A rhizosphere isolate from *Oryza sativa*, *Enterobacter cloacae* VITTPN2, as a potential plant growth promoting rhizobacteria; an *in vitro* study

Thahiya Naushad, Neethu Kamarudheen, Poorna Chandrika Gopal, Kokati Venkata Bhaskara Rao *

Molecular and Microbiology Research Laboratory, Division of Environmental Biotechnology, School of Bio Sciences and Technology, VIT University, Vellore, Tamil Nadu-632 014, India

Received 11th December 2018 / Accepted 22nd February 2019

Abstract. The increasing need for Plant Growth Promoting Rhizobacteria (PGPR) for biofertilizer development is warranted owing to the environmental hazards caused by chemical fertilizers. Our investigation was to isolate, screen and characterize PGPR from rhizospheric soil with potential PGPR properties. *Oryza sativa* and *Saccharum officinarum* rhizosphere were collected from the agricultural research station, Virinjipuram, Vellore (12.9202°N, 79.1333°E), Tamil Nadu, India for PGPR isolation. Eleven distinct isolates of bacteria were grown on Jensen's (seven) and Pikovskaya's media (four). Among these, four isolates (TPN1 to TPN4) showed phosphate solubilisation activity. And one isolate TPN2 particularly showed both nitrogen fixation and phosphate solubilization with other PGPR properties. Furthermore, the isolate TPN2 demonstrated promising results in Indole 3-Acetic Acid production ($99.29 \pm 0.945 \mu\text{g ml}^{-1}$). Since the isolate TPN2 displayed all PGPR characteristics under study, it was selected for pot culture studies. The seeds treated with TPN2 revealed an increase of 63.6% in shoot length and 14.63% in root length of the okra plant. There was a 74.6% increase in shoot length and a 16% increase in the root length of the tomato plant. Additionally, there was extensive development of lateral roots in okra plant. Henceforth TPN2 was identified as *Enterobacter cloacae* VITTPN2 (ku951582). This report produced remarkable results which promise the bacterial strain *Enterobacter cloacae* strain VITTPN2 can be further studied as a prospective biofertilizer.

Keywords: *Enterobacter cloacae*, IAA, nitrogen fixing, phosphate solubilisation, pot culture, shoot elongation

INTRODUCTION

The soil is one of the most exploited scientific frontiers and rhizosphere is the active part of that frontier as it contains many microorganisms. The rhizosphere is a fine sheet of soil around the roots of the plants which hold a large number of active bacteria including rapidly colonizing Plant Growth Promoting Rhizobacteria (PGPR) (Villacieros *et al.*, 2003; Suslow *et al.*, 1979).

Lately, a great deal of consciousness has been

given to PGPR as a valuable substitute for agrochemicals. Also, there has been much interest shown towards these bacteria to substitute chemicals, which promote the growth of plants in various number of ways, including production of plant growth promoting phytohormones, root growth enhancement, biocontrol of pathogens, degradation of organic pollutants, solubilization of unavailable forms of nutrients etc (Gupta *et al.*,

* Author for correspondence: K. V. Bhaskara Rao. Division of Environmental Biotechnology School of Bio Sciences and Technology, VIT University, Vellore TN-632 014, India. Email - kvbhaskararao@vit.ac.in

2015). The rhizospheric soil contains root exudates secreted by plants and rhizodeposits which act as chemical attractants for PGPR (Lowe *et al.*, 2012). The exudates include amino acids, sugars, vitamins, nucleosides, enzymes, inorganic ions and gaseous molecules. Activities of rhizobacteria are observed to be high in this region. Many bacteria like *Alcaligenes*, *Arthrobacter*, *Azospirillum*, *Azotobacter*, *Bacillus*, *Burkholderia*, *Enterobacter*, *Klebsiella*, *Pseudomonas*, *Rhizobium*, and *Serratia* are accounted as inhabitants of rhizosphere which enhances plant growth. (Bhattacharya and Jha 2012) The PGPR bacteria are characterised by (i) they must be capable of colonizing on the root surface (ii) during their expression of plant growth promoting activities, they should thrive, reproduce and be able to win over other microbes present around (iii) they must enhance the plant growth (Ahmad and Khan 2011).

