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ISOLATION AND SCREENING OF MULTIFUNCTIONAL RHIZOBACTERIA FROM THE SELECTED SITES OF MADHUPUR, NARSHINGDI AND MYMENSINGH, BANGLADESH

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ABSTRACT

A laboratory experiment was performed to isolate some native rhizobacteria that could be used as bioinoculants for sustainable crop production. A total of 43 rhizobacteria were isolated from undisturbed plant rhizosphere soils of three different locations of Bangladesh and evaluated their plant growth promoting traits, both direct and indirect. The study has screened out isolates on the basis of their phosphorous solubilization and nitrogen (N) fixation. The phosphate solubilization assay in National Botanical Research Institute of Phosphate (NBRIP) medium revealed that 12 bacterial isolates were able to solubilize tricalcium phosphate and the rhizobacteria M25 showed best performance with a PSI of 3.33 at 5 day. Exactly 47% (20 isolates) of the isolated rhizobacteria were able to grow in N-free Winogradsky's medium, which is an indication of potential N_2-fixers. Among the 20 potential N-fixers, 15 were able to grow within 24 hours of incubation indicating that they are more efficient in N-fixation. The present study successfully isolated and characterized 43 rhizobacteria. Some of these isolated rhizobacteria have potential plant growth promoting traits and are potential plant growth promoting rhizobacteria (PGPR) candidate. Considering all plant growth promoting traits, the isolate F37 was the best followed by M6. However, further experiments are needed to determine the effectiveness of these isolates under *in vitro* and different field conditions to understand the nature of interaction with the plant and environment.

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INTRUDUCTION

In recent years, focus has been on the use of plant growth promoting rhizobacteria (PGPR) as an alternative, environmentally friendly and effective strategy for plant control (Babalola and Glick, 2012; Patel *et al.,* 2012). Research on plants associated with microorganisms is currently expanding quite rapidly with the identification of new bacterial strains, which are more effective in promoting plant growth (Trivedi and Pandey, 2008). PGPR are among the most complex, diverse, and important assemblages in the biosphere (Khan, 2005). They are considered as a group of beneficial free living soil bacteria for sustainable agriculture and environment (Babalola, 2010).

PGPR are characterized by a number of activities, which include the capacity to colonize plant roots surfaces closely adhering to soil interface, increase mineral nutrient solubilization (i.e. P) and N- fixation (Khan, 2005; Shanab *et al.,* 2003) promote plant growth and yield, suppress plant diseases and soil borne pathogens by the production of hydrogen cyanide (HCN), siderophores, antibiotics, and/or competition for nutrients (Kamnev and Lelie, 2000; Shanab *et al.,* 2003; Idris *et al.,* 2007). Furthermore, PGPR improve plant stress tolerance especially to drought, salinity, metal toxicity and production of phytohormones such as indole-3-acetic acid (IAA) (Khan *et al.,* 2009; Verma *et al.,* 2010; Figueiredo *et al.,* 2010).

Research and development on beneficial rhizobacteriaas well as information on the exploitation of other plant growth promotional activities in Bangladesh is scanty. Though Atiqur *et al.,* (2010) have contributions relating those activities. The undisturbed forest flora of Madhupur and Narshingdi may harbour diverse rhizobacteria which may be rhizosphere competent, i.e. able to compete well with other rhizosphere microbes for nutrients secreted by the root. The present study was aimed to isolate and purify the rhizobacteria from the forest flora of Madhupur, Narshingdi and Mymensigh and to screen for P-solubilizing and N-fixing bacteria.

MATERIALS AND METHODS

Collection of rhizosphere plant samples

Fern (*Pteris* spp.) was collected from acidic soils of Madhupur forest, *Cyperus* spp. was collected from Rasulpur during the month of September 2013 and *Melastroma* samples were collected from red soils of Shivpur Upazilla, Narshingdi and Botanical Garden of Bangladesh Agricultural University, Mymensingh during January 2013. Plant samples were collected along with their root and carefully kept in plastic bags, labeled and sealed (in order to minimize the evaporation loss) and stored in a refrigerator at 4°C. A total of four plant samples were collected for the isolation of rhizosphere bacterial isolates.

