*British Microbiology Research Journal*
*6(1): 32-40, 2015, Article no.BMRJ.2015.056*
*ISSN: 2231-0886*

**SCIENCEDOMAIN** *international*
www.sciencedomain.org

# Characterization of Plant Growth Promoting Rhizobacteria Isolated from Chickpea (*Cicer arietinum*)

**Amjad Ali[1*], Rabia Khalid[1], Safdar Ali[1], Zahid Akram[2] and Rifat Hayat[1*]**

*[1]Department of Soil Science and SWC, PMAS Arid Agriculture University, Rawalpindi 46300, Pakistan.*
*[2]Department of Plant Breeding and Genetics, PMAS Arid Agriculture University, Rawalpindi 46300, Pakistan.*

***Authors' contributions***

*This work was carried out in collaboration between all authors. Authors AA, SA, RH and ZA help designed the study. Authors AA and RK wrote the first draft of the manuscript, managed the literature searches, analysis of the study and managed the experimental process. All authors read and approved the final manuscript.*

***Article Information***

DOI: 10.9734/BMRJ/2015/14496
Editor(s):
(1) Hung-Jen Liu, Director, Institute of Molecular Biology, National Chung Hsing University, Taiwan.
Reviewers:
(1) Anonymous, University of Waterloo, Canada.
(2) Anonymous, University of Piemonte Orientale, Italy.
(3) Anonymous, Universidad Nacional de Río Cuarto, Argentina.
(4) Anonymous, Universiti Putra Malaysia Bintulu Sarawak Campus, Bintulu, Sarawak, Malaysia.
(5) Gloria Regina Botelho, Universidade Federal de Santa Catarina, Brazil.
Complete Peer review History: http://www.sciencedomain.org/review-history.php?iid=827&id=8&aid=7284

***Original Research Article***

***Received 1st October 2014***
***Accepted 21st November 2014***
***Published 15th December 2014***

## ABSTRACT

**Aims:** To isolate bacterial strains from chickpea rhizospheric soil and nodules, to characterize and identify potential bacterial strains by using 16S rRNA gene sequencing.
**Place and Duration of Study:** Department of Soil Science & SWC, PMAS, Arid Agriculture University, Rawalpindi Pakistan between July 2010 and July 2011.
**Background:** Plant growth promoting rhizobacteria are being preferred nowadays as inoculants for influencing crops via multiple direct or indirect mechanisms but screening to find out the effective PGPR strains is one of the crucial steps. This research is aimed at keeping in view their potential for phosphate solubilization, indole acetic acid and ammonia production.
**Methodology:** Extensive survey was carried out in Pothwar (District, Rawalpindi, Attock and

**Corresponding author: E-mail: amjadalidewan@yahoo.com, hayat@uaar.edu.pk;*

Chakwal) for collection of chickpea rhizospheric soil and root nodules. The isolation of rhizospheric soil bacteria was performed by using dilution plate technique while the root nodules bacteria were isolated on yeast extract mannitol agar supplemented with congo red. Ten bacterial strains designated as AM-1 to AM10 were isolated, purified and characterized for phosphate solubilization, indole acetic acid (IAA) and ammonia production. These bacterial strains were identified as belonging to species of *Bacillus, Enterobacter, Pseudomonas, Rhizobium, Sphingobacterium, Pantoea* and *Chryseobacterium*. All bacterial strains solubilized phosphate and produced IAA. Two bacterial strains AM-5 (*Sphingobacterium canadense*) and AM-4 (*Rhizobium pusense*) solubilized the maximum amount of phosphate i.e. 273.84 µg $ml^{-1}$ and 262.83 µg $ml^{-1}$ respectively with a significant pH drop from 7 to 2.67. These strains proved positive for ammonia production. Six most potential and identified strains were selected on the basis of plant growth promoting activities.
**Conclusion:** AM-4 (*Rhizobium pusense*) and AM-5 (*Sphingobacterium canadense*) are efficient strains and there is a need of inoculation experiments under control and field conditions to use these stains as biofertilizer to enhance the growth and productivity of the chickpea.

