus sp. AK2, *Pseudomonas fluorescens* AK30, *Azotobacter diazotrophicus* AK14, Microbacterium sp. AK19, *Bacillus lentus* AK17, *Pseudomonas fluorescens* AK32, Bacillus sp. AK3 and *Bacillus halodurans* AK15.

All 15 bacterial strains produced IAA (Table 3). The highest concentration of IAA was produced by bacterial strain *Bacillus subtilis* AK31, *Pseudomonas fluorescens* AK32 and Bacillus sp. AK21.

Gnotobiotic assay or growth pouch study (GPS) with maize revealed that with control experiment less amount of IAA production were recorded (0.15, 0.99, 1.51 and 2.43 pmol/ml at 24h, 48h, 72h and 96h, respectively), while PGPR inoculated seeds

showed a good level of IAA production at different time intervals (Table 1). Similar results were also recorded with wheat seeds (Table 2). Furthermore, control experiments also revealed a deficient level of seed germination and radical length in maize (0.1, 0.4, 1.1 and 2.0 cm) and wheat (0.0, 0.2, 0.9 and 1.8 cm) at different time intervals (24h, 48h, 72h and 96h) and good germination ability as well as increased root length were recorded in maize and wheat (Tables 1 and 2) with bacterial inoculated seeds. These results may provide partial evidence that PGPRs and IAA play an important role in plant growth promotion.

Results of pot experiments showed that the bacterial strains increased the root dry weight and shoot dry weight of maize (30% and 26%) and wheat (40% and 10%) as compared to the control (Table 4).

DISCUSSION

Plant growth-promoting rhizobacteria colonize plant roots and exert beneficial effects on plant growth and development by a wide variety of mechanisms. To be an effective PGPR, bacteria must be able to colonize root environment because they need to establish themselves in the rhizosphere at population densities sufficient to produce the beneficial effects. Our results have demonstrated that selected plant growth-stimulating rhizosphere bacterial isolates were able to increase the growth of maize and wheat. Plant growth-promoting rhizobacteria promote plant growth by producing plant growth regulators or phytohormones [5]. These promote seed germination, root elongation, and stimulation of leaf expansion. Eighty percent of microorganisms, including pseudomonads, isolated from the rhizosphere of various crops have the ability to produce auxins as secondary metabolites [15, 16]. Researchers showed that root surface area of maize seedlings was significantly increased after inoculation with Azospirillum [17, 18, 19, 20, 21, 22]. Similar results revealed that *Pseudomonas* inoculation (*P. fluorescens* PsIA12 and *Agrobacterium rhizogenes* A1A4) stimulated wheat growth [23, 24].

Many scientists reported that plant growth promoting rhizobacteria might enhance plant height and productivity by synthesizing phytohormones [25, 26, 27]. In our experiments, all selected bacterial strains were able to produced IAA at different concentrations in comparison to controls. In summary, the bacteria-associated plant growth properties shown in our experiments suggested that PGPR bacteria were able to produce phytohormone auxin (IAA) that stimulated the growth of maize and wheat crops.

It was hypothesized that IAA and other plant hormones were responsible for increased growth of canola, tomato and wheat in non-sterile soil inoculated with *Azotobacter paspali* [28]. The experiment carried out in present work has demonstrated that Plant growth-promoting isolates have capacity to modify the growth of maize and wheat. Auxins produced by rhizobacteria can influence plant growth, including root development which improve uptake of essential nutrients thus increasing plant growth [29]. In the pot experiment, it was observed that inoculation with PGPR strains significantly promoted growth and increased the root-shoot dry weight of maize and

wheat. These results are consistent with the findings of other researchers who assessed the inoculation effect of PGPR *Azospirillum brasilense* on growth and dry weight of spring wheat [18, 30]. Similarly, promotion in growth parameters and yields of various crop plants in response to inoculation with PGPR were reported by other workers [19, 20, 21, 27].

CONCLUSION

The greater root and shoot dry weight response to all inoculants compared to control clearly showed the beneficial role of these rhizobacteria. Such an improvement might be attributed to produce growth promoting substances. Based on these results, it can be concluded that, inoculation of *Bacillus lentus*, *Bacillus* sp., *Azospirillum brasilense*, *Bacillus subtilis*, *Pseudomonas fluorescens*, *Azotobacter diazotrophicus*, *Bacillus halodurans* and *Pseudomonas* strains as biofertilizer will be useful in increasing cereal productivity and contribute to tackling food deficits in the world.