The PGPR increases plant growth by two different mechanisms (i) direct (ii) indirect. Direct means are, fixing the unavailable atmospheric nitrogen, phytohormone production like indole acetic acid, gibberellic acid, siderophores, reduction in ethylene and phosphate solubilisation (Glick *et al.*, 1998) whereas production of antibiotics, iron in the rhizosphere, antifungal metabolites and enzyme production and induced systemic resistance, are included in indirect mechanisms of growth enhancement by PGPR (Gupta *et al.*, 2015; Kloepper *et al.*, 1989).

Nitrogen (N_2) is a majorly important macronutrient essential for the growth of plants. Despite the presence of 78% nitrogen in the air, it is not available for the use of plants. PGPR helps convert N_2 to NH_3 by nitrogen fixation, thus making nitrogen available for plants. Phosphorus is the next major growth-limiting nutrient, that exists in both organically and inorganically in soil (Glick, 1995). A major amount of phosphorus is encountered in forms that are not soluble like apatite, inositol phosphate (soil phytate), phosphomonoesters, and phosphotriesters which lowers its uptake by plants (Khan *et al.*, 2009). Plants take up phosphorous in monobasic ($H_2PO_4^-$) and dibasic (HPO_4^{2-}) ion forms which are soluble (Glick, 2012). PGPR solubilises the soil phosphates by producing organic acids and phosphatase enzymes. This results in uptake of soluble phosphorous by plants.

Indole 3-Acetic Acid (IAA) is regarded as the major indigenous auxins (Bhattacharya and Jha 2012). IAA acts as signal molecules in regulating the growth of plants like organ formation, tropic and cellular response and gene regulation. PGPR strains are well documented for the biosynthesis of the IAA which subsequently increases plant root system and thereby enhances the nutrient uptake by plants. L- Tryptophan functions as a predecessor for IAA production, thus its presence in culture medium enhances the IAA production *in vitro* (Kloepper and Schroth 1978).

PGPR strains are protective agents against plant pathogens disseminated through soil by hydrogen cyanide (HCN) production (Glick, 2012). HCN inhibits the growth of plant pathogenic fungi. Biofertilizers have been increasingly recognized as substitutes to chemical fertilizers as these microbes help enhance the plant growth, yield and augment the nutrients in the soil they inhabit (Cappucino and Sherman 1992). A substantial rise in the productivity of crops is accounted after applying PGPR microbial inoculants (Lwin *et al.*, 2012).

In this study, we have screened and identified PGPR strains. Rhizospheric soil of *Oryza sativa* and *Saccharum officinarum* was used as a source to isolate PGPR. PGPR isolates obtained on various media were screened for PGPR properties such as nitrogen fixation, phosphate solubilisation, synthesis of IAA, HCN, ammonia synthesis and catalase production. The most active isolate was identified genomically and subjected to root and shoot elongation study for the primary conclusion of PGPR aiding plant growth.

MATERIALS AND METHODS

Chemicals and reagents. All the bacteriological media used in this study were purchased from Himedia (Mumbai, India). Every analytical grade chemicals and reagents such as indole acetic acid, L-tryptophan were purchased from Merck (Bangalore, India).

Sample collection and isolation of PGPR strains. Rhizosphere sample was obtained from the rhizosphere region of *Oryza Sativa* and *Saccharum officinarum* from the agricultural research

station, Virinjipuram of Vellore (12.9202°N, 79.1333°E) Tamil Nadu, India. The isolation of rhizospheric bacteria was carried out through traditional microbiological procedures (Cappucino and Sherman 1992). Jensen's agar medium and Pikovskaya's agar medium were employed for the selective isolation of nitrogen fixers and phosphate solubilizers respectively. The plates were incubated at an optimal temperature of $30\pm 2^{\circ}\text{C}$ for a period of 2-8 days with continuous monitoring for the presence of bacterial growth. The colonies with morphological differences were selected and preserved at 4°C on the corresponding medium.

Determination of dual nature of the isolates.

To evaluate the isolates in terms of both nitrogen fixation as well as phosphate solubilisation the isolates from Jensen's agar were grown on Pikovskaya's agar and vice-versa. The isolates which virtually exhibited both the properties were selected and preserved for further studies (Deolankar *et al.*, 2015).