*Keywords: PGPR; indole acetic acid; PSB; 16S rRNA gene sequencing; chickpea.*

## 1. INTRODUCTION

The rapid growing population has eventually raised the demand of food production. To meet these food demands, the use of chemical fertilizers was considered an important way, although their use enhanced the crop yield prominently but chemical fertilizers also caused a threat to health and environment. Therefore, the researchers emphasized the need to opt for alternative yield raising techniques without affecting the soil health and environmental quality [1]. Presently the application of biological techniques for plant nutrition and sustained plant productivity with a richer crop yield is being focused. Bacteria, with a vital role for plant growth known as plant growth promoting rhizobacteria (PGPR) make possible the improved plant growth by colonizing the plant roots. Seeds co-inoculated with rhizobia, PGPR and PSB (phosphorus solubilization bacteria) have been found out to enhance crop growth and productivity [2-7]. IAA produced by PGPR also accelerates elongation of root hairs and lateral roots thus so useful in improving crop growth [8]. Plant growth benefits due to the addition of PGPR, include increases in germination rate, root growth, yield, leaf area, chlorophyll content, nitrogen content, protein content, tolerance to drought, shoot and root weight [9,10]. Isolation and screening of efficient and competitive strains from native rhizobial population proves beneficial under field environment [11]. Rhizobial inoculants have contributed to increased nitrogen fixation and yield in legume crops that represent 70-80% of total nitrogen accumulated in plants [12]. Phosphorus is an essential macronutrient that is required in large quantity for good yield and quality crops. Phosphorus is added to soil as soluble inorganic phosphates and more than 50% of it is converted to insoluble form and becomes unavailable to crops [13]. Many rhizospheric bacteria including *Bacillus, Pseudomonas* and *Rhizobium* etc are involved in phosphate solubilization [14]. PGPR synthesizes and exports phytohormones which are called plant growth regulators (PGRs). These PGRs may play regulatory role in plant growth and development and include auxins, gibberellic acid, ethylene, cytokinins, and abscisic acid [15,16]. Indole-3-acetic acid (IAA) is well-known to accelerate both rapid (e.g., increases in cell elongation) and lasting (e.g., cell division and differentiation) effects in plants and is physiologically most effective auxin [17-18]. The most prevalent and best depicted phytohormone in plants is IAA and it has been projected that 80% of bacteria isolated from the rhizosphere are able to synthesize IAA [19]. With the advent of PCR and DNA sequencing, differentiating of the gene sequences of bacterial species revealed that 16S rRNA gene is highly conserved for some species belonging to same genus. It helps in identification of bacteria to the species level [20]. Pakistani soils are scarce in plant nutrients [21]. The remarkable input cost for crop production relates to expensive inorganic fertilizers. Hence, there is a dire need to employ rhizobacteria as biofertilizers to mobilize the unavailable nutrients from soil. In Pakistan the bacterial inoculants are use as biofertilizer, but their application is limited throughout the country, revealing the little awareness and knowledge of bacterial validation from rich ecology of Pakistan. Efforts are needed to validate the soil beneficial bacteria by using molecular technique (DNA sequence) and use as bioinoculant for sustainable crop production [22]. The present

study was designed with the objectives to characterize soil bacteria based upon 16SrRNA gene sequencing and plant growth promoting traits i.e., phosphate solubilization and IAA production for enhancing crops yield.

## 2. MATERIALS AND METHODS

### 2.1 Isolation of Rhizobacteria

Extensive survey was carried out in three Pothwar district (Rawalpindi, Attock and Chakwal) in order to collect rhizospheric soil and root nodules samples from chickpea. The plant growth promoting rhizobacteria (PGPR) were isolated from the rhizospheric soil of chickpea by using dilution plate technique [23] in which phosphate buffer saline solution (PBS, 1X) was used. Bacterial growth was obtained on nutrient media i.e. tryptic soy agar (TSA) contained in sterilized petri plates and in incubator at 28°C for at least 48 hours. Individual colonies were picked and streaked on plates containing TSA media for purification and screening under sterilized conditions in laminar air flow cabinet. Re-streaking of single colonies was done repeatedly in order to get pure cultures [22]. The isolates were stored in glycerol (35%, w/v) at -80ºC. For isolation from nodules, healthy, pink and undamaged nodules were detached from roots (cut root 0.5 cm on each side of the nodule). Nodules were surface sterilized by immersing them in 95% ethanol for 5-10 seconds, washed with sterilized distilled water for 3-4 times then nodules were dipped in 3% hydrogen peroxide for 3-4 minutes [24]. Nodules were crushed with blunt tipped sterilized forceps and dipped in sterile water and then one loop full of the nodule suspension was streaked on yeast extract mannitol (YEM) agar plates supplemented with congo red as an indicator [25]. These plates were incubated at 28ºC for 2-3 days. Individual bacterial colony was picked with sterilized loop and was streaked on the media plate for purification [26].

