http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

OPEN ACCESS Pakistan Journal of Biological Sciences

ISSN 1028-8880
DOI: 10.3923/pjbs.2021.773.779

Research Article
Native Plant Growth-Promoting Rhizobacteria for Growth Promotion of Lettuce from Qassim, Saudi Arabia

[1]Ahmad Al-Turki and [1,2]Reda R.H. Abdullah

[1]Promising Research Center in Biological Control and Agricultural Information, College of Agriculture and Veterinary Medicine, Qassim University, P.O.Box 6622, Buraidah 51452, Qassim, Saudi Arabia
[2]Plant Protection Research Institute, Agriculture Research Center, Dokki, Giza, Egypt

Abstract

Background and Objective: Plant Growth-Promoting Rhizobacteria (PGPR) are a group of bacteria that colonize plant roots and enhance the growth and productivity of plants. However, only those PGPR that is acclimatized to the local soil conditions performs well. The present study aims to pick up effective PGPR isolates from local soil and utilize them as potential bio-inoculants to enhance lettuce plant growth. **Materials and Methods:** Rhizospheric soil samples were obtained from each of six desert plant species in the Qassim region and 45 bacterial isolates were obtained. Four of them were identified and tested for growth-promoting activities by application to the soil in which lettuce was grown under greenhouse conditions. **Results:** The selected bacterial isolates were identified as *Bacillus cereus* BW-201B, *Pseudomonas aeruginosa* AMU1, *Pseudomonas putida* CNE30 and *Enterobacter* sp. CZGRY7. Application of these four isolates to the soil in which lettuce was grown under greenhouse conditions resulted in significant increases in shoot height, shoot weight, chlorophyll levels and the percentages of N, P and K compared with those of control treatment. **Conclusion:** These findings suggest that local soil bacterial strains represent excellent bioinoculants for growth and yield increase in lettuce under local agro-climatic conditions in Saudi Arabia. Our approach might offer a good alternative for the chemical-free farming of lettuce.

Key words: Plant growth-promoting rhizobacteria (PGPR), biofertilizers, bioinoculants lettuce, agro-climatic, greenhouse, rhizosphere, iron solubilization, phytohormones

Citation: Al-Turki, A. and R.R.H. Abdullah, 2021. Native plant growth-promoting rhizobacteria for growth promotion of lettuce from Qassim, Saudi Arabia. Pak. J. Biol. Sci., 24: 773-779.

Corresponding Author: Reda R. H. Abdullah, Plant Protection Research Institute, Agriculture Research Center, Dokki, Giza, Egypt

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The rhizosphere hosts a large and active population of microbes capable of having positive, neutral and harmful effects on plants. The Plant Growth-Promoting Rhizobacteria (PGPR) are root-colonizing bacteria, that exert a beneficial effect on the growth of their host[1]. Plant and PGPR interactions in the rhizosphere are responsible for promoting plant health and increasing soil fertility. PGPR strains use one or more direct or indirect mechanisms to enhance the growth and health of plants and crop yield. These mechanisms can be active simultaneously or at different times and stages of plant growth[2]. Direct mechanisms of plant growth promotion include fixation of atmospheric nitrogen, solubilization of minerals such as phosphorus and iron (through siderophores) and synthesis of phytohormones[3]. N_2 fixation after, Phosphate (P) solubilization is the next most important factor in plant growth promotion. Several types of soil bacteria, particularly those belonging to the genera *Bacillus* and *Pseudomonas*, solubilize the insoluble forms of P by secreting organic acids and enzyme phosphatases[4,5]. Siderophores, which are low-molecular-weight compounds produced under low-iron stress, can solubilize iron and make it available to crop plants for various metabolic activities. Besides, many PGPR secretes a variety of hormones that play vital roles in the growth and development of plants. The production of phytohormones, such as Indole-3-Acetic Acid (IAA), by PGPR, has been seen as one of the vital mechanisms underlying growth and yield increases in crop plants. PGPR capable of N_2 fixation, P solubilization, iron solubilization, siderophore production and phytohormone production can thus improve the growth and health of crop plants[6].

The indirect mechanisms of plant growth promotion by PGPR involve biological control of phytopathogens through the production of siderophore antibiotics, lytic enzymes and hydrogen cyanide and nutrient and niche competition. Siderophores inhibit the iron nutrition of phytopathogens, thus restricting their growth and HCN also inhibits the growth of phytopathogens[7].

