Malaysian Journal of Microbiology, Vol 13(2) June 2017, pp. 124-131

Malaysian Journal of Microbiology
Published by Malaysian Society for Microbiology
(In SCOPUS since 2011)

Screening and characterization of plant growth promoting traits of phosphate solubilizing bacteria isolated from wheat rhizosphere of Algerian saline soil

Chibani Hiba Rahman[1*], Bouznad Ahcene[1], Bellahcene Miloud[2], Djibaoui Rachid[1]

[1]Laboratory of Microbiology and Plant Biology, Faculty of Natural and Life Sciences, University of Mostaganem, Algeria.
[2]University Center of Ain Temouchent, Algeria.
Email: hiba.chibani@univ-mosta.dz

Received 16 August 2016; Received in revised form 10 September 2016; Accepted 7 November 2016

ABSTRACT

Aims: The capacity of some soil microorganisms to solubilize in soil is an important activity exhibited by plant growth promoting rhizobacteria (PGPR) to increase plant performance. This study aimed at isolation and selection of phosphate solubilizing bacteria from saline soil and *in vitro* evaluation of their plant growth promoting traits.
Methodology and results: Phosphate solubilizing bacteria isolated from wheat rhizosphere, of saline soil in western region of Algeria were tested for their plant growth promoting traits such us indole acetic acid (IAA), hydrogen cyanide (HCN), siderophore and ammonia production and their ability to fix nitrogen. Among 104 bacterial isolates, 41 were selected for their phosphate solubilizing activity using tricalcium phosphate (TCP) as a sole phosphorus source. IAA production was shown by almost all the bacterial isolates. Twelve isolates were recorded positive for HCN production, 32 produced siderophore and 31 were able to fix nitrogen. The most dominant phosphate solubilizing bacteria found were identified as *Pseudomonas* followed by *Aeromomas hydrophila Bacillus* sp. and *Burkholderia cepacia*.
Conclusion: Phosphate solubilizing bacteria that were isolated from saline soil showed a high potential in to producing growth promoting traits and can be used as inoculants to increase the phosphorus uptake by plants.

Keywords: Phosphate solubilising bacteria, saline soil, tricalcium phosphate, plant growth promoting traits

INTRODUCTION

Phosphorus is considered to be the most important element in plant nutrition of, after nitrogen. It is an essential component in all main metabolic procedures in plants for example energy transfer, photosynthesis, signal transduction and respiration (Khan *et al.*, 2010). Inorganic phosphorus is found in soils, mostly in insoluble mineral complexes such as tricalcium phosphate $Ca_3 (PO_4)_2$, ion phosphate $FePO_4$ and aluminium phosphate $AlPO_4$ (Barber, 1995), which appear after repeated applications of chemical fertilizers. Plants have not the capacity to absorb these insoluble forms besides only 0.1% of total phosphorus is in soluble form and it is available for plant nutrition (Zhou *et al.*, 1992).

It is for this reason that the available phosphorus levels have to be supplemented in most agricultural soil by adding chemical phosphorus fertilizers. The frequent and imprudent applications of chemical phosphorus fertilizers lead to the decrease of soil fertility by perturbing microbial population–thus reduce crops yield (Gyaneshwar and Naresh, 2002).

Among chemical environmental stress soil salinity is the most important stress factor for plants (Idikut *et al.*, 2012). Salinity leads to osmotic stress and causes the formation of reactive oxygen species (ROS) thus disturbs cellular structures which and consequently damage mitochondria and chloroplast (Mittler, 2002). Soil salinity considerably reduces absorption of mineral nutrients, particularly phosphorus for the reason that phosphate ions precipitate with calcium ions in saline soil and become inaccessible to plants (Grattan and Grieve, 1999). Utilization of phosphate solubilizing bacteria to solve this problem for raison of their ability to solubilize phosphate in soil is supported by many researchers (Khan *et al.*, 2007). Mechanisms like organic acid production, chelation, and ion exchange reactions are implemented by these bacteria to solubilize inaccessible phosphorus forms and make them available to plants (Vessey, 2003). Phosphate solubilizing bacteria (PSB) are part of plant growth promoting rhizobacterias (PGPRs) and are capable of solubilizing inorganic phosphate from different compounds, such as dicalcium phosphate, tricalcium phosphate and rock phosphate. Moreover, PSMs may also showed plant growth promoting traits such as indole acetic acid (IAA), cytokinins, gibberellic acid and ethylene production, hydrogen cyanide (HCN) and siderophore

***Corresponding author**

ISSN (print): 1823-8262, ISSN (online): 2231-7538

production, nitrogen fixation and resistance to soil pathogens etc (Cattelan *et al.*, 1999).

The main objectives of this study were the isolation and screening of phosphate solubilizing bacteria from wheat rhizosphere of salt affected soil and *in vitro* assessment of their plant growth promoting activities.

MATERIALS AND METHODS

Soil sampling

Saline soil samples were collected randomly from the rhizosphere regions of wheat plants growing at different sites at Relizane (western Algeria). All samples were kept in plastic bags and transported to the laboratory and stored at 4 °C. Total of fifteen soil samples were air-dried, ground and passed through 2 mm sieve before chemical analyses pH, soil moisture and conductivity of the soil samples were measured.