### 2.2 Phosphate Solubilization

For quantitative estimation of inorganic phosphate solubilization, bacterial isolates were grown in Nautiyal [27] broth containing 0.5% tricalcium phosphate. The flasks having 100 ml broth were inoculated with 200 µg ml-1 bacterial cultures in 3 replications. The bacterial isolates were allowed to grow for 8 days in shaker. pH of the broth medium was noted before inoculation and after eight days of incubation. Bacterial culture was centrifuged at 3000 rpm for 25 minutes [27]. The available phosphorus in supernatant was determined by Watanabe and Olsen [28]. Optical density was recorded at 700 nm.

### 2.3 Production of Indole Acetic Acid (IAA)

For the capacity of the bacterial isolates to synthesize indole acetic acid (IAA), bacterial cultures were grown for 48 h in tryptic soy broth (TSB) at 28±2ºC. A bacterial suspension (100 µl each) of fully grown bacterial culture was inoculated in 5 ml Luria Broth (LB) medium in absence and presence of 500 µg/ml of tryptophan and, placed for 48 h in an incubating shaker at 28±2ºC. Centrifugation of bacterial culture was done at 3000 rpm for 15 minutes and the supernatant (2ml) was mixed with two drops of orthophosphoric acid and 4ml of the Salkowski reagent (50ml, 35% of perchloric acid, 1ml 0.5M FeCl3 solution). The development of a pink color indicated IAA production, and the optical density (O.D) 530 nm was read using a spectrophotometer [29].

### 2.4 Ammonia Production

Freshly grown cultures were inoculated in 10 ml peptone water and incubated at 28ºC for 48 h. Nessler's reagent (0.5 ml) was added to each tube and the appearance of brown to yellow color was a positive test for ammonia production [30].

### 2.5 Identification of PGPR Using 16S rRNA Gene Sequencing

DNA template was prepared by picking an individual colony and dissolving it in 1X Tris-EDTA buffer solution [31]. Partial 16S rRNA gene sequences of the strains was acquired after PCR amplification of the genes by using the protocol described by Katsivila [32] using universal forward and reverse primers: 9F (5'-GAGTTTGATCCTGGCTCAG-3') and 1510R (5'-GGCTACCTTGTTACGA-3'). Reaction mixture (50 µL) was denatured at 94ºC for 2 min, followed by primer annealing at 55ºC for 1 min and primer extension at 72ºC for 2 min and finally extension was obtained at 72ºC for 10 min in a thermocycler [31]. Separation of amplified PCR products of 16S ribosomal gene was carried out on 1% agarose gel in 0.5X TE (Tris-EDTA) buffer containing 2 µl ethidium bromide (20 µg ml-1). λ Hind-III ladder was used as a size

marker. The gel was examined under UV light and photographed by gel documentation system [22]. The purified PCR products were sent to MACROGEN (Seoul, Korea) for sequencing by using four universal forward and reverse primers. The results of sequence attained from MACROGEN, Korea were BLAST in search engine of the EzTaxon server [33] and the sequences of closely related species were retrieved to establish the exact nomenclature of the bacterial isolates. The sequences were aligned with the relevant sequences retrieved from GenBank by using CLUSTAL W program in the MEGA 5.2 software [34]. Aligned sequences were analyzed using the same software to construct unrooted phylogenetic trees by using the neighbor-joining [35] method, with bootstrap values based on 1000 replications [36].

## 3. RESULTS

### 3.1 Phosphorus Solubilization

The quantitative estimation of insoluble mineral phosphate solubilization by bacterial isolates revealed that all bacterial strains solubilized an effective amount of inorganic mineral phosphate and their range was between 145.06 to 273.84 µg ml-1 (Table 1). The lowest quantity of insoluble mineral phosphate was solubilized by AM-10 (*Bacillus oceanisediminis*) and highest by AM-5 (*Sphingobacterium canadense*) as compared to control (no inoculums) that solubilized only 4.63 µg ml-1 of Ca3(Po4)2. The results showed that the phosphate solubilized by bacterial strains is significant as compared to control. The pH of the Nautiyal broth medium was initially adjusted to 7.0 ± 0.05, which was reduced to 2.67 during eight days of incubation for P-solubilization and the higher pH drop was associated with higher P-solubilization by bacterial strains. AM-5 and AM-4 (Rhizobium pusense) solubilized phosphate upto 273.84 µg ml-1and 262.83 µg ml-1, respectively found out to be effective phosphate solubilizers.

### 3.2 Indole Acetic Acid (IAA)

All bacterial strains were capable of producing IAA with or without tryptophan, but every strain showed the trend to produce less IAA without tryptophan (Table 1). Great variation was observed in the IAA production ability among tested strains. The significantly raised amount of IAA was produced by AM-5 as 42.24 µg ml-1 with addition of tryptophan followed by AM-4 as 37.76 u µg ml-1 and minimum amount of IAA with tryptophan was noticed in AM-7 as 15.52 µg ml-1 in their broth culture.