Based on these observations, PGPR strains capable of direct plant growth promotion and biocontrol of phytopathogens have been regarded as a green solution to improve the nutrient status of host plants and have been accepted as alternative agents to chemical fertilizers and chemical fungicides[8,9]. However, there is little information available regarding the screening and use of PGPR in Saudi Arabia.

Therefore, the present study was carried out to isolate and characterize native plant growth-promoting bacterial strains from the rhizospheric soils of the Qassim region, Saudi Arabia and to test them as potential bio-inoculants to enhance lettuce plant growth.

MATERIALS AND METHODS

Study area: The study was carried out at the Department of Plant Production and Protection, Faculty of Agriculture and Veterinary, Qassim University, Qassim, Saudi Arabia from March, 2017-January, 2019

Sample collection and isolation of bacteria from the root rhizosphere of plants: Six rhizospheric soil samples were collected from six desert plants *Rhanterium epapposum*, *Atriplex leucoclada*, *Cyperus conglomerates*, *Lasiurus hirsutus*, *Cenchrus ciliaris* and *Stipagrostis obtusa* in four locations of the Qassim region, North-central Saudi Arabia. Each sample was put in sterilized saline solution then serially diluted to a 10^{-7} dilution. An aliquot (0.1 mL) of this suspension was individually spread on Pikovskaya agar and incubated at 30°C for 3 days. Colonies showing halo zones were considered P-solubilizers. The P-solubilizers bacteria were purified by repeated subculturing on Pikovskaya agar medium and stocked on nutrient agar slants until further use.

Assessment of plant growth-promoting traits

Phosphate solubilization: The colonies of bacteria showing a P solubilization zone on Pikovskaya agar were grown in Pikovskaya broth at 30°C for 5 days. The soluble P concentration was measured colourimetrically at 750 nm on a spectrophotometer (U3310 Spectrophotometer, Hitachi, Tokyo) by the ascorbic-molybdate-blue method[10]. Total soluble phosphorus was calculated from a regression equation of the standard curve (Standard curve was prepared by dissolve serial concentrations of K_2HPO_4 and measured their optical density by the ascorbic-molybdate-blue method then draw regression line between concentration and optical density on excel program to obtain straight-line equation). The soluble phosphate that was liberated was expressed as μg mL^{-1}.

Production of IAA: Total 1 mL aliquot of 24 hrs grown P-solubilizers bacterial cultures were separately grown in 10 mL Nutrient Broth (NB) containing 0.1% L-tryptophan at 30°C and 180 rpm for 48 hrs in the dark. After an incubation

period, each broth was centrifuged at 10,000 rpm for 10 min at 4°C and the supernatant was assayed for the estimation of IAA. For this purpose, 1 mL of supernatant was mixed with 2 mL of Salkowski reagent (2% of 0.5 M $FeCl_3$ in 35% $HClO_4$ solution), which resulted in pink colour; the absorbance of the sample was read at 535 nm in a UV/visible spectrophotometer (U3310 Spectrophotometer, Hitachi, Tokyo)[11,12] IAA production was calculated from a regression equation of the standard curve and expressed as μg mL^{-1}. (Standard curve was prepared by dissolving serial concentrations of IAA and measured their optical density by the same previous method then draw regression line between concentration and optical density on excel program to obtain straight-line equation).

Ammonia production: Overnight-grown bacterial cultures were inoculated in 10 mL of peptone and incubated at 30°C for 48 hrs. Following incubation, 0.5 mL of Nessler's reagent (0.09 mol L^{-1} solution of potassium tetraiodomercurate (II) ($K_2[HgI_4]$) in 2.5 mol L^{-1} potassium hydroxide) was added to 1 mL of broth culture, which was then observed for the development of a faint yellow to dark brown colour as an indication of ammonia production[13].

Nitrogen fixation: The P-solubilizers bacteria were screened for nitrogen fixation on the nitrogen-free Ashby medium. A loopful of each isolate was streaked on Ashby medium and incubated at 30°C for 3 days and the medium was observed for growth, to indicate nitrogen fixation[14].