Isolation of total phosphate solubilizing bacteria

Isolation of PBS bacteria was carried out by serial dilution method. Ten grams of rhizosphere soil was dissolved in 90 mL of saline phosphate buffer, then shook for 30 min. One mL of rhizosphere soil suspension was added to 9 mL of sterile saline water to obtain a suspension with a 10^{-2} dilution. Subsequently 0.1 mL of the suspension diluted to 10^{-5} was grown on Pikovskaya (Pikovskaya 1948) PVK agar supplemented by 5 g of tricalcium phosphate (TCP) as a sole phosphorus source. Inoculated plates were incubated for 7 days at 28± 2 °C. Appearance of clear halo zone on Pikovskaya's agar plates indicates positive phosphate solubilization ability. Bacterial colonies surrounded by a transparent halo on PVK agar were picked off, checked for purity and classified as putative P-solubilizers.

Quantitative estimation of phosphate solubilization in culture broth

The isolated bacteria presenting halo zones on solid PVK medium were used to measure phosphate solubilization in liquid medium. The *in vitro* phosphate solubilizing capacity of each strain was determined on National Botanical Research Institute's Phosphate growth medium (NBRIP) (Nautiyal, 1999) supplemented by 5 g of TCP as sole phosphorus source. The flasks containing 50 mL of NBRIP medium was inoculated with 1 mL of bacterial suspension (2×10^9 CFU/mL) in triplicates and incubated at 28±2 °C on a rotary shaker 180 rpm for 7 days. The cultures were harvested by centrifugation at 6000 rpm for 30 min. The phosphorus in supernatant was estimated by vanado-molybdate-yellow colour method (Jackson, 1973). The total soluble phosphorus was calculated from the regression equation of standard curve. The values of soluble phosphate liberated were expressed as µg/ml over control. The pH of culture supernatants were also measured using a pH Meter.

Production of indole acetic acid (IAA)

Production of auxin indole-3-acetic acid (IAA) by bacteria was tested using Lauria Bertani (LB) and Salkowski reagent. Fifty milliliter of Lauria Bertani (LB) containing (0.1 g/L of L-tryptophan was inoculated with 1 mL of bacterial suspension (approximately 10^9 CFU/mL) in triplicates and incubated in incubator Shaker at 28± 2 °C and 180 rpm for 4 days in dark. The bacterial cultures were centrifuged at 6,000 rpm for 30 min. Estimation of indole-3-acetic acid (IAA) in the supernatants was done using colorimetric assay (Brick *et al.*, 1991). From a standard curve prepared with known concentration of IAA, the quantity in the culture was determined and expressed as µg/mL.

Hydrogen cyanide production

Screening of bacterial isolates for hydrogen cyanide (HCN) production was carried out following the method described by Bakker and Schippers (1987). Bacterial cultures were streaked on nutrient agar medium containing 4.4 g/L of glycine. A Whatman filter paper No. 1 soaked in 0.5% picric acid solution (in 2% sodium carbonate) was placed inside the plate lid. Plates were sealed with parafilm and incubated at 28±2 °C for 4 days. HCN production was evaluated according to the colour change, ranging from yellow to orange.

Siderophore production

Siderophore production by rhizobacterial isolates was detected according to Schwyn and Neilands (1987). Autoclaved CAS agar medium was poured in each Petri dish. The bacterial inoculum was placed in the centre of a Petri dish. The plates were incubated in the dark at 28± 2 °C for 5 days. The change of CAS agar colour from blue to orange around bacterial colony was considered as positive production of siderophore.

Nitrogen fixing activity

The visual detection of nitrogen-fixing activities of the selected isolates were observed by using nitrogen-free malate-mannitol medium (NF-MM) (Herman *et al.*, 1994), containing bromothymol blue (BTB) as an indicator. The medium was inoculated by the bacterial isolates and incubated at 28± 2 °C for 24 h. The blue coloured zone producing isolates were marked as nitrogen fixers in the solid culture conditions. The colouring zone was calculated by deducting the colony diameter from the colouring zone diameter (Dobereiner and Day, 1975).

Ammonia production

All the bacterial isolates were tested for the production of ammonia as described by Cappuccino and Sherman (1992). Overnight grown bacterial cultures were inoculated in 10 mL of peptone broth and incubated at 28± 2 °C for 48 h on a shaker. After incubation 0.5 mL of

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Nessler's reagent was added. The development of faint yellow to dark brown colour indicated the production of ammonia.

Biochemical identification of bacterial isolates

The bacterial isolates were further characterized by Gram staining, catalase, oxidase, starch hydrolysis activity and motility followed by biochemical identification using API 20NE kit (BioMérieux, France). API 20 NE is a standardized system for the identification of non-fastidious, non-enteric Gram-negative rods combining 8 conventional tests, 12 assimilation tests. The results were interpreted with the API WebTM software (version 7.0).

Statistical analysis

The data obtained in this study was subjected to analysis of variance (ANOVA) and comparisons of means were performed by Newman and Keuls test at $p \leq 0.05$ using statbox. The correlation between phosphate solubilization and pH was explored by Excel.

RESULTS

The pH of soil samples ranged from 7.82 to 8.46, moisture content from 10.52 to 18.6% and electrical conductivity from 6.1 to 13.3 ds/m. A total of 104 phosphate solubilizing isolates were obtained from different saline soil sites at Relizane (western Algeria). Out of these isolates 41 were selected for their phosphate solubilizing activity. These were screened for their plant growth promoting activities such as indole acetic acid (IAA), Siderophore production, HCN production, nitrogen fixing ability and ammonia production.