Isolation and purification of bacterial strains

All the glassware including petridishes were sterilized for 20 minutes at 120°C by autoclave (Model: JSAC-80 JSR). A small part of roots from each sample were separately taken in a test tube. After pouring 10 mL of sterilized distilled water, vigourous shaking was done in bio-safety cabinet (Model: JSCB-900SB JSR). At the end of shaking, the samples were serially diluted upto 10^{-1} and 10^{-2} with sterilized distilled water. Then, 2-3 drops from dilutions and originals were placed on nutrient broth agar (NBA) by Spread Plate Technique and incubated at 28°C for 48h in a microbial incubator (Model: EN-120, Nuve) (Atiqur, 2010). Several screenings were done to isolate pure cultures. Pure colonies were isolated and maintained on NBA plates at 4°C for further studies. For long term storage, the pure isolates were preserved in eppendorf tubes and stored in 10% glycerol solution at -20°C.

Characterization of bacterial strains

Morphological characterization of bacterial strains

The bacterial isolates were grown in NBA medium (containing nutrient broth 1%, sucrose (1%, agar 1.5% and pH=6.5) and incubated in microbial incubator at 28°C for 24 hours to study their colony structure, shape, color, elevation and pigmentation. All the glassware and media were sterilized before incubation.

Biochemical characterization of bacterial strains

Gram reaction test

To study the biochemical characteristics, all the bacterial isolates were grown in NBA medium and incubated in microbial incubator at 28°C for 48 hours. On glass slide a loopful of bacteria from a well grown colony was mixed with a drop of 3% KOH aqueous solution. Mixing was continued for less than l0 seconds. A toothpick was used for picking as well as mixing bacteria from a colony. The toothpick was raised a few centimeters from the glass slide. Strands of viscid material confirmed that the bacterium was gram-negative. Producing thread with a toothpick indicates that it was gram (+ve) bacteria (Ahmed, 2011).

Catalase test

A small amount of bacterial isolate was placed from culture onto a clean microscope slide. A few drops of H_2O_2 were added onto the smear. A positive result is the rapid evolution of O_2 as evidenced by bubbling. A negative result is no bubbles or only a few scattered bubbles (Wheelis, 2008).

Screening for Phosphate Solubilizing Bacteria

All bacterial strains were tested by an agar assay using National Botanical Research Institute Phosphate (NBRIP) containing sucrose = 10 gL^{-1}, $Ca_3(PO_4)_2$ =5 g L^{-1}, $MgCl_2.6H_2O$ = 5 g L^{-1}, $MgSO_4.7H_2O$ = 0.25 g L^{-1}, KCl = 0.2 g L^{-1}, $(NH_4)_2SO_4$ = 0.1 g L^{-1}, pH=7.0 medium supplemented with 1.5% agar (Nautiyal, 1999). Six strains per plate were stabbed in triplicate using sterile toothpicks. The halo and colony diameters were measured after 5 day of incubation of the plates at 25°C. The ability was described by the following equation (Edi *et al.,* 1996).

$$\text{Solubilization index} = \frac{\text{Colony diameter} + \text{Clearing zone}}{\text{Colony Diameter}}$$

Screening for N-fixing bacteria

To study nitrogen fixation ability of the bacteria, isolates were grown in modified Winogradsky's medium (Hashidoko *et al.,* 2002) which is an N-free medium. (Winogradsky's medium without tryptophan and yeast extract) and kept for 48 hours in microbial incubator at 28°C.

RESULTS

Collection and isolation of bacteria

A total of 43 rhizobacterial strains as shown in Table 1 were successfully isolated from the rhizosplane of four plant root samples collected from red soils of Rasulpur area of Madhupur forest, Sonaimuri Upazila of Narshingdi and Botanical Garden of BAU, Mymensingh.

Morphological characteristics

The morphological characteristics of PGPR isolates varied widely. All the isolates produced different size, shape and elevation and pigment production capacity. Some of the rhizobacterial isolates showed clear pigment producing ability, while others are creamy, colorless or white in colour. The morphological characteristics of the isolated bacteria have been presented in Table 2.

Biochemical characteristics

All the isolates were studied for their biochemical characteristics. Both gram reaction and catalase tests were done. Among the 43 rhizobacteria, 17 isolates were gram negative and the rests were gram positive (Table 3). In catalase test, only 3 isolates were catalase negative (*viz.* M10, M11 and M31). Catalase positive bacteria was indicated by the production of bubble with 3% H_2O_2.