### 3.3 Ammonia Production

All the ten isolated strains proved positive for ammonia production as shown in Table 1. which differentiated them as potential PGPR.

### 3.4 Identification of Bacterial Isolates by 16S rRNA Gene Sequencing

Identification of bacterial strains based on 16S rRNA gene sequences is shown in Table 2. BLAST search results through NCBI and Eztaxon server showed highest (99.9% and 99.6%) similarity of AM-1 and AM-10 with Bacillus safensis AF234854 and *Bacillus oceanisediminis* GQ292772, respectively. Similarly, the highest sequence similarity of AM-2 matched with Enterobacter clocae Z96079 (99.8%). AM-3, AM-6 and AM-7 belonged to genus Pseudomonas having highest similarity with Pseudomonas beteli (99.6%), Pseudomonas plecoglossicida (99.8%) and Pseudomonas lini (99.2%), respectively. AM-4, AM-5, AM-8 and AM-9 belonged to Rhizobium pusense (99.9%), *Sphingobacterium canadense* (97.6%), Pantoea rodasii (98.9%) and Chryseobacterium gleum (99.2%), respectively.

Fig. 1 reveals a phylogenic tree of AM-5 and sequences of some closely related species of *Sphingobacterium* obtained from Eztaxon. In phylogenetic tree, AM-5 form a clade with *Sphingobacterium* halpophilum, *Sphingobacterium canadense* and *Sphingobacterium* multivorum. The sequence similarity of AM-5 (97.6%) with closely related bacterial species retrieved from gene bank databases and its phylogenetic analysis illustrated the strain as a candidate novel species which need to be validated by further studies including the minimal standard necessary for the validation of novel taxa [37].

## 4. DISCUSSION

PGPR mediated phosphate solubilization through biosynthesis of organic acids that create acidification conditions in the media [38]. PGPR solubilized more inorganic phosphate through gene modification followed by their expression in specific bacterial strains [39]. Phytase genes have been cloned from number of PGPR [40]. In our study, significance drop in pH of broth culture

was observed after eight days of incubation and was associated with efficient P-solubilizers. A similar pH drop in broth media by different PGPR is reported in other studies [41,42]. Phosphorus is an important nutrient, which contributes for many metabolic processes [43]. Phosphorus solubilization enhances phosphorus availability for the host plant. The low phosphorus availability to the plant in vivo is because of insoluble forms of the soil phosphate. The plants attain the monobasic and dibasic phosphate ions which are changed through insoluble forms by the bacteria [44]. Other studies revealed the similar results as this study that the solublilization of insoluble mineral phosphate ranging from 1.53 to 360 µg ml-1 with decreased pH values that was initially 7.1 and ultimately was found in varied values from 4.16 to 6.45 [45]. Our results are also in line with the findings of other researchers [46], who concluded that *Enterobactor* sp. and *Pseudomonas* sp. out of 14 isolated strains solubilized the highest amount of tricalcium phosphate and phosphate solubilization was found associated with obvious decline in the pH that was upto 4.43 and was initially sustained upto 7.

Auxin production by bacteria is improved by the addition of tryptophan [47], since it is the precursor, it significantly boosts the production of IAA which was observed comparatively low without tryptophan. Many soil bacteria that interact with plants can synthesize hormones similar to those produced by the plant as growth regulator, such as auxins, gibberellins and cytokines. Different physiological processes in crops starting from root initiation and extension to phototropism can be controlled by the hormone auxin IAA, which is synthesized from tryptophan [48]. Like phosphate solubilization, bacteria that produce auxins are likely to have strong influence on plant growth. Different species of PGPR are conducive to varying amounts of IAA production [49,50]. Greater amount of IAA production by rhizobacteria was reported by many researchers [51]. In our findings, the highest production of IAA was found in the two strains AM-5 and AM-4, i.e. 42.24 µg ml-1 and 37.76 µg ml-1 with tryptophan and 9.28 µg ml-1 and 12.64 µg ml-1 without tryptophan respectively. The bacterial strains generate good amount of IAA in presence of the main physiological precursor tryptophan. PGPR isolates have been studied as producer of IAA in presence or absence of tryptophan in culture media [52,53]. A significant increase in the production of IAA in the presence of 1, 2 and 5 mg L-1 of tryptophan, i.e. 1.47-11.88 mg L-1, 5.99-24.8 mg L-1 and 3-32.8 mg L-1, respectively was also observed [54].