Table 1: Effect of isolated PGPRs (plant growth-promoting rhizobacterias) on maize root growth and IAA production in Gnotobiotic study (Growth Pouch Study)

Bacterial Strains	Experiment Detail	Incubation Time (h)			
		24	48	72	96
Control	IAA Conc. (pmol/ml)	0.15	0.99	1.51	2.43
	Root length (cm)	0.10	0.40	1.10	2.00
AK1	IAA Conc. (pmol/ml)	1.90	2.78	4.24	6.86
	Root length (cm)	0.40	1.30	2.50	4.10
AK21	IAA Conc. (pmol/ml)	2.00	2.88	4.40	7.11
	Root length (cm)	0.42	1.35	2.59	5.00
AK11	IAA Conc. (pmol/ml)	0.80	1.65	2.52	4.08
	Root length (cm)	0.17	0.77	1.49	4.50
AK31	IAA Conc. (pmol/ml)	2.00	2.88	4.40	7.11
	Root length (cm)	0.42	1.35	2.59	5.00
AK8	IAA Conc. (pmol/ml)	2.10	2.99	4.56	7.36
	Root length (cm)	0.44	1.40	2.68	3.80
AK2	IAA Conc. (pmol/ml)	1.10	1.96	2.99	4.83
	Root length (cm)	0.23	0.92	1.76	4.00
AK30	IAA Conc. (pmol/ml)	1.40	2.27	3.46	5.59
	Root length (cm)	0.29	1.06	2.04	3.00
AK14	IAA Conc. (pmol/ml)	1.00	1.86	2.84	4.58
	Root length (cm)	0.21	0.87	1.67	5.20
AK19	IAA Conc. (pmol/ml)	0.90	1.76	2.68	4.33

	Root length (cm)	0.19	0.82	1.58	5.00
AK17	IAA Conc. (pmol/ml)	1.30	2.17	3.31	5.34
	Root length (cm)	0.27	1.01	1.95	4.50
AK32	IAA Conc. (pmol/ml)	1.50	2.37	3.62	5.85
	Root length (cm)	0.32	1.11	2.13	5.30
AK3	IAA Conc. (pmol/ml)	1.00	1.86	2.84	4.58
	Root length (cm)	0.21	0.87	1.67	4.80
AK15	IAA Conc. (pmol/ml)	0.90	1.76	2.68	4.33
	Root length (cm)	0.19	0.82	1.58	3.30
AK9	IAA Conc. (pmol/ml)	1.50	2.37	3.62	5.85
	Root length (cm)	0.32	1.11	2.13	3.60
AK25	IAA Conc. (pmol/ml)	1.90	2.78	4.24	6.86
	Root length (cm)	0.40	1.30	2.50	4.80

Table 2: Effect of isolated PGPRs (plant growth-promoting rhizobacterias) on wheat root growth and IAA production in Gnotobiotic study (Growth Pouch Study)

Bacterial Strains	Experiment Detail	Incubation Time (h)			
		24	48	72	96
Control	IAA Conc. (pmol/ml)	0.10	0.97	1.47	2.25
	Root length (cm)	0.00	0.20	0.90	1.80
AK1	IAA Conc. (pmol/ml)	0.60	1.63	2.49	3.80
	Root length (cm)	0.20	0.70	1.70	3.50
AK21	IAA Conc. (pmol/ml)	0.90	2.03	3.10	4.73
	Root length (cm)	0.30	0.87	2.12	4.36
AK11	IAA Conc. (pmol/ml)	0.30	1.23	1.88	2.87
	Root length (cm)	0.10	0.53	1.28	2.64
AK31	IAA Conc. (pmol/ml)	1.50	2.83	4.32	6.59
	Root length (cm)	0.50	1.21	2.95	6.07
AK8	IAA Conc. (pmol/ml)	1.00	2.17	3.31	5.04
	Root length (cm)	0.33	0.93	2.26	3.60
AK2	IAA Conc. (pmol/ml)	1.20	2.43	3.71	5.66
	Root length (cm)	0.40	1.04	2.53	4.00
AK30	IAA Conc. (pmol/ml)	0.90	2.03	3.10	4.73
	Root length (cm)	0.30	0.87	2.12	3.00
AK14	IAA Conc. (pmol/ml)	1.10	2.30	3.51	5.35
	Root length (cm)	0.37	0.99	2.39	5.20
AK19	IAA Conc. (pmol/ml)	1.00	2.17	3.31	5.04

	Root length (cm)	0.33	0.93	2.26	4.60
AK17	IAA Conc. (pmol/ml)	0.40	1.37	2.09	3.18
	Root length (cm)	0.13	0.59	1.42	2.90
AK32	IAA Conc. (pmol/ml)	1.80	3.23	4.93	7.53
	Root length (cm)	0.60	1.39	3.37	6.90
AK3	IAA Conc. (pmol/ml)	0.80	1.90	2.90	4.42
	Root length (cm)	0.27	0.81	1.98	4.10
AK15	IAA Conc. (pmol/ml)	1.20	2.43	3.71	5.66
	Root length (cm)	0.40	1.04	2.53	5.20
AK9	IAA Conc. (pmol/ml)	1.60	2.97	3.50	5.34
	Root length (cm)	0.53	1.27	2.39	3.20
AK25	IAA Conc. (pmol/ml)	1.20	2.43	3.71	5.66
	Root length (cm)	0.40	1.04	2.53	4.80