Phosphate solubilization ability is marked by the formation of transparent halos around bacterial colony on solid Pikovskaya medium supplemented with tricalcium phosphate ($Ca_3(PO_4)_2$) as a only source of phosphorus (Figure 1). All the 104 isolates were able to produce transparent halo around the colony with different diameter which indicates a positive phosphate solubilisation ability and they were also capable to solubilize inorganic phosphorus ($Ca_3(PO4)_2$) in liquid medium. The results showed that the phosphate solubilizing ability in NBRIP liquid medium of test isolates varied from 24.29 to 738.57 µg/mL using TCP as a source of insoluble P (data of all PSB are not given). The PSB showing the highest amount of solubilized phosphorus are chosen to be tested for their plant growth promoting traits (41 isolates). The isolate that showed the best capability in solubilizing phosphate was PSB91 (738.57 µg/mL). pH values of the bacterial cultures decreased from initial value of 7.0 to values ranged from 3.9 to 4.61. A highly significant negative (r = -0, 86) correlation was observed between the amounts of solubilized P and pH values (Table 1).

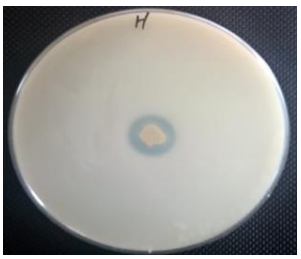

Figure 1: Phosphate solubilisation by bacterial isolate.

Table 1: Phosphate solubilization by selected bacterial isolates.

Isolate	Concentration of phosphate (µg/ml)	Final pH of P solubilization
PSB3	605.34±15.91^{d}	4.11±0.15
PSB5	525.60±14.73^{j}	4.35±0.26
PSB8	652.83±25.28^{b}	3.90±0.56
PSB11	515.90±12.78^{l}	4.38±0.89
PSB13	584.17±13.78^{e}	4.27±0.21
PSB16	553.62±24.17^{h}	4.32±0.72
PSB18	555.86±16.60^{gh}	4.31±0.41
PSB22	642.49±22.74^{c}	3.92±0.46
PSB24	587.05±19.65^{e}	4.18±0.27
PSB27	566.17±17.14^{fg}	3.97±0.82
PSB29	562.90±21.92^{fgh}	4.18±0.63
PSB31	410.89±16.62^{p}	4.35±0.51
PSB34	453.28±16.95^{n}	4.26±0.32
PSB36	564.74±11.5f^{g}	4.26±0.65
PSB38	402.58±15.71^{pq}	4.47±0.12
PSB41	610.96±13.87^{d}	4.12±0.43
PSB43	571.22±18.70^{f}	4.22±0.74
PSB44	541.59±23.67^{i}	4.32±0.75
PSB45	601.85±22.60^{d}	4.11±0.95
PSB49	561.33±12.03^{fgh}	4.35±0.31
PSB52	370.04±10.61^{r}	4.53±0.45
PSB56	570.63±16.51^{f}	4.22±0.15
PSB58	398.00±11.76^{q}	4.57±0.61
PSB64	487.71±15.35^{l}	4.44±0.34
PSB66	502.25±14.83^{k}	4.46±0.85
PSB67	481.71±16.49^{l}	4.42±0.22
PSB70	568.05±25.89^{f}	4.25±0.12
PSB71	447.20±17.94^{n}	4.33±0.41
PSB72	466.94±18.60^{m}	4.34±0.33
PSB74	435.52±20.87^{o}	4.50±0.23
PSB78	348.05±15.24^{s}	4.55±0.29
PSB82	327.50±16.50^{t}	4.61±0.57
PSB85	398.70±12.42^{q}	4.52±0.45
PSB87	538.37±12.70^{i}	4.23±0.11
PSB89	521.9±14.66^{J}	4.30±0.15
PSB91	738.56±16.07^{a}	4.01±0.77
PSB93	455.13±13.18^{n}	4.35±0.67
PSB95	410.35±22.16^{p}	4.42±0.81
PSB97	521.77±23.66^{j}	4.27±0.36
PSB99	407.67±15.57^{pq}	4.47± 0.37
PSB102	604.37±17.85^{d}	4.14±0.41
r	-0.86	

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Phosphate solubilizing bacteria were assayed for their capacity to produce IAA in LB medium supplemented with L-tryptophan as precursor (1%) by the appearance of pink colour after addition of Salkowski reagent to the culture (Figure 2). IAA production was revealed by almost all the bacterial isolates and IAA quantities of estimated ranged from 10.90 to 63.35 µg/mL as shown in Table 2. A relatively higher content of IAA was found in the culture of bacterial isolate PSB45 followed by PSB85 and PSB91 with 37.43 and 32.85 µg/mL respectively compared to other isolates.

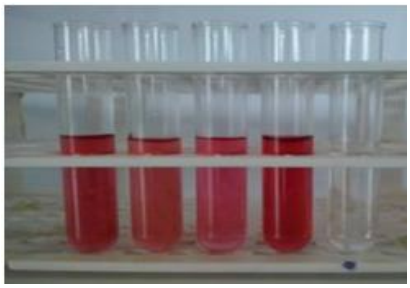

Figure 2: IAA production by bacterial isolates.

Among the 41 isolates, only twelve between them were recorded positive for HCN production by changing filter paper from yellow to orange (Table 2, Figure 3). The ability of the tested bacterial isolates to produce siderophores *in vitro* was assessed qualitatively using the CAS-agar plate assay. Of the 41 bacterial isolates tested, 32 strains produced siderophores. Siderophore production capacity of the isolates was evaluated according to the diameter of the orange halo around bacterial colonies as shown in Figure 5, as weak (4-10 mm), moderate (11- 20 mm) and strong (higher than 21mm). Amongst the siderophore producing isolates, 18 were weak siderophore producers, 10 isolates were moderate siderophore producers while 7 isolates were strong siderophore producers (Table 2).