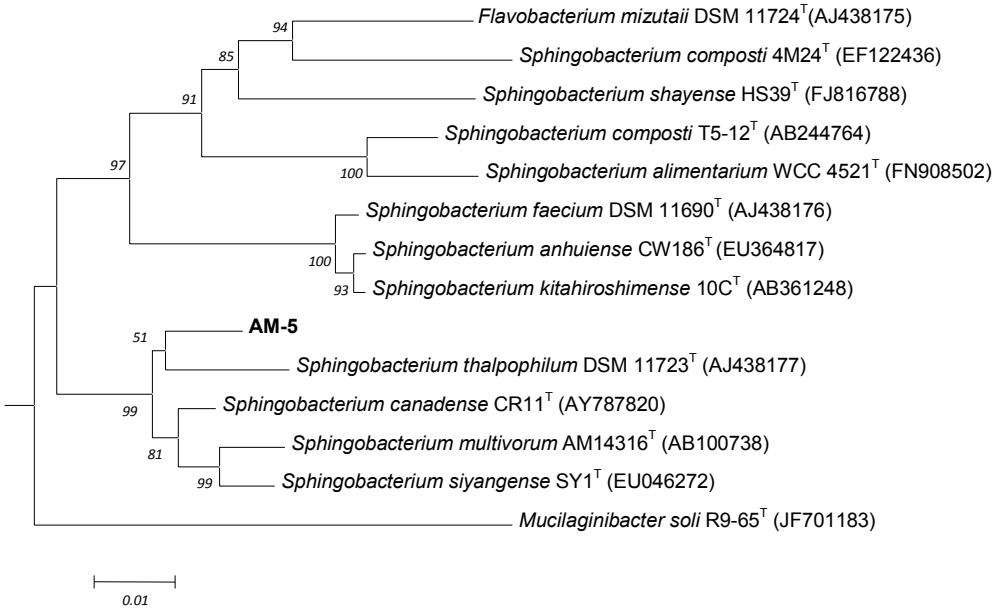


**Fig. 1. Neighbor-joining tree based on nearly complete 16S rRNA gene sequences of strain AM-5 and the recognized species of genus *Sphingobacterium.* Bootstrap values >50 % are indicated at the nodes**

**Table 1. Plant growth promoting traits of bacterial strains**

| Strains ID | P-Solubilization ($\mu gmL^{-1}$)±S.E | pH (7.0) | IAA with tryptophan ($\mu gmL^{1}$)±S.E | IAA Without tryptopan ($\mu gmL^{1}$)±S.E | $NH_3$ |
|---|---|---|---|---|---|
| Control | 4.63 ±1.17 | 6.73 | 1.43±0.18 | 0.28±0.06 | _ |
| AM-1 | 249.93±4.83 | 3.10 | 17.58±0.25 | 5.10±0.27 | + |
| AM-2 | 193.51±4.51 | 4.85 | 18.81±0.00 | 9.29±0.07 | + |
| AM-3 | 216.38±7.63 | 2.67 | 17.42±0.23 | 8.43±0.22 | + |
| AM-4 | 262.83±3.29 | 3.01 | 37.76±0.10 | 12.64±0.19 | + |
| AM-5 | 273.84±9.31 | 3.39 | 42.24±0.10 | 9.28±0.12 | + |
| AM-6 | 195.99±4.10 | 3.76 | 18.34±0.23 | 4.82±0.26 | + |
| AM-7 | 172.54±4.95 | 5.04 | 15.52±0.06 | 5.46±0.19 | + |
| AM-8 | 177.52±16.43 | 5.36 | 17.43±0.14 | 8.82±0.15 | + |
| AM-9 | 179.32±16.76 | 5.01 | 35.12±0.06 | 10.60±0.11 | + |
| AM-10 | 145.06±3.65 | 5.94 | 17.31±0.14 | 5.89±0.15 | + |

**Table 2. 16S rRNA gene sequence similarity of isolated strains with closely related taxa**

| Isolated strains ID | Closely related Taxa | 16S rRNA gene (ntd) | DDBJ accession | Highest similarity(%) |
|---|---|---|---|---|
| AM-1 | *Bacillus safensis* | 1440 | AF234854 | 99.9 |
| AM-2 | *Enterobacter clocae* | 1425 | Z96079 | 99.8 |
| AM-3 | *Pseudomonas beteli* | 1426 | AB021406 | 99.6 |
| AM-4 | *Rhizobium pusense* | 1417 | FJ969841 | 99.9 |
| AM-5 | *Sphingobacterium canadense* | 1439 | AY787820 | 97.6 |
| AM-6 | *Pseudomonas plecoglossicida* | 1433 | AB009457 | 99.8 |
| AM-7 | *Pseudomonas lini* | 1437 | AY035996 | 99.2 |
| AM-8 | *Pantoea rodasii* | 1442 | JF295053 | 98.9 |
| AM-9 | *Chryseobacterium gleum* | 1420 | ACKQ01000057 | 99.2 |
| AM-10 | *Bacillus oceanisediminis* | 1457 | GQ292772 | 99.6 |