Hydrogen cyanide (HCN) production: Screening of P-solubilizers bacterial isolates for Hydrogen Cyanide (HCN) production was carried out as per Castric[15]. Bacterial isolates were plated on nutrient agar containing 4.4 g L^{-1} glycine. Whatman filter paper No. 1 (Whatman Clifton, NJ, USA). soaked in 0.5% picric acid solution (in 2% sodium carbonate) was placed inside the lid of a plate. Plates were sealed with parafilm, incubated at 30±0.1°C for 4 days and monitored for the development of light to dark brown colour as an indication of HCN production.

Siderophore production: Siderophore production was tested by using Chrome Azurol S (CAS) medium according to Husen[16] and Patel[9]. Ten microliters of each isolate were placed on the centre of the surface of CAS agar plates and incubated at 30°C for 2 days. Plates were monitored for the appearance of orange halos around colonies.

Identification of isolates: The identification of the bacterial isolates was based on 16S rRNA gene sequence analysis as previously described[17]. The genomic DNA of the best bacterial isolates in the present experiments was extracted by the phenol-chloroform method[17]. Isoplant II DNA extraction kit (Nippon Gene, Toyama, Japan). The 16S rRNA nucleotide sequences were determined by PCR using a thermal cycler system. The reaction volume was 30 μL and contained 15 μL of PCR Master Mix, 5 μL of template DNA and 5 μL of each primer. The forward primer F (5'-AGA GTT TGA TCC TGG CTC AG-3') and the reverse primer R (5'-TGA CTG ACT GAG GCT ACC TG-3') were used.

Thermal cycling conditions were as follows: one cycle of 95°C for 5 min followed by 35 cycles of denaturation at 94°C for 30 sec, annealing at 61°C for 30 sec and extension at 72°C for 5 min, followed by a final cycle of extension of 72°C for 7 min and incubation at 4°C until stopped. The nucleotide sequences of the PCR products of the 16S rRNA of the strains were determined by using an ABI PRISM® 3500XL DNA Sequencer (Applied Biosystems) at the Macrogen Company, Korea.

The partial sequences that were obtained were compared with the sequences from DNA databases and sequences with a similarity above 95% were retrieved by the nucleotide Basic Local Alignment Search Tool (BLAST) program at the National Center for Biotechnology Information (NCBI) BLAST server (www.ncbi.nlm.nih.gov/BLAST).

Plant growth promotion studies under greenhouse conditions: The four most active bacterial isolates were selected and grown separately on NB medium for 72 hrs at 30°C and 180 rpm. These broth cultures were centrifuged and the cell mass (10^9 CFU mL^{-1}) was washed with a sterile saline solution and mixed with a sticker solution containing 1% Arabic gum, 1% sucrose and 20% corn powder. The lettuce roots were soaked in the above bacterial solution for 10 min and transferred to test pots; plants without bacterial treatment were grown in positive control pots and negative control pots. All pots contained a mixture of sand and sterile compost (4:1) and 1% phosphate rock powder and 1% feldspar powder. Positive control pots contained mineral fertilizer, while negative control pots contained sand soil only, i.e., no fertilizer. Growth parameters were recorded four weeks after planting.

Statistical analysis: All experiments were performed in triplicate and the averages of the three replicates were used. Data were analyzed using one-way ANOVA. Means comparisons were conducted according to Duncan's multiple range test using a probability threshold of 5% for significance (CoStat Software 2005)[18].

RESULTS

Different plant growth-promoting activities by bacterial isolates: Results of the effects of bacterial isolates on factors affecting plant growth are presented in Table 1. The activities studied included soluble phosphate levels, IAA, Ammonia, HCN and Siderophores production. Twelve of the isolates showed the development of sharp and large phosphate solubilization zones. In a quantitative estimation, between 252 and 506 ppm, tri-calcium phosphate was solubilized by these twelve isolates (Table 1). The isolate RF showed the highest phosphate solubilization (506 ppm) in Pikovskaya broth. While only the isolates R155, RA, RH, RF, R186 and R159 were considered as N fixers.

All the isolates except R155 produced IAA, at levels between 18 and 117 ppm (Table 1); the isolates RA and R186 produced the highest amounts of IAA (102 and 117 ppm, respectively).

All twelve P-solubilizing isolates except RC and R159 produced ammonia (Table 1). Among the various isolates, five of them: RA, RH, RF, R186 and R9p, produced HCN (Table 1), but all the isolates except R159 produced siderophores (Table 1, Fig. 1a-c). The negative control group with no fertilizer is presented in Fig. 1a, lettuce response in a positive group with mineral fertilizer (Fig. 1b) has been compared with the use of isolated bacteria fertilizer in Fig. 1c.