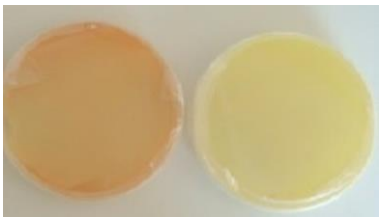

Figure 3: HCN production by isolate.

Phosphate solubilizing bacteria were also tested for their ability to fix nitrogen on nitrogen free medium (NF-MM) supplemented by BTB used as colour indicator to detect the release of ammonium in the culture as shown in Figure 5. Among the 41 bacterial isolates 31 were able to fix nitrogen by changing medium colour from green to blue colour. The diameter of coloration zone ranged from 7 to 26 mm. The highest value of diameter of coloration zone was obtained with the isolate PSB41 (Table 2). All our selected isolates were capable to produce ammonia (Table 2).

Table 2: Characterization of bacterial isolates for multiple plant growth promoting traits.

Isolate	Concentration of IAA (µg/mL)	HCN production	SDR production	Zone diameter of N fixation	Ammonia production
PSB3	13.80±2.63^{p}	+	+++	17	+
PSB5	14.37±3.15^{n}	-	-	14	+
PSB8	14.84±1.12^{l}	-	-	16	+
PSB11	18.05±3.83^{g}	-	+	11	+
PSB13	11.59±4.35^{vw}	-	+	09	+
PSB16	12.62±3.16^{s}	-	+	13	+
PSB18	17.54±5.10^{h}	-	++	12	+
PSB22	11.57±2.36^{vw}	-	+	-	+
PSB24	11.48±1.94^{w}	-	-	07	+
PSB27	11.56±3.57^{vw}	-	-	12	+
PSB29	10.90±4.67^{z}	+	+++	11	+
PSB31	10.77±2.45^{z}	-	-	11	+
PSB34	11.91±3.65^{u}	-	+	10	+
PSB36	16.90±3.05^{i}	+	+	-	+
PSB38	14.74±2.05^{m}	-	-	-	+
PSB41	22.67±3.55^{d}	-	+	26	+
PSB43	12.36±3.69^{t}	+	+++	25	+
PSB44	22.73±2.54^{cd}	+	+	11	+
PSB45	63.35±5.28^{a}	+	++	07	+
PSB49	11.95±3.54^{u}	-	+	12	+
PSB52	11.66±3.45^{v}	-	+	-	+
PSB56	11.22±2.66^{x}	-	+	-	+
PSB58	13.60±3.67^{q}	-	+	21	+
PSB64	13.84±3.54^{p}	-	+	18	+
PSB66	15.49±3.05^{k}	+	++	17	+
PSB67	22.81±7.69^{c}	-	+	10	+
PSB70	20.21±6.76^{f}	-	-	16	+
PSB71	18.05±4.11^{g}	-	++	17	+
PSB72	15.45±3.84^{k}	-	+	-	+
PSB74	14.02±3.58^{o}	+	++	18	+
PSB78	20.60±5.84^{e}	+	++	13	+
PSB82	12.95±4.64^{r}	+	+++	22	+
PSB85	37.43±2.57^{a}	-	+	14	+
PSB87	27.26±3.38^{ab}	-	+	11	+
PSB89	27.10±1.99^{b}	-	-	-	+
PSB91	32.85±2.32^{a}	-	-	-	+
PSB93	16.08±4.56^{j}	-	++	07	+
PSB95	16.12±4.03^{j}	+	++	09	+
PSB97	12.31±5.54^{t}	+	++	-	+
PSB99	11.05±2.69^{y}	-	++	13	+
PSB102	12.64±2.76^{s}	-	+	-	+

+, positive; ++, moderately positive; +++, strongly positive; -, negative

A total of forty one isolates of rhizobacteria obtained were biochemically identified by several tests. 20 bacterial isolates were identified as genus *Pseudomonas* which is the most dominant isolate found including Gram-negative, rod shaped *Pseudomonas luteola* and *P. fluorescens*, followed by *Aeromomas hydrophila* (10 isolates). Some of these are Gram-positive spore forming bacteria identified as *Bacillus* sp. (8 isolates). Only 3 isolates were Gram-negative and rod shaped identified as *B. cepacia* (Table 3).

ISSN (print): 1823-8262, ISSN (online): 2231-7538

Table 3: Identification of selected bacterial isolates.