Phytohormone studies - Indole Acetic Acid (IAA) assay. One ml of overnight bacterial culture was inoculated into Luria Bertani broth with $5\text{ }\mu\text{g ml}^{-1}$ of L-tryptophan and placed in an orbital shaker (120 rpm) at room temperature for 48 hours. Upon incubation period, 2 ml was withdrawn and centrifuged at 4307 xg for 30 mins. IAA production was estimated by UV-Vis Spectrophotometer by Salkowski's reagent at 530 nm as mentioned by Patten and Glick, 2000. IAA produced by isolated strain was calculated in comparison to indole acetic acid curve and the highest IAA producer was established (Ashrafuzzman *et al.*, 2009).

Phosphate solubilisation activity. For determination of solubilisation capacity of the isolates, they were incubated on modified Pikovskaya's medium at $37\pm 2^{\circ}\text{C}$ for 5 days. The plates were observed continuously until 5 days for the appearance of the zone of clearance indicative of the potency of the isolate (Vessey, 2003; Salamone 2000).

Hydrogen cyanide and catalase production. Isolates were screened for the ability to produce hydrogen cyanide (HCN) according to the

method of Castric (Castric *et al.*, 2010). For the purpose of detection of HCN production, the selected isolates were inoculated on nutrient agar plates with glycine concentration of 4.4 g ml^{-1} . A strip of Whatman filter paper no. 1 soaked in sodium carbonate in 0.5% picric acid solution and was placed inside sticking on to the surface of the lid of the agar plate. The plate was sealed with paraffin film and incubated at $36\pm 2^{\circ}\text{C}$ for 72 hours. The filter paper turning orange indicates the production of HCN.

For assessing catalase activity, 250 μl of 48 hr old bacterial isolates was placed on the surface of a glass slide and mixed with an equal volume of 3% H_2O_2 with a sterile applicator stick. The effervescence indicates the catalase activity (Schaad, 1992).

Ammonia production. To investigate the production of ammonia, peptone water was used to grow the isolate. 5-10 ml of peptone water was inoculated with 100 μl of overnight culture and incubated at $37\pm 2^{\circ}\text{C}$ for 2-3 days. Production of ammonia was detected by addition of Nessler's reagent (Colins and Lyne 1980). Production of ammonia was indicated by the brown to yellow colouration.

Characterization and identification of the potential isolate. The isolate showing all the activities viz., nitrogen-fixing ability, phosphate solubilisation, maximum IAA production and catalase activity were identified by following microscopic, biochemical and molecular analyses. The potential isolate TPN2 was identified through 16S rRNA sequence analysis. InstaGene Matrix Genomic DNA isolation kit (Bio-Rad) was used to isolate bacterial genomic DNA. The segments were amplified using the universal primers 27F 5'-AGAGTTTGATCMTGGTCAG-3' and 1492 R 5'-TACGGYTACCTTGTTACGACTT-3'. The PCR product was purified with the help of montage PCR clean-up kit (Millipore). Sequencing was performed on ABI 3730xl sequencer. The resultant 16S rRNA sequence was observed for similarity using NCBI BLAST similarity sequence tool. The sequence was further analysed for phylogeny with the highest similar sequences. Multiple alignments of the sequences was performed by MUSCLE 3.7. Mega 5 was used for constructing the phylogenetic tree by

neighbour joining method with interior branch test.

Pot culture study for determining plant growth promotion. The most potential isolate exhibiting all the above plant growth promoting properties were selected for the *in vitro* studies in pot cultures. Seeds of two important vegetable crops, tomato and Okra plant were obtained from the agricultural research station, Virinjipuram, Vellore, Tamil Nadu, India. Surface sterilization of the seeds was performed using sodium hypochlorite for 10-20 mins and they were washed thoroughly with distilled water. The seeds were immersed in contact with the suspension of bacteria prepared prior to the experiment. The suspension was prepared by inoculating a loopful of bacterial suspension in 100 ml of Luria Bertani broth on an orbital shaker at 180 rpm for 48 hours. The treated seeds were planted in pots as deep as 4 to 5 cm of 300 g sterile soil for a period of 15 days. A control pot was also maintained along with the experimental pots.

The plants were maintained and observed daily for a period of 3 weeks through seed sowing to harvesting. The elongation (cm plant⁻¹) of shoot and root of each plant (n=8) was studied after the period of growth. The comparison was made to the control set of plants (Yadav *et al.*, 2010).