Screening phosphate solubilizing bacteria

The isolated rhizobacteria were screened for P solubilizing capability using tricalcium phosphate. After five days of incubation in NBRIP agar medium, only 12 out of 43 isolates were able to grow and showed

phosphate solubilizing capacity in the form of $Ca_3(PO_4)_2$ with various phosphate solubilizing indexes. Out of 43 isolates, 12 isolates grew and solubilize phosphate on NBRIP medium as evidenced by hallow zone around the colony (Figure 1). The phosphate solubilizing capacity of the isolated rhizobacteria have been shown in Table 4.

The remaining isolates were unable to grow in NBRIP medium. Among the solubilizers, 3 isolates showed weak solubilization (PSI<1.5, 5 showed moderate solubilization (PSI upto 2.5) and 5 isolates have high phosphate solubilizing capacity (PSI >2.5). Maximum phosphate solubilizing activity was shown by M25 (PSI 3.33) followed by F39 (PSI 3.0). Least P solubilization was exhibited by M35 (PSI 1.11).

Screening N-fixing Bacteria

To screen nitrogen fixing bacteria, N-free modified Winogradsky's medium was used. The bacteria able to grow in N-free medium were identified as nitrogen fixing bacteria. Among 43 isolates, 20 grew in N-free Winogradsky's medium with 15 isolates showing high growth only after 24 hours (Table 5).

Table 1. List of isolated Rhizobacteria from collected plant roots

Source	Sampling location	Isolate code
Melastroma spp.	Narshingdi	M1, M2, M3, M4, M5, M6, M7, M8, M9, M10, M11, M12, M13, M14, M15, M16, M17, M18
*Melastroma*spp.	Botanical garden	M19, M20, M21, M22, M23, M24, M25, M26, M27, M28, M29, M30, M31, M32, M33, M34, M35
*Pteris*spp.	Madhupur	F36, F37, F38, F39
*Cyperus*spp.	Rasulpur	FR40, FR41, FR42, FR43

Table 2. Morphological characteristics of isolated Rhizobacteria

Morphological properties	Isolate No.
Shape	
Round	M1, M2, M3, M4, M5, M7, M8, M9, M12, M16, M17, M19, M23, M25, M26, M27, M28, M31, M32, M34, M35, F36
Irregular	M6, M11, M13, M14, M18, M20, M21, M22, M24, M29, M30, M33, F37, F38, F39, FR40, FR41, FR42, FR43
Oval	M10, M15
Colour	
Deep red	M9, M12
Grey	M5
Greyish	M1, M2, M7, M11, M40
Pink	M26
Reddish	M23
Red	M28, M30, M34
Slight green	M17, F37, F38
Slight red	M27
Transparent	M32
Whitish	M10, M13, M18, M20, M21, M25, FR41
White	M3, M4, M8, M14, M15, M19, M22, M31, M33, M35, F39, FR42
Yellowish	M16, M24
Yellow	M29, F36, FR43
Elevation	
Raised	All except M18, M22, M26
Depressed	M18, M22, M26

Table 3. Biochemical characteristics of isolated Rhizobacteria

Biochemical Characterization	Isolate No.
Gram test	
Gram (-)	M1, M2, M11, M17, M19, M21, M22, M24, M27, M28, M34, M35, F37, F38, F39, FR40
Gram (+)	M3, M4, M5, M6, M7, M8, M9, M10, M12, M13, M14, M15, M16, M18, M20, M23, M25, M26, M29, M30, M31, M32, M33, FR41, FR42, FR43
Catalase test	
Catalase (+)	M1, M2, M3, M4, M5, M6, M7, M8, M9, M12, M13, M14, M15, M16, M17, M18, M19, M20, M21, M22, M23, M24, M25, M26, M27, M28, M29, M30, M32, M33, M34, M35, F36, F37, F38, F39, FR40, FR41, FR42, FR43
Catalase (-)	M10, M11, M31

Table 4. Phosphate solubilization capacity of isolated Rhizobacteria with PSI

PSI value	Isolate No.
0.0 – 1.0	M3, M5, M7, M8, M9, M10, M12, M13, M14, M15, M18, M19, M20, M21, M22, M23, M24, M26, M27, M28, M29, M30, M31, M32, M33, M34, , F36, FR40, FR41, FR42, FR43
1.0 – 2.0	M4, M11, M16, M35
2.0 – 3.0	M1, M2, M6, M17, F37, F38, F39
3.0 – 4.0	M25