Isolate	Gram's staining	Shape	Catalase	Oxydase	Amylase	Motility	ID
PSB3	-	rod	+	-	+	+	*P. luteola*
PSB5	+	rod	+	-	+	+	*Bacillus* sp.
PSB8	+	rod	+	+	+	+	*Bacillus* sp.
PSB11	-	coccibacilli	+	-	-	+	*A. hydrophila*
PSB13	-	rod	+	-	+	+	*P. fluorescens*
PSB16	-	rod	+	-	+	+	*P. luteola*
PSB18	-	rod	+	-	-	+	*P. luteola*
PSB22	-	rod	+	+	+	+	*B. cepacia*
PSB24	+	rod	+	-	+	+	*Bacillus* sp.
PSB27	+	rod	+	+	+	+	*Bacillus* sp.
PSB29	-	coccibacilli	+	+	-	+	*A. hydrophila*
PSB31	+	rod	+	-	+	+	*Bacillus* sp.
PSB34	-	rod	+	-	+	+	*P. luteola*
PSB36	-	rod	+	-	+	+	*P. luteola*
PSB38	+	rod	+	+	+	+	*Bacillus* sp.
PSB41	-	rod	+	-	+	+	*P. luteola*
PSB43	-	coccibacilli	+	+	-	+	*A. hydrophila*
PSB44	-	coccibacilli	+	+	-	+	*A.hydrophila*
PSB45	-	rod	+	-	+	+	*P. luteola*
PSB49	-	rod	+	-	+	+	*P. luteola*
PSB52	-	rod	+	-	+	+	*P. luteola*
PSB56	-	rod	+	-	+	+	*P. luteola*
PSB58	-	rod	+	-	+	+	*P. luteola*
PSB64	-	rod	+	-	+	+	*B. cepacia*
PSB66	-	rod	+	+	+	+	*P. fluorescens*
PSB67	-	rod	+	-	+	+	*P. luteola*
PSB70	+	rod	+	-	+	+	*Bacillus* sp.
PSB71	-	rod	+	-	+	+	*P. luteola*
PSB72	-	coccibacilli	+	+	-	+	*A. hydrophila*
PSB74	-	Rod	+	+	+	+	*B. cepacia*
PSB78	-	coccibacilli	+	+	-	+	*A.hydrophila*
PSB82	-	rod	+	+	-	+	*A.hydrophila*
PSB85	-	rod	+	-	+	+	*P. luteola*
PSB87	-	rod	+	-	+	+	*P. luteola*
PSB89	-	coccibacilli	+	+	-	+	*A. hydrophila*
PSB91	+	Rod	+	-	+	+	*Bacillus* sp.
PSB93	-	rod	+	-	+	+	*P. luteola*
PSB95	-	rod	+	-	+	+	*P. fluorescens*
PSB97	-	coccibacilli	+	+	-	+	*A. hydrophila*
PSB99	-	rod	+	+	-	+	*A.hydrophila*
PSB102	-	rod	+	-	+	+	*P. luteola*

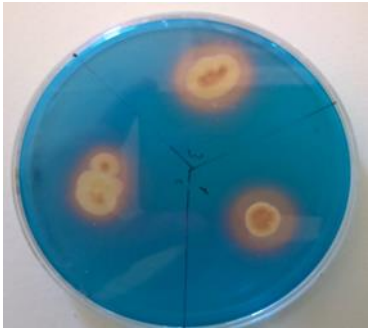

Figure 4: Siderophore production by bacteria.

In this study the highest amounts of released phosphorus were solubilized by *Pseudomonas* with 24.39% followed by *Bacillus* sp. (12.19%), *Aeromomas* (9.75%) and finally *Burkholderia* with 0.41%. Bacteria producing the highest concentration of IAA were isolates belonging to the genus *Pseudomonas* (24.39%) followed by *Aeromonas* (9.75%), *Bacillus* (8.33%) and *Burkholderia* with 0.41%. For HCN production *Aeromonas* was the higher producer (14.63%) followed by *Pseudomonas* (12.19%) *Burkholderia* (0.41%), and finally *Bacillus* with 0%. Isolates belonging to *Pseudomonas* were able to produce siderophore with 48. 78% followed by *Aeromonas* (14.63%), *Burkholderia* (7.31%) and *Bacillus* with 0%. The majority of isolates belonging to genus *Pseudomonas* were able to fix nitrogen with

ISSN (print): 1823-8262, ISSN (online): 2231-7538

19.51% followed by *Aeromonas* and *Bacillus* (19.51% and 12.19% respectively) and finally *Burkholderia* with 4.87%

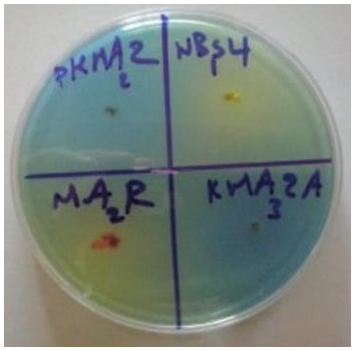

Figure 5: Nitrogen fixation by bacterial isolates.

DISCUSSION

Plant growth promoting rhizobacteria (PGPR) are a group containing a large number of bacteria number found in the rhizosphere, at root exteriors and in association with them, which can enhance directly and/or indirectly yield and growth of agricultural crops. The capability of some rhizospheric microorganisms to solubilize soil phosphate and make it available for plants by converting it into accessible forms like orthophosphate, is an important activity in a PGPB for improving plant growth (Chen *et al.*, 2006). In our study 104 phosphate solubilizing bacterial isolates were isolated from wheat plant rhizosphere of salt affected soil; these bacteria are more effective in phosphorus solubilization than fungi as reported by Alam *et al.* (2002). Our rhizobacterial isolates were biochemically identified as *Pseudomonas*, *A. hydrophila, Bacillus* sp. and *B. cepacia.* Srinivasan *et al.* (2012) also isolated *Pseudomonas* and *Bacillus* as phosphate solubilizers from salt affected soil. Moreover *A. hydrophila* and *Bacillus* sp. were isolated from the rhizospheric soil of wheat grown in saline soil by Ashraf *et al.* (2004). In quantitative estimation, all isolates showed diverse levels of phosphate solubilizing activity. The higher concentration of released phosphorus in cultures was exhibited by genus *Pseudomonas* followed by *Bacillus*. The same results were described by Ahmad *et al.* (2008) who reported that solubilization of phosphate was commonly detected in the isolates of *Bacillus* and *Pseudomonas*. Prior reports defined some *Burkholderia* strains as competent phosphate solubilizers (Peix *et al.*, 2001; Caballero-Mellado *et al.*, 2007). pH of bacterial cultures dropped significantly compared to the control. Similar results were observed by Mardad *et al.* (2013) and Alam *et al.* (2002). A negative correlation ($r = -0.86$) was detected between the amounts of solubilized phosphorus of bacterial cultures and their pH values. The same negative correlation was reported by Xiao *et al.* (2009). Mainly, the mode of action of phosphate solubilising bacteria in soil is by the secreting of acids into the medium (Khan *et al.*, 2014; Oteino *et al.*, 2015). Hence, various genus of bacteria including *Bacillus*, *Pseudomonas*, *Enterobacter*, *Serratia* and *Azotobacter* sp. employ many solubilization reactions, such as acidification, exchange reactions, chelation and production of acids to release phosphorus from mineral complexes (Pandey and Maheshwari, 2007).