Table 3: Indole-3-acetic acid (IAA) production by isolated bacterial strains (µg ml^{-1} filtrate)

Origin	Bacterial strains	IAA (µg/ml filtrate)
Wheat rhizosphere	*Bacillus lentus* AK1	1.45
Wheat rhizosphere	*Bacillus* sp. AK21	1.90
Wheat rhizosphere	*Azospirillum brasilense* AK11	0.60
Wheat rhizosphere	*Bacillus subtilis* AK31	2.62
Wheat rhizosphere	*Bacillus lentus* AK8	1.36
Wheat rhizosphere	*Bacillus* sp. AK2	1.50
Wheat rhizosphere	*Pseudomonas fluorescens* AK30	1.30
Maize rhizosphere	*Azotobacter diazotrophicus* AK14	1.49
Maize rhizosphere	*Microbacterium* sp. AK19	1.30
Maize rhizosphere	*Bacillus lentus* AK17	1.11
Maize rhizosphere	*Pseudomonas fluorescens* AK32	2.70
Maize rhizosphere	*Bacillus* sp. AK3	1.40
Maize rhizosphere	*Bacillus halodurans* AK15	1.55
Maize rhizosphere	*Pseudomonas* AK9	1.25
Maize rhizosphere	*Pseudomonas* AK25	1.50

Table 4: Influence of isolated bacterial strains on shoot and root dry weight of maize and wheat

Bacterial strains	Dry matter (g/pot) of Maize		Dry matter (g/pot) of Wheat	
	Root	**Shoot**	**Root**	**Shoot**
Control	100	100	100	100
	(0.2021)	(0.1623)	(0.1956)	(0.2205)
Bacillus lentus AK1	110	101	120	105
Bacillus sp. AK21	123*	116	130*	105
Azospirillum brasilense AK11	115	110	106	100
Bacillus subtilis AK31	130*	126*	135*	106
Bacillus lentus AK8	110	101	116	108
Bacillus sp. AK2	116	108	120	101
Pseudomonas fluorescens AK30	104	100	109	100
Azotobacter diazotrophicus AK14	121	116*	125*	110*
Microbacterium sp. AK19	120	114	118	102
Bacillus lentus AK17	119	114	110	101
Pseudomonas fluorescens AK32	124*	120*	140*	110*
Bacillus sp. AK3	116	110	119	102
Bacillus halodurans AK15	109	101	123*	106
Pseudomonas AK9	109	101	115	101
Pseudomonas AK25	116	100	120	103

* Significantly different from the control for $P < 0.05$

REFERENCES

1 **Tripathi AK, Nagarajan T and SC Verma** Inhibition of biosynthesis and activity of nitrogenase in *Azospirillum brasilense* Sp7 under salinity stress. *Curr. Microbiol.* 2002; **44**:363-367.

2 **Kloepper JW, Leong J, Teintze M and MN Scrhorth** Enhanced plant growth by siderophores produced by plant growth promoting rhizobacteria. *Nature* 1980; **286**:885–886.

3 **Sylvia DM, Fuhrmann JJ, Hartel PG and DA Zuberer** Principles and applications of soil microbiology. Prentice-Hall, Inc. Upper Saddle River, NJ, 1998:550.

4 **Kloepper JW and CJ Beauchamp** A review of issues related to measuring of plant roots by bacteria. *Can. J. Microbiol.* 1992; **8**:1219-1232.

5 **Glick BR, Karaturovi DM and PC Newell** A novel procedure for rapid isolation of plant growth-promoting pseudomonads. *Can. J. Microbiol*. 1995; **41**:533-536.

6 **Glick BR, Patten CL, Holguin G and DM Penrose** Biochemical and genetic mechanisms used by plant growth promoting bacteria. Imperial College Press, London, United Kingdom, 1999: 267.

7 **Dunne C, Crowley JJ, Loccoz YM, Dowling DN, de Bruijn F and F O'Gara** Biological control of *Pythium ultimum* by *Stenotrophomonas maltophilia* W81 is mediated by an extracellular proteolytic activity. *Microbiology* 1993; **143**:3921- 3931.

8 **Aslantas R, Cakmakci R and F Sahin** Effect of plant growth promoting rhizobacteria on young apple tree growth and fruit yield under orchard conditions. *Sci. Hort.* 2007; **111**: 371-377.