Identification of the active bacterial isolates: The most active bacterial isolates identified using 16S rRNA sequencing are shown in Table 2.

The 16S rRNA sequences of the strains RA, RH, RF and R186 shared 99, 99, 96 and 99% similarity with those of *Pseudomonas aeruginosa* AMU1, *Enterobacter* sp. CZGRY7, *Bacillus cereus* BW-201B, and *Pseudomonas putida* CNE30 (Table 2).

Growth properties of lettuce plant after treatment with a bacterial mixture: Growth properties of the lettuce plant after 4 weeks of post-treatment with bacterial mixture under greenhouse conditions are shown in Table 3. It can be noticed that application of PGPR isolates resulted in a significant

Table 1: Bacterial isolates showing different plant growth-promoting activities

Isolate code	Soluble phosphorus (ppm)	Indol acetic acid (IAA) (ppm)	Growth on nitrogen free medium	Ammonia production	Hydrogen cyanide (HCN) production	Siderophore production
R4i	315	33	-	++	-	+
R155	326	0	+++	+++	-	+
RA	329	117	++	++++	++	+
RJ	361	60	-	++	-	+
RC	454	18	-	-	-	+
RH	475	72	+	+++	+++	+
RI	488	39	-	+++	-	+
RG	493	48	-	++	-	+
RF	506	84	+	+++	+	+
R9p	252	45	-	++	+	+
R186	253	102	+++	+++	++	+
R159	265	30	++	-	-	-

Table 2: Identification of the most active bacterial isolates by 16S rRNA sequencing

Isolate code	Identification	Similarity in 16S rRNA sequence (%)
RA	*Pseudomonas aeruginosa* AMU1	99
RH	*Enterobacter* sp. CZGRY7	99
RF	*Bacillus cereus* BW-201B	96
R186	*Pseudomonas putida* CNE30	99

Table 3: Growth properties of lettuce plants after four weeks of post-treatment with bacterial mixture under greenhouse conditions

	Plant parameters					
Treatments	Shoot height (cm)	Shoot weight (g)	Chlorophyll (ppm)	Nitrogen N (%)	Phosphorus P (%)	Potassium K (%)
Negative control	10^{b}	84^{b}	30^{c}	1.6^{c}	0.43^{c}	1.6^{c}
Positive control	14^{a}	125^{a}	45^{a}	3.7^{a}	0.91^{a}	3.4^{a}
PGPR isolates	15^{a}	120^{a}	39^{b}	2.3^{b}	0.76^{b}	2.9^{b}
F-test	**	***	***	***	***	***
LSD 5%	1.99	14.13	4.89	0.50	0.02	0.19

Negative control is without any fertilizer, Positive control is a mineral fertilizer. Soil content in all treatments = Sand: Sterile compost (4:1), 1% rock phosphate and 1% feldspar, LSD: Least significant difference, PGPR: Isolated plant growth-promoting rhizobacteria

Fig. 1(a-c): Shoots and roots of lettuce plants after four weeks of treatment
(a) Shoot and root growth in the negative control (without any fertilizer), (b) Shoot and root growth in the positive control (mineral fertilizer) and (c) Shoot and root growth in the treatment by isolated bacteria strains

increase in the shoot height of lettuce when compared with the group that received no fertilizers and non-significantly higher than the shoot height of lettuce that received mineral fertilizers. Also, there was a significant increase in the shoot weight in grams in plants treated with PGPR isolates when compared with those without any mineral fertilizer. As shown in Table 3 there were significant increases in Chlorophyll, N, P and K (%) in PGPR isolates treated group.

DISCUSSION

PGPR colonizes plant roots and promotes plant growth and development through a variety of mechanisms, such as providing mineral nutrition (P and Fe), phytohormones, ammonia, or nitrogen and suppressing the growth of deleterious organisms through HCN or siderophore production. Activation of phosphate solubilization and promotion of mineral nutrient uptake is usually believed to be involved in plant growth promotion[19,20]. Of the 45 isolates obtained from the six rhizosphere samples in this study, four of them were identified. The most active bacterial isolates identified using 16S rDNA sequencing are *Pseudomonas aeruginosa* AMU1, *Enterobacter* sp. CZGRY7, *Bacillus cereus* BW-201B and *Pseudomonas putida* CNE30 (Table 2). They were found to be the most efficient PGPR, as they exhibited all the plant growth-promoting traits tested, including phosphate solubilization and IAA, ammonia, HCN and siderophore production.