Recently many researchers have studied production of phytohormones by PGPR (Rajkumar and Freitas, 2008; Ahmad and Khan, 2012). The most important plant growth regulators produced by phosphate solubilizing microorganisms are auxins (Oves *et al.,* 2013). Approximately 75% of bacteria isolated from saline soil have the capacity to produce IAA which concluded that saline soil is a rich source of IAA producing bacteria. A high level of IAA production by *Pseudomonas* was noted in our study and by other researchers (Ahmad *et al.*, 2008; Kumar *et al.*, 2012). Other IAA producing bacteria belongs to *Aeromonas* (Halda-Alija, 2003) and *Bacillus* (Swain *et al.*, 2007) were also reported.

HCN is a volatile, secondary metabolite that suppresses the development of microorganisms. An important role of HCN produced by bacteria from rhizosphere in biological control of pathogens has been described (Siddiqui *et al.*, 2006). In the current study isolates belonging to genus of *Aeromonas*, *Burkholderia* and *Pseudomonas* were able to produce HCN. To date various bacterial genera are able to produce HCN, including *Pseudomonas*, *Aeromonas*, *Alcaligenes* and *Rhizobium* (Devi *et al.*, 2007; Ahmad *et al.*, 2008).

Siderophores produced by PGPR can promote plant growth either directly or indirectly, using radiolabeled ferric siderophores (Sujatha and Ammani, 2013). Our results showed that isolates belonging to genus *Pseudomonas* and *Burkholderia* were the best siderophore producers but isolates belonging to *Bacillus* sp. were unable to produce them. Also García-Gutiérrez *et al.* (2012) found that all *Pseudomonas* strains isolated from soil were able to produce siderophore, while only one strain among *Bacillus* was able to produce such compounds. Luvizotto *et al.* (2010) reported that *B. cepacia* exhibit a high levels of siderophore production.

The presence of nitrogen-fixing bacteria in soil along with its isolation and conversion into PGPR biofertilizer is an important strategy reducing the use of expensive chemical fertilizers especially in nutrient poor and degraded soils (Cakmakci *et al.*, 2006). The majority of our rhizobacterial isolates have the ability to fix nitrogen. Currently there are evidences that plant stimulation effect by PGPR such as *Azoarcus* sp., *Burkholderia* sp., *Gluconacetobacter* is related to their ability to fix nitrogen (Vessey, 2003). In the study of Cakmakci *et al,* (2006), the quality and yield of wheat, spinach and sugar beet was improved using nitrogen fixing bacterial isolates *Pseudomonas* RC06 and *Bacillus* OSU-142 as biofertilizers. Zhang *et al.* (1996) reported that *A. hydrophila* have the ability to fix atmospheric nitrogen.

Ammonia production is an essential trait exhibited by PGPR, which can effect indirectly plants growth (Yadav *et al.*, 2010). All our isolates were able to produce ammonia. These results are similar with those of Ahmed et al. (2008) who revealed the production of ammonia commonly

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

detected in all the isolates of *Pseudomonas*, *Bacillus* and *Azotobacter.* Similarly, all bacteria identified as *Bacillus* and *Pseudomonas* isolated by Yadav *et al.* (2010) from chickpea rhizosphere in India have the ability to produce ammonia.

CONCLUSION

Phosphate solubilizing bacteria isolated from wheat rhizosphere from saline soil located in western region of Algeria showed a high potential to produce growth promoting traits. Isolates belonging to the *Bacillus* and *Pseudomonas* showed a high phosphate solubilisation. Pseudomonas sp. was the highest producer of IAA and siderophore and had the capacity to fix nitrogen. *A. hydrophila* and *B. cepacia* showed high potential produce HCN and other PGP traits also. Those bacterial isolates can be used as inoculants to enhance the phosphorus uptake by plants and reduce the utilization of phosphorus fertilizers and increase yield of crop production.

ACKNOWLEDGEMENT

This study was funded by the Laboratory of Microbiology and Plant Biology, Faculty of Natural and Life Sciences, University of Mostaganem, Algeria.

The authors wish to thank Dr. CHIBANI Abdelwaheb for his help in the finalization of the English version of this paper.