Statistical analysis. Experiments involved were performed in triplicates. Pot culture experiment result was examined statistically by Two-way Analysis of Variance (ANOVA) using GraphPad Prism 6.0 (GraphPad Software Inc. San Diego, USA). The two independent variables root and shoot length were found to have an interaction with the dependent variable total height and the study was found to be statistically significant.

RESULTS AND DISCUSSION

Isolation and screening for dual nature. The process of isolation yielded four bacterial colonies showing nitrogen fixing ability and seven phosphate solubilising colonies on Jensen's medium and Pikovskaya's medium respectively. Upon the cross-media inoculation studies, four bacterial isolates which showed the dual nature of

phosphate solubilisation and nitrogen fixation were selected for further studies. These isolates were designated as TPN1 to TPN4. These isolates were found potent to fix atmospheric nitrogen and also to make available the phosphate present in the soil to plants by exhibiting phosphate solubilisation.

Phytohormone studies- IAA Production. IAA is obtained at the stationary phase of the bacterial culture as IAA as the secondary metabolite and varies with species, strains and their culture conditions (Yadav *et al.*, 2010; Gupta *et al.*, 1994). The isolates TPN2 and TPN4 showed higher IAA production at tryptophan concentrations 5 µg/ml. The synthesis of auxin in plants and microbes are regulated by tryptophan as it is the main precursor (Vincent, 1970). This variation in concentration determines the PGPR bacterial ability to produce IAA. Previous reports of some rhizosphere isolate studied by Lwin *et al.* shows 121 µg ml⁻¹ of IAA at a concentration of 0.5 µg ml⁻¹ whereas TPN2 exhibited 97.24 µg ml⁻¹ of IAA production at a concentration of 5 µg ml⁻¹ of tryptophan (Lwin *et al.*, 2012) (Figure 1). The production was indicated by dark pink. IAA synthesis by *Pseudomonas putida* has been reported as 25.65 µg ml⁻¹ and 48.46 µg ml⁻¹ at a tryptophan concentration of 100 µg ml⁻¹ and 200 µg ml⁻¹ respectively while *Bacillus subtilis* 16.23 µg ml⁻¹ at 100 µg ml⁻¹ and 36.38 µg ml⁻¹ at 200 µg ml⁻¹ (Yadav *et al.*, 2010).

Figure 1. Quantification of IAA produced by the isolates

Phosphate solubilization. Phosphate solubilization is another vital characteristic of PGPR bacteria, a mechanism of making available the insoluble phosphates by the release of any

dissolving compounds, extracellular enzymes or release of phosphate on degradation (Rodriguez and Reynaldo 1999). It is a characteristic of members of *Arthrobacter*, *Bacillus*, *Rhizobium*, *Flavobacterium*, *Enterobacter* and few other genera (Khalid *et al.*, 2004). Determination of phosphate solubilization was performed on Pikovskaya's media and the zone of clearance was noted. Isolates exhibited different degree of clearance with TPN2 been the best of the isolates showing an inhibition of 11.2 mm followed by TPN4 with 9.4 mm. The other two isolates exhibited low inhibition of 3.2 mm and 1.7 mm by TPN1 and TPN3 respectively. Thus, TPN2 and TPN4 were selected for further studies.

Hydrogen cyanide, catalase and ammonia production. HCN is one of the metabolites that inhibit the growth of microbes and may also deter plant growth (Gupta *et al.*, 1994; Vincent, 1970). HCN activity was reported negative for TPN4 whereas the isolate TPN2 was positive.

Catalase activity is critical for the regulation of oxidative stress. The isolate TPN2 demonstrated catalase activity but it was absent in TPN4. This adds on to the importance of TPN2 as a biofertilizer.

For the above reasons TPN2 was tested for another PGPR property namely, ammonia production. If produced ammonia helps in inducing plant growth as it is the most common assimilatory form of nitrogen. The ammonia released by a rhizobacterial strain plays a signalling role in interaction between PGPR and plants and also increase the glutamine synthetase activity (Chitra *et al.*, 2002). The isolate was potentially showing ammonia production after 72 hours of incubation. In a similar fashion, Ahmed and Khan (2010) reported the production of ammonia by *Enterobacter asburiae*.