The effects of the mixture of these four isolates on the growth and vigour of lettuce under greenhouse conditions were investigated and compared with untreated plants grown with mineral fertilizer (positive control) or without mineral fertilizer (negative control). It was found that the growth and vigour of lettuce plants treated with the bacterial mixture were nearly equal to those of lettuce plants treated with mineral fertilizer but higher than those of the non-treated plants, as shown in Table 3. The data of Fig. 1 shows the shoots and roots of lettuce plants after four weeks of treatment under conditions of the mineral and bacterial fertilizer and no-fertilizer treatments. The increase in the levels of parameters studied enhanced the overall lettuce growth-promoting factors because IAA is one of the most

important phytohormones and functions as an important signal molecule in the regulation of plant development. IAA production by PGPR varies among different species and strains and is influenced by culture conditions, growth stage and substrate availability[21]. A high level of IAA production by *Pseudomonas* was reported by other researchers[22]. Accordingly, Ammonia production is another important trait of PGPR that indirectly influences plant growth.

The production of HCN (which acts as an inducer of plant resistance) and siderophores has been postulated to play an important role in the biological control of pathogens[23]. A large number of bacteria, including species of *Pseudomonas*, *Azospirillum*, *Azotobacter*, *Klebsiella*, *Enterobacter*, *Alcaligenes*, *Arthrobacter*, *Burkholderia*, *Bacillus*, *Rhizobium* and *Serratia*, have been reported to enhance plant growth[24].

This study discovered that only locally adapted PGPR can enhance the growth and productivity of plants. Little information is present about the four isolates RA, RH, RF and R186 that were found to be efficient PGPR, as they exhibited all the plant growth-promoting traits tested. They were identified by gene sequences as *Pseudomonas aeruginosa* AMU1, *Enterobacter* sp. CZGRY7, *Bacillus cereus* BW-201B and *Pseudomonas putida* CNE30. These isolates were submitted to GenBank and may have potential commercial significance in the future.

CONCLUSION

It can be concluded that only locally adapted PGPR enhanced the growth and productivity of plants. Four isolates RA, RH, RF and R186 were found to be efficient PGPR, as they exhibited all the plant growth-promoting traits tested, including phosphate solubilization and IAA, ammonia, HCN and siderophore production. The 16S rRNA sequences of the strains RA, RH, RF and R186 shared 99, 99, 96 and 99% similarity with those of *Pseudomonas aeruginosa* AMU1, *Enterobacter* sp. CZGRY7, *Bacillus cereus* BW-201B and *Pseudomonas putida* CNE30, respectively and were identified as such Gene sequences of these isolates were submitted to GenBank have potential commercial significance.

SIGNIFICANCE STATEMENTS

This study discovered that only locally adapted PGPR can enhance the growth and productivity of plants. Little information is present about the four isolates RA, RH, RF and R186 that were found to be efficient PGPR, as they exhibited all the plant growth-promoting traits tested. They were identified by gene sequences as *Pseudomonas aeruginosa* AMU1, *Enterobacter* sp. CZGRY7, *Bacillus cereus* BW-201B and *Pseudomonas putida* CNE30. These isolates were submitted to GenBank and may have potential commercial significance in the future.

ACKNOWLEDGMENTS

The authors thank the Promising Research Center in Biological Control and Agricultural Information (BCARC), Qassim University, Saudi Arabia, for funding this work.