Table 5. Bacterial isolates response in N free media

Growth in N free Medium*	Isolate No.
-	M3, M4, M5, M6, M8, M9, M12, M19, M21, M24, M26, M27, M28, M29, M30, M32, M33, M34, M35, F38, F39, FR41, FR42, FR43
+	M7, M22, M25, FR40
++	M1, M2, M10, M11, M13, M14, M15, M16, M17, M18, M20, M23, M31, F36, F37

*(-), (+) and (++) designate low, medium and high bacterial growth, respectively in N-free Winogradsky's medium after 24 hrs.

DISCUSSION

Plants provide a nutrient rich habitat for the growth and development of various groups of microorganisms, especially bacteria. Bacteria profit from plants because of the enhanced availability of nutrients and plants. In turn, plant benefit from the bacterial associations by growth enhancement, stress reduction or protection from pathogens. In present study, 43 rhizobacteria were isolated from 4 root samples collected from undisturbed red soils of Madhupur, forest soil of Narshingdi and one samples collected from Botanical Garden of Bangladesh Agricultural University, Mymensingh. The morphological and biochemical characteristics of the isolates were studied which revealed that the undisturbed rhizosphere was a rich source of diverse group of rhizobacteria. The isolated rhizobacteria varied in shape, colour, pigment production and other morphological features studied.

Identification and characterization of plant growth promoting traits of the rhizobacteria is an important step in determining their potential utilization in sustainable soil management and crop production system. The beneficial effect of rhizobacteria on plant growth stimulation may be due to a number of mechanisms. To determine the potential of plant growth stimulation, all the 43 isolated rhizobacteria were studied for their biofunctionalities. The isolates were tested for their mineral phosphate solubilization and growth under N-free conditions and the results were discussed in following sections.

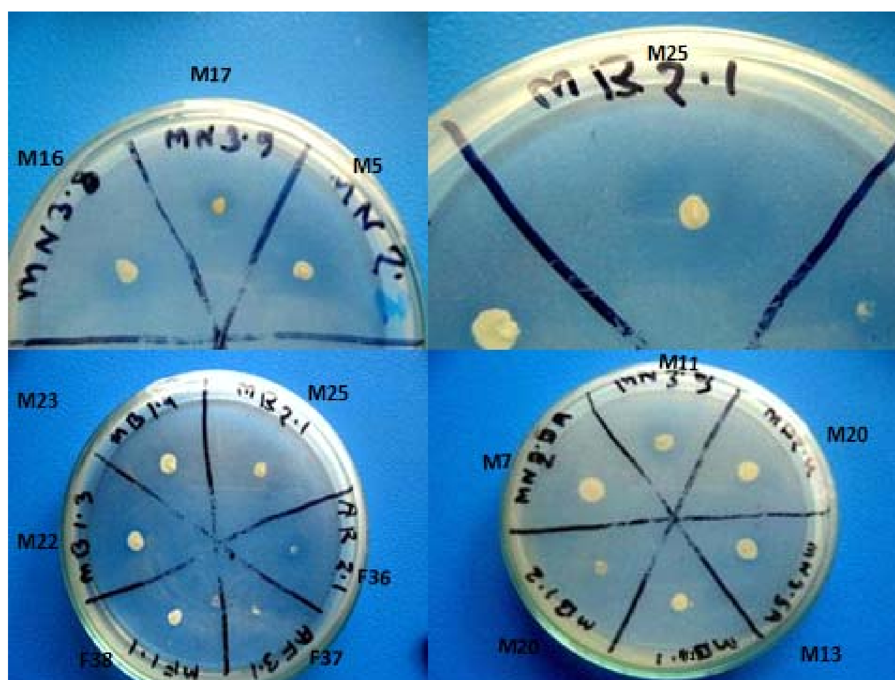

Figure 1. Bacterial isolates showing P-solubilization in NBRIP media supplemented with tricalcium phosphate. Hallow zones around the bacterial colony indicates phosphate solubilization.