REFERENCES

Ahmad, F., Ahmad, I. and Khan, M. S. (2008). Screening of free-living rhizobacteria for their multiple plant growth promoting activities. *Microbiological Research* **163, 173-181.**

Ahmad, M. and Khan, M. S. (2012). Effect of fungicides on plant growth promoting activities of phosphate solubilizing *Pseudomonas putida* isolated from mustard (*Brassica campestris*) rhizosphere. *Chemosphere* **86, 945-950.**

Alam, S. S., Khalil, N., Ayub, B. and Rashid, M. (2002). *In vitro* solubilization of inorganic phosphate by phosphate solubilizing microorganism (PSM) from maize rhizosphere. *International Journal of Agricultural Biology* **4, 454-458.**

Ashraf, M., Hasnain, S., Berge, O. and Mahmood, T. (2004). Inoculating wheat seedlings with exopolysaccharide-producing bacteria restricts sodium uptake and stimulates plant growth under salt stress. *Biology and Fertility of Soils* **40, 157-162.**

Bakker, A. W. and Schippers, B. (1987). Microbial cyanide production in the rhizosphere to potato yield reduction and *Pseudomonas* spp. mediated plant growth stimulation. *Soil Biology and Biochemistry* **19, 451-457.**

Barber, S. A. (1995). Soil nutrient bioavailability: A mechanistic approach, 2nd Edn. John Wiley. New York, USA. **pp. 245-248.**

Brick, J. M., Bostock, R. M. and Silverstone, S. E. (1991). Rapid *in situ* assay for indole acetic acid production by bacteria immobilized on nitrocellulose membrane. *Applied and Environmental Microbiology* **57, 535-538.**

Caballero-Mellado, J., Onofre-Lemus, J., Estrada-de Los Santos, P. and Martínez-Aguilar, L. (2007). The tomato rhizosphere, an environment rich in nitrogen-fixing *Burkholderia* species with capabilities of interest for agriculture and bioremediation. *Applied and Environmental Microbiology* **73, 5308-5319.**

Cakmakci, R., Donmez, F., Aydın, A. and Sahin, F. (2006). Growth promotion of plants by plant growth promoting rhizobacteria under greenhouse and two different field soil conditions. *Soil Biology and Biochemistry* **38, 1482-1487.**

Cappuccino, J. G. and Sherman, N. (1992). Biochemical activities of microorganisms. *In:* Microbiology, A Laboratory Manual. The Benjamin/Cummings Publishing Co. California, USA. **pp.188-247.**

Cattelan, A. J., Hartel, P. G. and Fuhrmann, J. J. (1999). Screening of plant-growth promoting rhizobacteria to promote early soybean growth. *Soil Science Society of America Journal* **63, 1670-1680.**

Chen, Y. P., Rekha, P. D., Arun, A. B, Shen, F. T., Lai, W. A. and Young, C. C. (2006). Phosphate solubilizing bacteria from subtropical soil and their tricalcium phosphate solubilizing abilities. *Applied Soil Ecology* **34, 33-41.**

Devi, K. K., Seth, N., Kothamasi, S. and Kothamasi, D. (2007). Hydrogen cyanide producing rhizobacteria kill subterranean termite *Odontotermesobesus* (Rambur) by cyanide poisoning under *in vitro* conditions. *Current Microbiology* **54, 74-78.**

Dobereiner, J. and Day, J. M. (1976). Associative symbiosis in tropical grasses: Characterization of microorganisms and dinitrogen fixing sites. *In:* Newton, W.E. and Nyman, C.J. (eds.). Proceedings of the 1st International Symposium on Nitrogen Fixation, Washington State University Press, Pullman, USA. **pp. 518-538.**

García-Gutiérrez, L., Romero, D., Zeriouh, H., Cazorla, F. M., Torés, J. A., Vicente, A. and Pérez-García, A. (2012). Isolation and selection of plant growth promoting rhizobacteria as inducers of systemic resistance in melon. *Plant and Soil* **358, 201-212.**

Grattan, S. R. and Grieve, C. M. (1999). Salinity mineral nutrient relations in horticultural crops. *Scientia Horticulturae* **78, 127-157.**

Gyaneshwar, P. and Naresh, K. G. (2002). Role of soil microorganisms in improving P nutrition of plants. *Plant Soil* **245, 83-93**.

Halda-Alija, L. (2003). Identification of indole-3- acetic acid producing freshwater wetland rhizosphere bacteria associated with *Juncus effusus L*. *Canadian Journal of Microbiology* **49, 781-787.**

Herman, R. P., Provencio, K., Torrez, T. and Seager, G. M. (1994). Seasonal and spatial population dynamics of the nitrogen efficient guild in a desert bajada grassland. *Applied and Environmental Microbiology* **60, 1160-1165**.

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Idikut, L. Z., Dumlupinar, S. N., Kara, C., Yururdurmaz, A. and Çolkese, M. (2012). The effect of different temperatures and salt concentrations on some popcorn landraces and hybrid corn genotype germinations. *Pakistan Journal of Botany* **44, 579-587.**

Jackson, M. L. (1973). Soil chemical analysis. Prentice Hall of Englewood cliffs. New Jersey, USA. **pp. 378-398.**

Khan, M. S., Zaidi, A. and Ahmad, E. (2014). Mechanism of phosphate solubilisation and physiological functions of phosphate-solubilizing microorganisms. *In*: Phosphate Solubilizing Microorganisms. Khan, M. S., Zaidi, A. and Mussarrat, J. (eds.). Springer International Publishing, Switzerland. **pp. 34-45.**

Khan, M. S., Zaidi, A., Ahmed, M., Oves, M. and Wani, P. M. (2010). Plant growth promotion by phosphate solubilizing fungi-current respective. *Archives of Agronomy and Soil Science* **56, 73-98.**