## 5. CONCLUSION

The present study has exhibited the molecular characterization of 10 bacterial strains isolated from chickpea rhizospheric soil and nodules and recommended that isolates AM-4 and AM-5 identified as Rhizobium and *Sphingobacterium* sp. have the potential to solubilize tricalcium phosphate and synthesize IAA, therefore these two strains found out to be the most promising PGPR among the isolated strains. Additional inoculation experiments are recommended under control and field conditions on different crops to confirm these stains as patent potential biofertilizer. The sequence similarity of AM-5 (97.6%) has illustrated it as a candidate novel species when compared with closely related species retrieved from gene bank databases. Therefore there is a need to validate AM-5 by using the minimal standards of genotypic and molecular studies requisite for the validation of new taxa.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

## REFERENCES

1. Kantar F, Hafeez FY, Kumar BGS, Sundram SP, Tejera NA, Aslam A, Bano A, Raja P. Chickpea: *Rhizobium* management and nitrogen fixation. Chickpea Breeding Management. Microbiol Res. 2007;153:113-117.
2. Dashti N, Zhang F, Hynes R, Smith DL. Plant growth promoting rhizobacteria accelerate nodulation and increase nitrogen fixation activity by field grown soybean [*Glycin max* (L. Merr.)] under short season conditions. Pantl Soil. 1998;200:205-213.
3. Rodelas B, Gonzalez-Lopez J, Martinez-Toledo MV, Pozo C, Salmeron V. Influence of *Rhizobium/ Azotobacter* and *Rhizobium/ Azospirillum* combined inoculation on mineral composition of faba bean (*Vicia Faba* L). Biol Fertil Soils. 1999;9:165-169.
4. Chebotar VK, Asis Jr CA, Akao S. Production of plant growth promoting substances and high colonization ability of rhizobacteria enhance the nitrogen fixation of soybean when coinoculation with

*Bradyrhizobium japonicum*. Biol Fertil Soils. 2001;34:427-432.