REFERENCES

1. Kumar, A., A. Kumar, S. Devi, S. Patil, C. Payal and S. Negi, 2012. Isolation, screening and characterization of bacteria from rhizospheric soils for different Plant Growth Promotion (PGP) activities: An *in vitro* study. Recent Res. Sci. Technol., 4: 1-5.
2. Ahmad, F., I. Ahmad and M.S. Khan, 2008. Screening of free-living rhizospheric bacteria for their multiple plant growth promoting activities. Microbiol. Res., 168: 173-181.
3. Nelson, L.M., 2004. Plant growth promoting rhizobacteria (PGPR): Prospects for new inoculants. Online Crop Manage., 3: 1-7.
4. Sharma, A.D., M. Thakur, M. Rana and K. Singh, 2004. Effect of plant growth hormones and abiotic stresses on germination, growth and phosphatase activities in *Sorghum bicolor* (L.) Moench seeds. Afr. J. Biotechnol., 3: 308-312.
5. Vazquez, P., G. Holguin, M.E. Puente, A. Lopez-Cortes and Y. Bashan, 2000. Phosphate-solubilizing microorganisms associated with the rhizosphere of mangroves in a semiarid coastal lagoon. Biol. Fert. Soils, 30: 460-468.
6. Rodriguez, H. and R. Fraga, 1999. Phosphate solubilizing bacteria and their role in plant growth promotion. Biotechnol. Adv., 17: 319-339.
7. Shaikh, S.S., R.Z. Sayyed and M.S. Reddy, 2016. Plant Growth-Promoting Rhizobacteria: An Eco-friendly Approach for Sustainable Agroecosystem. In: Plant, Soil and Microbes, Hakeem, K.R., M.S. Akhtar and S.N.A. Abdullah (Eds.), Springer International Publishing, Switzerland, ISBN: 978-3-319-27453-9, pp: 181-201.
8. Vessey, J.K., 2003. Plant growth promoting rhizobacteria as biofertilizers. Plant Soil, 255: 571-586.
9. Patel, J.S., M.I.C. de Novaes, M. Barbier and S. Zhang, 2013. Evaluation of biopesticides for managing downy mildew of basil. Plant Dis. Manag. Rep., Vol. 7.
10. Mehta, S. and C.S. Nautiyal, 2001. An efficient method for qualitative screening of phosphate-solubilizing bacteria. Curr. Microbiol., 43: 51-56.
11. Gordon, S.A. and R.P. Weber, 1951. Colorimetric estimation of indoleacetic acid. Plant Physiol., 26: 192-195.

12. Loper, J.E. and M.N. Schroth, 1986. Influence of bacterial sources of indole-3-acetic acid on root elongation of sugar beet. Phytopathology, 76: 386-389.
13. Cappuccino, G.J. and N. Sherman, 2001. Biochemical Activities of Microorganisms. In: Microbiology: A Laboratory Manual, Cappuccino, G.J. and N. Sherman (Eds.)., 6th Edn., Benjamin-Cummings Publishing Company, USA., ISBN-13: 978-0805376487, pp: 133-194.
14. Jensen, E.S., 1987. Inoculation of pea by application of Rhizobium in the planting furrow. Plant Soil, 97: 63-70.
15. Castric, P.A., 1975. Hydrogen cyanide, a secondary metabolite of *Pseudomonas aeruginosa*. Can. J. Microbiol., 21: 613-618.
16. Husen, E., 2003. Screening of soil bacteria for plant growth-promoting activities *in vitro*. Indones. J. Agric. Sci., 4: 27-31.
17. Tamura, K., J. Dudley, M. Nei and S. Kumar, 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Mol. Biol. Evol., 24: 1596-1599.
18. Costat, 2005. Microcomputer Program Analysis Version 6.303. Cohort Software, Monterey, CA., USA.
19. Lalande, R., N. Bissonnette, D. Coutlee and H. Antoun, 1989. Identification of rhizobacteria from maize and determination of their plant-growth promoting potential. Plant Soil, 115: 7-11.
20. Glick, B.R., 1995. The enhancement of plant growth by free-living bacteria. Can. J. Microbiol., 41: 109-117.
21. Mirza, M.S., W. Ahmad, F. Latif, J. Haurat, R. Bally, P. Normand and K.A. Malik, 2001. Isolation, partial characterization and the effect of Plant Growth-Promoting Bacteria (PGPB) on micro-propagated sugarcane *in vitro*. Plant Soil, 237: 47-54.
22. Xie, H., J.J. Pasternak and B.R. Glick, 1996. Isolation and characterization of mutants of the plant growth-promoting rhizobacterium *Pseudomonas putida* GR12-2 that overproduce indoleacetic acid. Curr. Microbiol., 32: 67-71.
23. Voisard, C., C. Keel, D. Haas and G. Defago, 1989. Cyanide production by *Pseudomonas fluorescens* helps suppress black root rot of tobacco under gnotobiotic conditions. Eur. Mol. Biol. Organiz. J., 8: 351-358.
24. Okon, Y. and C.A. Labandera-Gonzalez, 1994. Agronomic applications of *Azospirillum*: An evaluation of 20 years worldwide field inoculation. Soil Biol. Biochem., 26: 1591-1601.