Khan, M. S., Zaidi, A., Ahmed, M., Oves, M. and Wani, P. M. (2010). Plant growth promotion by phosphate solubilizing fungi-current respective. *Archives of Agronomy and Soil Science* **56, 73-98.**

Kumar, A., Kumar, A., Devi, S., Patil, S., Payal, C. and Negi, S. (2012). Isolation, screening and characterization of bacteria from Rhizospheric soils for different plant growth promotion (PGP) activities: An *in vitro* study. *Recent Research in Science and Technology* **4, 01-05.**

Luvizotto, D. M., Marcon, J., Andreote, F. D, Neves, A. A. C., Araújo, W. L. and Pizzirani-Kleiner, A. A. (2010). Genetic diversity and plant-growth related features of *Burkholderia spp.* from sugarcane roots. *World Journal of Microbiology and Biotechnology* **26, 1829-1836.**

Mardad, I., Serrano, A. and Soukri, A. (2013). Solubilisation of inorganic phosphate and production of organic acids by bacteria isolated from a Moroccan mineral phosphate deposit. *African Journal of Microbiology Research* **7, 626-635.**

Mittler, R. (2002). Oxidative stress, antioxidants and stress tolerance. *Trends in Plant Sciences* **7, 405-410.**

Nautiyal, C. S. (1999). An efficient microbiological growth medium for screening phosphate solubilizing microorganisms. *FEMS Microbiology Letters* **170, 265-270.**

Oteino, N., Lally, R. D., Kiwanuka, S. K, Lloyd, A., Rayan, D., Germaine, K. J. and Dowling, D. N. (2015). Plant growth promotion induced by phosphate solubilizing endophytic *Pseudomonas* isolates. *Frontiers in Microbiology* **6, 745-755.**

Oves, M., Khan, M. S. and Zaidi., A. (2013). Chromium reducing and plant growth promoting novel strain *Pseudomonas aeruginosa* OSG41 enhance chickpea growth in chromium amended soils. *European Journal of Soil Biology* **56, 72-83.**

Pandey, P. and Maheshwari, D. K. (2007). Two species microbial consortium for growth promotion of Cajanus Cajan. *Current Science* **8, 92-25.**

Peix, A., Mateos, P. F., Rodriguez-Barrueco, C., Martinez-Molina, E. and Velazquez, E. (2001). Growth promotion of common bean *(Phaseolus vulgaris L.)* by a strain of Burkholderia cepacia under growth chamber conditions. *Soil Biology and Biochemistry* **33, 1927-1935.**

Pikovskaya, R. I. (1948). Mobilization of phosphorus in soil inconnection with vital activity of some microbial species. *Microbiologia* **17, 362-370.**

Rajkumar, M. and Freitas, H. (2008). Influence of metal resistant-plant growth-promoting bacteria on the growth of *Ricinuscommunis* in soil contaminated with heavy metals. *Chemosphere* **71, 834-842.**

Schwyn, B. and Neilands, J. B. (1987). Universal assay for the detection and determination of siderophores. *Analytical Biochemistry* **160, 47-56.**

Siddiqui, I. A, Shaukat, S. S, Hussain Sheikh, I. and Khan, A. (2006). Role of cyanide production by *Pseudomonas fluorescens* CHAO in the suppression of root-knot nematode, *Meloidogyne javanica* in tomato. *World Journal of Microbiology and Biotechnology* **22, 641-650.**

Srinivasan, R., Yendigeri, M. S., Kashyap, S. and Alagawadi, A. R. (2012). Effect of salt on survival and P solubilization potential on phosphate solubilizing microorganisms from salt affected soils. *Saudi Journal of Biological Sciences* **19**, **427-434.**

Sujatha, N. and Ammani, K. (2013). Siderophore production by the isolates of fluorescent Pseudomonads. *International Journal of Current Research and Academic Review* **5**, **1-7.**

Swain, M. R., Naskar, S. K. and Ray, R. C. (2007). Indole-3-acetic acid production and effect on sprouting of Yam (*Dioscorea rotundata* L.) minisetts by *Bacillus subtilis* isolated from culturable cowdung microflora. *Polish Journal of Microbiology* **56**, **103-110.**

Vessey, J. K. (2003). Plant growth promoting rhizobacteria as biofertilizers. *Plant and Soil* **255**, **571-586.**

Xiao, C., Chi, R., Huan, H. E. and Zhang, W. (2009). Characterization of tricalcium phosphate solubilization by *Stenotrophomonas maltophilia* YC isolated from phosphate mines. *Journal of Central South University of Technology* **16**, **0581-0587.**

Yadav, J., Verma, J. P. and Tiwari, K. N. (2010). Effect of plant growth promoting rhizobacteria on seed germination and plant growth chickpea (*Cicerarietinum* L.) under *in vitro* conditions. *Biological Forum An International Journal* **2(2)**, **15-18.**

Zhang, F., Dashti, N., Hynes, R. K. and Smith, D. L. (1996). Plant growth promoting rhizobacteria and soybean [Glycine max (L.) Merr.] nodulation and nitrogen fixation at suboptimal root zone temperatures. *Annals of Botany* **77**, **453-459.**

Zhou, K., Binkley, D. and Doxtader, K.G. (1992). A new method estimating gross phosphorus mineralization and immobilisation rates in soils. *Plant and Soil* **147**, **243-250.**

 ISSN (print): 1823-8262, ISSN (online): 2231-7538