Phenotypical and genotypical identification of the isolate. Similar to many reported PGPR bacteria, isolate TPN2 was also identified as a Gram-negative motile rod. All the basic biochemical characteristics of the isolate were determined (as shown in Table 1). The ability of motility becomes beneficial being a PGPR bacterium. The 16S rRNA sequencing is a technique for determining bacterial phylogeny and defining the taxonomy. Therefore, the

potential isolate was subjected to 16S rRNA sequencing and the sequence was checked for similarity using BLAST search which displayed 99% similarity with *Enterobacter cloacae*. Based on morphological, biochemical and phylogenetic character similarities, the isolate was thus identified as *Enterobacter cloacae* VITTPN2 and was deposited in GenBank under the accession no: **ku951582** (see Figure 2).

Table 1. Biochemical characterization of TPN2.

Test	TPN2
Morphology	Rod
Gram stain	-
Motility	+
Indole	-
Methyl Red	-
Voges-Proskauer	+
Catalase	+
Oxidase	-
Citrate	+
Urease	-
H ₂ S	-
Starch hydrolysis	-
Glucose utilization	+
Sucrose	+
Mannitol	+

(+) presence; (-) absence

Hydrogen cyanide, catalase and ammonia production. Our study demonstrates that seeds of the plants appear to have been affected by the PGPR isolate TPN2 as the growth was observed 4 days prior to the untreated control seeds. The PGPR isolate also significantly affected the height of both tomato and Okra plant (as shown in Table 2 and Figure 3). Results indicate that the height of the plant has increased in the PGPR treated plants over un-inoculated control.

The treatment with PGPR isolates considerably augmented the root and shoot length of both tomato and Okra. Root length of tomato and okra was recorded to be 3.50 ± 0.721 cm plant⁻¹ and 5.60 ± 0.361 cm plant⁻¹. The shoot length of tomato was observed to be 7.7 ± 1.908 cm plant⁻¹ whereas in Okra it was 16.00 ± 2.784 cm plant⁻¹ (Figures 4 and 5). In a previous study, the root and shoot elongation was found to be 8.4 ± 1.1 cm plant⁻¹ and 15.6 ± 1.1 cm plant⁻¹ by *P. putida* and 12.2 ± 1.6 cm plant⁻¹ and 6.7 ± 1.2 cm plant⁻¹ for *B. subtilis* (Yadav *et al.*, 2010). The differences can

vary among *in vitro* set up. Also, TPN2 would require optimization in terms of production of higher amounts of IAA and ammonia for being a better PGPR. On contrary to the control plants, plants treated with TPN2 isolate showed commendable root and shoot elongation. The growth and advancement of the lateral root system were also observed in the system of the okra plant. The increase of shoot and length can be attributed to the overexpression of H-NS genes as reported by English *et al.* (2010).

Nitrogen fixation is yet another important element for the enhancement of soil fertility. Both free-living, as well as symbionts, are capable of the above due to the presence of *nif* genes. Members

of genera *Enterobacter*, *Acetobacter*, *Azotobacter* and a few more are able to fix nitrogen (Siddiqui, 2006). TPN1, TPN2, TPN3 and TPN4 showed the dual nature of being good nitrogen fixers and phosphate solubilizers. In 2011, Shankar *et al.* reported the efficacy of *Enterobacter cloacae* strain GS1 as PGPR by direct mechanisms exhibited by the bacterium whereas in our study we explain both direct and indirect mechanisms involving catalase production (Shankar *et al.*, 2011). Therefore the two isolates although of the same species exhibited different capabilities as PGPR. Our isolate is concluded to have more diverse PGPR characteristics.

Table 2. The root and shoot elongation of tomato plant.

	Shoot length plant ⁻¹ (cm)	Root length plant ⁻¹ (cm)	Total height plant ⁻¹ (cm)
Untreated Control	5.40±0.100	3.50±0.721	8.43±0.066
Treated seeds	7.7±1.908	2.80±0.721	10.5±1.24

The values are represented as mean ± SD

Table 3. The root and shoot elongation of okra plant.