5. Sindhu SS, Suneja S, Goel AK, Parmar N, Dadarwal KR. Plant growth promoting effects of *Pseudomonas* sp. on coinoculation with *Mesorhizobium* sp*. cicer* strain under sterile and "wilt sick" soil conditions. Appl Soil Ecol. 2002;19:57-64.
6. Zaidi A, Khan MS, Amil M. Interactive effect of rhizotrophic microorganisms on yield and nutrient uptake of chickpea (*Cicer arietinum* L.). Europian J Agron. 2003;19:15-2.
7. Rudresh DL, Shivaprakash MK, Prasad RD. Effect of combined application of *Rhizobium,* phosphate solubilizing bacterium and *Trichoderma* spp. on growth, nutrient uptake and yield of chickpea (*Cicer aritenium* L.). Appl Soil Ecol. 2005;28:139-146.
8. Okon Y, Kapulnik Y. Development and function of *Azospirillum* inoculated roots. Plant Soil. 1986;90:3-16.
9. Çakmakçi R. The importance of phosphate-degrading bacteria in plant development. Selçuk University. Journal of the Faculty of Agriculture. 2005;35:93-108.
10. Çakmakçi R. Use of plant growth promoting rhizobacteria in agriculture. Atatürk University. Journal of the Faculty of Agriculture. 2005;36:97-107.
11. Chatel DL, Greenwood RM. The location and distribution in soil of *Rhizobia* under senesced annual legume pastures. Soil Biol Biochem. 1973;5:799-808.
12. Catroux G, Hartmann A, Revellin C. Trends in *Rhizobial* inoculant production and use. Plant Soil. 2001;230:21-30.
13. Singh S, Kapoor K. Solubilization of insoluble phosphates by *Bacteria* isolated from different sources. Environ Ecol. 1994;12:51-55.
14. Rodríguez H, Fraga R. Phosphate solubilizing bacteria and their role in plant growth promotion. Biotechnol Adv.1999;17:319-339.
15. Zahir AZ, Arshad M, Frankenberger WT. Plant growth promoting rhizobacteria: application and perspectives in Agriculture. Adv Agron. 2004;81:97-168.
16. Hayat R, Ali S, Amara U, Khalid R, Ahmed I. Soil beneficial bacteria and their role in plant growth promotion: A review. Ann Microbiol. 2010;60:579-598.
17. Cleland RE. Auxin and cell elongation. In: Davies PJ (ed) Plant hormones and their role in plant growth and development. Kluwer Dordrecht. 1990;132-148.
18. Hagen G. The control of gene expression by auxin. In: Davies PJ (ed) Plant hormones and their role in plant growth and development. Kluwer Dordrecht. 1990;149–163.
19. Patten CL, Glick BR. Bacterial biosynthesis of indole-3-acetic acid. Can J Microbiol. 1996;42:207-220.
20. Seklenska-Pobell S, Evguenieva E, Radeva G, Squartini A. Characterization of *Rhizobium* 'hedysari' by RELP analysis of PCR amplified rDNA and by genomic PCR fingerprinting. J App Bacteriol. 1996;80:517-528.
21. Rashid A, Rafique E, Ali N. Micronutrient deficiencies in rainfed calcareous soils of Pakistan. II. Boron nutrition of the peanut plant. Communications in Soil Science and Plant Analy. 1997;28(1-2):149-159.
22. Hayat R, Khalid R, Ehsan M, Ahmed I, Yokota A, Ali S. Molecular characterization of soil bacteria for improving crop yield in Pakistan. Pak J Bot. 2013;45(3):1045-1055.
23. Brierly WB, Jewson ST, Brierly M. The quantitative study of soil fungi. Int Congr Soil Sci Trans 1st Washington DC. 1928;3:48-71.
24. Somasegaran P, Reyes VG, Hoben HJ. The influence of high temperatures on the growth and survival of *Rhizobium* spp. in peat inoculants during preparation, storage and distribution. Can J Microbiol. 1984;30:23-30.
25. Nelson LM, Child JJ. Nitrogen fixation and hydrogen metabolism in *Rhizobium leguminosarum* isolates in pea *Pisum sativum* root nodules. Can J Microbiol. 1981;27:1028-1034.
26. Vincent JM. The cultivation, isolation and maintenance of *Rhizobia*. In: Vincent JM (ed) A manual for the practical study of the root-nodule *Bacteria*. Blackwell Scientific, Oxford. 1970;1-13.
27. Nautiyal CS. An efficient microbiological growth medium for screening phosphate solubilizing microorganisms. FEMS Microbiol Lett. 1999;170:265-270.
28. Watanabe FS, Olsen SR. Test of an ascorbic acid method for determining phosphorus in water and $NaHCO_3$ extracts from soil. Soil Sci Amer Proceed. 1965;29:677-678.
29. Brick JM, Bostock RM, Silverstone SE. Rapid in situ assay for indole acetic acid