9 **Allison LE** Wet-combustion apparatus and procedure for organic and inorganic carbon in soil. *Soil Sci. Sot. Am. Proc.* 1960; **24:**36-40.

10 **Overman RR and AK Davis** The Application of Flame Photometry to Sodium and Potassium Determinations in Biological Fluids. *J. Biol. Chem.* 1947; 641-649.

11 **Zhongqi H and CW Honeycutt** A Modified Molybdenum Blue Method for Orthophosphate Determination Suitable for Investigating Enzymatic Hydrolysis of Organic Phosphates. *Comm.Soil. Sci. and Plant. Analy.* 2005; **36:**1373–1383.

12 **Gordon SA and RP Weber** Colorimetric estimation of indole acetic acid. *Plant Physiol.* 1951; **26**:192-195.

13 **Lifshitz R, Kloepper JW, Scher FM, Tipping EM and M Laliberté** Nitrogen-fixing pseudomonads isolated from the roots of plants grown in the Canadian high arctic. *Appl. Environ. Microbiol.* 1986; **51**:251-255.

14 **Hoagland DR and TC Boyer** General nature and process of salt accumulation by roots with description of experimental methods. *Plant Physiol.*1936; **11**: 471-507.

15 **Kampert M, Strzelczyk E and A Pokojska** Production of auxins by bacteria isolated from the roots of pine seedlings (*Pinus silvestris* L.) and from soil. *Pol. J. Microbiol.* 1975; **7**:135-143.

16 **Patten CL and BR Glick** Role of *Pseudomonas putida* indole acetic acid in development of the host plant root system. *Appl. Environ. Microbiol.* 2000; **68**:3795-3801.

17 **Fallik E and Y Okon** Identification and quantification of IAA and IBA in *Azospirillum brasilense* inoculated maize roots. *Soil Biol. Biochem.* 1989; **21**:147-153.

18 **Dobbelaere S, Croonenborghs A, Thys A, Ptacek D, Okon Y and J Vanderleyden** Effect of inoculation with wild type *Azospirillum brasilense* and *A. irakense* strains on development and nitrogen uptake of spring wheat and grain maize. *Biol. Fert. Soils.*2002; **36(4):**284–297.

19 **Kozdroja J, Trevorsb JT and JD van Elsasc** Influence of introduced potential biocontrol agents on maize seedling growth and bacterial community structure in the rhizosphere. *Soil. Biol. Biochem.*2004;**36:**1775–1784.

20 **Shaharoona B, Arshad M, Zahir ZA and A Khalid** 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.* 2006; **38:** 2971–2975.

21 **Pandy A, Sharma E and LKS Plani** Influence of bacterial inoculation on maize in upland farming systems of the sikkim himalaya. *Soil. Biol. Biochm.* 1998; **30(3):** 379-384.

22 **Barbieri P and E Galli** Effect on wheat root development of inoculation with an *Azospirillum brasilense* mutant with altered indole-3-acetic acid production. *Res. Microbiol.* 1993; **144**:69-75.

23 **Defreitas JR and JJ Germida** Growth promotion of winter wheat fluorescent *Pseudomonas* under field conditions. *Soil Biol. Biochem.* 1992; **24**:1137-1146.

24 **Egamberdiyeva D and G Höflich** Influence of growth promoting bacteria from Uzbekistan and Germany on the growth and nutrient uptake of cotton and wheat on different soils. In, *Plant nutrition-Food security and sustainability of agro-ecosystems.* W.J. Horst et al., (Eds), 2001: 674-675.

25 **Prokryl Z, Vancura V and M Wurst** Auxin formation by rhizosphere bacteria as a factor of root growth. *Biol. Plant.* 1985; **27**:159- 163.

26 **Burd GI, Dixon DG and BR Glick** Plant growth promoting rhizobacteria that decrease heavy metal toxicity in plants. *Can.J.Microbiol.*2000; **33:**237-245.

27 **Gravel V, Antoun H and RJ Tweddell** 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.* 2007**; 39:**1968–1977.

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

29 **A Vikram** Efficacy of phosphate solubilizing bacteria isolated from vertisols on growth and yield parameters of sorghum. *Res. J. Microbiol.* 2007; **2(7):**550-559.

30 **Dobbelaere S, Croonenborghs A, Thys A, Ptacek D, Vanderleyden J, Dutto P, Labendera-Gonzalez C, Caballero-Mellado J, Aguirre F, Kapulnik Y, Brener S, Burdman S, Kadouri D, Sarig S and Y Okon** Response of Agronomically important crops to inoculation with *Azospirillum*. *Aust. J. Plant. Physiol.* 2001; **28:**871–879.