	Shoot length plant ⁻¹ (cm)	Root length plant ⁻¹ (cm)	Total height plant ⁻¹ (cm)
Untreated Control	11.44±0.087	5.10±0.100	16.50±0.015
Treated seeds	16.00±2.784	5.60±0.361	22.60±1.682

The values are represented as mean ± SD

Figure 2. Molecular phylogenetic tree of TPN2 constructed by Neighbour joining tree with interior branch analysis.

Figure 3. Harvested plant upon elongation by pot culture experiment. (A) Tomato plant (B) Okra plant.

Figure 4. Effect in length of Okra plant after treatment with *E. cloacae* VITTPN2.

Figure 5. Effect in length of Tomato plant after treatment with *E. cloacae* VITTPN2.

CONCLUSION

Plant growth promoting rhizobacteria colonize in plant roots have many beneficial roles in plant growth. These may vary with types and nature of the soil and depends on the bacterial species. The

isolate TPN2 (*Enterobacter cloacae* VITTPN2) showed good IAA production, phosphate solubilisation, ammonia production and catalase activity. It also showed good activity in the root and shoot elongation. This shows that TPN2 can be a prospective biofertilizer.

ACKNOWLEDGEMENTS

The authors are grateful to the management and staff of VIT University, Vellore, Tamil Nadu, India for providing the facilities to complete the study successfully.

REFERENCES

- Ahmad, M. & Khan M. S. 2010. Influence of selective herbicides on plant growth promoting traits of phosphate solubilizing *Enterobacter asburiae* strain PS2. *Research Journal of Microbiology* 5: 849-857.
- Ahmad, M. & Khan M. S. 2011. Assessment of plant growth promoting activities Rhizobacterium, *Pseudomonas putida* under insecticide stress. *Microbiology Journal* 1: 54-64.
- Ashrafuzzaman, M., Hossen, F. A., Ismail, M. R., Hogue, M. A., Islam, M. Z., Shahidullah, S. M., & Meon, S. 2009. Efficiency of plant growth-promoting rhizobacteria (PGPR) for the enhancement of rice growth. *African Journal of Biotechnology* 8: 1247-1252.
- Bhattacharyya, P. N & Jha, D. K. 2012. Plant growth-promoting rhizobacteria (PGPR): emergence in agriculture. *World Journal Microbiology Biotechnology* 28: 1327-1350.
- Cappuccino, J. C. & Sherman, N. 1992. Microbiology: "A Laboratory Manual", 10th Ed. Benjamin/Cummings, New York, NY, USA.
- Castric, V., Bechsgaard, J., & Grenier, S. 2010. Molecular evolution within and between self-incompatibility specificities. *Molecular Biology and Evolution* 27: 11-20.
- Chitra, R. S., Sumitra, V. C., & Yash, D. S. 2002. Effect of different nitrogen sources and plant growth regulators on glutamine synthetase and glutamate synthase activities of radish cotyledons. *Bulgarian Journal of Plant Physiology* 28: 46-56.
- Colins C. H. & Lyne, P. M. 1980. Microbiological methods. 8th Ed. Butterworth & Co. London. pp. 405.
- Deolankar, S., Chakarborthy, I., Kamarudheen, N., & Rao, K. V. B. 2015. Evaluation of plant growth promoting activity of rhizosphere bacteria of different crop plants. *Research Journal of Pharmaceutical, Biological and Chemical Sciences* 6 : 683-689.
- English, M. M., Coulson, T. J. D., Horsman, S. R., & Patten, C. L. 2010. Overexpression of *bms* in the plant growth-promoting bacterium *Enterobacter cloacae* UW5 increases root colonization. *Journal of Applied Microbiology* 108: 2180-2190.
- Glick, B. R. 1995. The enhancement of plant growth by free-living bacteria. *Canadian Journal of Microbiology* 41: 109-117.
- Glick, B. R. 2012. Plant growth-promoting bacteria: mechanisms and applications. *Scientifica* 1: 1-15.
- Glick, B. R., Penrose, D. M., & Li, J. 1998. A model for the lowering of plant ethylene concentrations for by plant growth promoting bacteria. *Journal of Theoretical Biology* 190:

- 63-68.
- Gupta, G., Parihar, S. S., Ahirwar, N. K., Snehi, S. K., & Singh, V. 2015. Plant Growth Promoting Rhizobacteria (PGPR): current and future prospects for development of sustainable agriculture. *Journal of Microbial and Biochemical Technology* 7: 96-102.
- Gupta, R., Single, R., Sankar, A., Chandar, R. M., & Kumar, R. S. 1994. A modified assay for screening phosphate solubilizing microorganisms. *Journal of General and Applied Microbiology* 40: 255-260.
- Khalid, A., Arshad, M and Zahir, Z.A. 2004. Screening plant growth promoting rhizobacteria for improving growth and yield of wheat. *Journal of Applied Microbiology*. 96:473-480.
- Khan, A. A., Jilani, G., Akhtar, M., S., Naqvi, S. M. S., & Rasheed, M. 2009. Phosphorus solubilizing bacteria: occurrence, mechanisms and their role in crop production. *Research Journal of Agriculture and Biological Sciences* 1: 48-58.
- Kloepper, J. W., Lifshitz, R., & Zablotowicz, R. M. 1989. Free-living bacterial inocula for enhancing crop productivity. *Trends in Biotechnology* 7: 39-44.
- Kloepper, J. & Schroth, W. M. N. 1978 Plant growth promoting rhizobacteria on radishes In: station de Pathology vegetable et Phyto-bacteriologie, editor. *Proceedings of 4th International conference on plant pathogenic bacteria vol II. Tours: Gilbert-Clary. France.* pp .879-82.
- Lowe, A., Susan, M., McArdle, R., Alan, C., & Cassells. 2012. Effects of AMF and PGPR root inoculation and a foliar chitosan spray in single and combined treatments on powdery mildew disease in strawberry. *Agricultural and Food Science* 21: 28-38.
- Lwin, K. M., Myint, M. M., Tar, T., & Aung, W. Z. M. 2012. Isolation of plant hormone (Indole-3-Acetic Acid - IAA) producing rhizobacteria and study on their effects on maize seedling. *Engineering Journal* 16: 138-144.
- Patten, C. & Glick, B. 2000. Role of *Pseudomonas putida* indole acetic acid in development of host plant root system. *Applied and Environmental Microbiology* 68: 3795-3801.
- Rodriguez, H. & Reynaldo, F. 1999. Phosphate solubilizing bacteria and their role in plant growth promotion. *Biotechnology advances* 17: 319-339.
- Salamone, I. E. G. 2000. Direct beneficial effects of cytokinin producing rhizobacteria on plant growth. Ph.D. Thesis, University of Saskatchewan, Saskatoon, SK, Canada.
- Schaad, N. W. 1992. Laboratory guide for identification of plant pathogenic bacteria, 2nd Ed. International Book Distributing Co, Lucknow. pp. 44-58.
- Shankar, M., Ponraj, P., Ilakkiam, D., & Gunasekaran, P. 2011. Root colonization of a rice growth promoting strain of *Enterobacter cloacae*. *Journal of Basic Microbiology* 51: 523-530.
- Siddiqui, Z. 2006. PGPR: prospective biocontrol agents of plant pathogens. PGPR: *Biocontrol and Biofertilization*, Springer, The Netherlands. pp. 111-142.
- Suslow, T. V., Kloepper, J. W., Schroth, M. N, Burr T. J. 1979. Beneficial bacteria enhance plant growth. *California Agriculture Online* 11: 15-17.
- Vessey, J. K. 2003. Plant growth promoting rhizobacteria as biofertilizers. *Plant Soil* 255: 571-586.
- Villacieros, M., Power, B., Sanchez-Contreras, M., Loret, J., Oruzabal, R. I., Martin, M., Franandez-Pinas, F., Bouile, I., Whelan, C., Dowling, D. N., & Rivilla, R. 2003. Colonization behaviour of *Pseudomonas fluorescence* and *Sinorhizobium meloti* in the alfalfa (*Medicago sativa*) rhizosphere. *Plant Soil* 25: 47-54.
- Vincent, J. M. 1970. A manual for the practical study of root-nodule bacteria. Blackwell Scientific Publ., Oxford, UK.
- Yadav, J., Verma, J. P., & Tiwari, K. N. 2010. Effect of plant growth promoting Rhizobacteria on seed germination and plant growth Chickpea (*Cicer arietinum* L.) under *in vitro* conditions. *Biological Forum - An International Journal* 2: 15-18.