production by bacteria immobilized on nitrocellulose membrane. Appl Environ Microbiol. 1991;57:535-538.
30. Cappuccino JC, Sherman N. In: Microbiology: A laboratory manual, third ed. Benjamin/cummings Pub. Co. New York.1992;125-179.
31. Hayat R, Ahmed I, Peak J, Ehsan M, Iqbal M, Chang YH. A moderately boron tolerant candidates novel soil bacterium *Lysinibacillus Pakistanensis* sp. Nov. Cand, isolated from soybean (*Glycine max* L) rhizosphere. Pak J Bot. 2013;45:41-50.
32. Katsivila ED, Bonse A, Kruger C, Strompl A, Luingston, Mittich R. An extraction membrane boifilm reactor for degredation of 1,3- dichloropropene in industrial water. Appl Microbiol Biotechnol. 1999;52:853-862.
33. Chun J, Lee JH, Jung Y, Kim M, Kim S, Kim BK, Lim YW. Ez Taxon: A web-based tool for the identification of prokaryotes based on 16S ribosomal RNA gene sequences. Int J Syst Evol Microbiol. 2007;57:2259-2261.
34. Tamura KJ, Dudley M, Kumar S. MEGA4: Molecular evolutionary genetics analysis (MEGA) software version 4.0. Mol Biol Evol. 2007;24:1596-1599.
35. Saitou N, Nei M. The neighbor-joining method: A new method for reconstructing phylogenetic trees. Mol Biol Evol. 1987;4:406-425.
36. Felsenstein J. Confidence limits on phylogenies: An approach using the bootstrap. Evolution. 1985;39:783-791.
37. Logan NA, Berge O, Bishop AH, Busse HJ, De Vos P, Fritze D, Heyndrickx M, Kämpfer P, Rabinovitch L, Salkinoja-Salonen MS, Seldin L, Ventosa A. Proposed minimal standards for describing new taxa of aerobic, endospore-forming bacteria. IJSEM. 2009;59:2114-2121.
38. Goldstein AH. Future trends in research on microbial phosphate solubilization: One hundred years of insolubility. In: Velázquez E, Rodríguez-Barrueco C (eds). First international meeting on microbial phosphate solubilization. Springer, Dordrecht. 2007;91-96.
39. Rodríguez H, Fraga R, Gonzalez T, Bashan T. Genetics of phosphate solubilization and its potential applications for improving plant growth-promoting bacteria. Plant Soil. 2006;287:15-21.
40. Tye AJ, Siu FK, Leung TY, Lim BL. Molecular cloning and the biochemical characterization of two novel phytases from *Bacillus subtilis* and *Bacillus licheniformis*. Appl Microbiol Biotechnol. 2002;59:190-197.
41. Illmer P, Schinner F. Solubilization of inorganic phosphates by microorganisms isolated from forest soil. Soil Biol Biochem. 1992;24:389-395.
42. Yu X, Liu X, Zhu TH, Liu GH, Mao C. Isolation and characterization of phosphate-solubilizing bacteria from walnut and their effect on growth and phosphorus mobilization. Biol Fert Soils. 2011;47:437-446.
43. Mikkelsen R. Managing phosphorus for maximum alfalfa yield and quality. In: Proceedings, National Alfalfa Symposium. UC Cooperative Extension (eds) University of California San Diego, California, USA; 2004.
44. Vessey JK. Plant growth promoting rhizobacteria as biofertilizers. Plant Soil. 2003;255:571-586.
45. Abd El-Azeem SA, Mehana MTA, Shabayek AA. Some plant growth promoting traits of rhizobacteria isolated from Suez Canal region Egypt. African Crop Sci Conference Proceedings. 2007;8:1517-1525.
46. Hayat R, Ahmed I, Sheirdil RI. An overview of plant growth promoting rhizobacteria (PGPR) for sustainable agriculture. In Crop Production for Agricultural Improvement. Muhammad Ashraf, Münir Öztürk, Muhammad Sajid Aqeel Ahmad, Ahmet Aksoy. Springer Dordrecht Heidelberg New York London. 2012;557-579. ISBN: 978-94-007-4115-7. ISBN: 978-94-007-4116-4. (eBook), DOI: 10.1007/978-94-007-4116-4.
47. Tien TM, Gaskin MH, Hubbel DH. Plant growth substances produced by *A. brasilense* and their effect on the growth of pearlmillet (*Pennisetum americanum* L.). Appl Environ Microbiol. 1979;37:1016-1024.
48. Khan MS, Zaidi A, Wani PA, Ahemad M, Oves M. Functional diversity among plant growth-promoting rhizobacteria. In: Khan, MS, Zaidi A, Musarrat J. (eds): Microbial Strategies for Crop Improvement. Springer, Berlin Heidelberg. 2009;105-132.
49. Brown ME. Plant growth substances produced by microorganism of soil and rhizosphere. Appl Bacteriol. 1972;35:443-451.
50. Vijila K. Estimation of IAA production in nitrogen fixing microorganisms. Partial

manual microbial interaction in soil. Tamil Nadu Agricultural University Coimbatore. 2000;38-39.
51. Xie H, Pasternak JJ, Glick BR. Isolation and characterization of mutants of the plant growth-promoting rhizobacterium *Pseudomonas putida* GR12-2 that overproduce indoleacetic acid. Curr. Microbiol. 1996;32:67-71.
52. Lebuhn MT, Heulin Hartmann A. Production of auxin and other indolic and phenolic compounds by *Paenibacillus polymyxa* strains isolated from different proximity to plant roots. FEMS Microbiol Ecol. 1997;22:325-334.
53. Mehnaz S, Lazarovits G. Inoculation effects of *Pseudomonas putida*, *Gluconacetobacter azotocaptans* and *Azospirillum lipoferum* on corn plant growth under green house conditions. Microbial Ecol. 2001;51:326-335.
54. Ahmad F, Ahmad I, Khan MS. Indole acetic production by indigenous isolates of *Azotobacter* and *Fluorescent pseudomonas* in the presence and absence of tryptophan. Turk J Biol. 2005;29:29-34.



*Peer-review history:*
*The peer review history for this paper can be accessed here:*
*http://www.sciencedomain.org/review-history.php?iid=827&id=8&aid=7284*