Phosphate solubilization

Phosphorus is a major essential macronutrient for biological growth and development. Plant growth is often limited by insufficient phosphate availability. The low solubility of common phosphates such as $Ca_3(PO_4)_2$, hydroxyapatite and aluminium phosphate may cause low phosphate availability in agricultural soils. Microorganisms offer a biological rescue system capable of solubilizing the insoluble inorganic P of soil and make it available to the plants. The ability of some microorganisms to convert insoluble P to an accessible form, like orthophosphate, an important trait in a PGPB for increasing plant yields (Chen *et al.,* 2006). High proportion of phosphate solubilizing microbes is concentrated in the rhizosphere and they are metabolically more active than other sources (Vazquez *et al.,* 2000).

Qualitative P-solubilization potential estimated by observing the large clear/halo zones on agar media revealed that out of 43 bacterial isolates tested, 12 isolates had P-solublizing ability (Table 4) in agar medium supplemented with insoluble $Ca_3(PO_4)_2$. The isolates exhibited different degrees of phosphate solublizing capacity as revealed by PS index ranging from 1.11 to 3.33 (at day 5). The isolated rhizobacteria showed variation in their phosphate solubilization capacity. This variation in P-solubilization by the rhizobacteria might be due to difference in their organic acids production capacity both in terms of amount and type of acids. Similar results were reported by Rashid *et al*. (2004). The rhizobacterial isolates M25 and F39 showed the highest P solubilization (PSI >3) in plate culture which is generally a reliable method for preliminary screening and characterization of P-solubilizing microorganisms. In order to precisely characterize the isolates for their P-solubilization ability, liquid culture and field trial of the P-solubilizers identified through plate assay. The cause of P solubilization could probably be due to secretion of organic acids, such as gluconic, 2-ketogluconic, oxalic, citric, acetic, malic, and succinic acid (Zaidi *et al.* 2009).

The ability of several isolates to solubilize tricalcium phosphate *in vitro* suggested the application of those isolates in crop fields. Rodriguez and Fraga (1999) studied that *Pseudomonas* and other PSB like *Bacillus* and *Rhizobium* were capable of increasing the availability of P in soil. Specifically, all those isolates might be potential inoculants for alkaline soil based on the ability to solubilize phosphate bounded by calcium which mostly exists in alkaline soils (Goldstein, 1995). Soil inoculation with PSB has been shown to improve solubilization of fixed soil P and applied phosphates resulting in higher crop yields (Nautiyal and Mehta, 2001).

Several PSB could also promote plant growth by rendering phosphate into solution more than they need for their metabolism and the surplus can be absorbed by plant (Kloepper et al., 1980).

Nitrogen fixation

Nitrogen is the most significant yield-limiting element in many agricultural production systems. The nitrogen-fixing bacteria have stimulating effect on the plant; they are able to fix the nitrogen in symbiosis with leguminous plants using the nitrogenase enzyme. Biological N_2 fixation is gaining importance in rice ecosystem because of current concern on the environmental and soil health that are caused by the continuous use of nitrogenous fertilizers and the need for improved sustainable rice productivity.

Therefore, the isolated rhizobacteria were tested for their response in N-free medium. Of the 43 isolates, 19 grew in the medium indicating that these bacteria were able to utilize the atmospheric nitrogen. Fifteen rhizobacterial isolates showed high growth response only after 24 hours, while others grew after 48 hours of incubation. The remaining isolated bacteria (24) could not grow in N-free medium indicating that these bacteria were unable to utilize atmospheric N_2 and therefore, these bacteria are non N_2-fixing bacteria. However, to confirm their N-fixing ability, acetylene reduction assay and NifH gene identification is necessary before their intended utilization as PGPR.

CONCLUSION

Further study is needed to evaluate microbe-microbe and plant-microbe-environment interactions and their plant growth-promoting activities to ensure their use in a sustainable manner in crop production system.

COMPETING INTEREST

The authors declare that they have no competing interests.

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REFERENCES

1. Ahmed I, 2011. Isolation and characterization of As resistant bacteria from the contaminated soil and their effects on seed germination of rice, MS Thesis, Department of Agricultural Chemistry, Bangladesh Agricultural University.
2. Atiqur R, IR Sitepu, S Tang, and Y Hashidoko, 2010. Salkowski's reagent test as a primary screening index for functionalities of rhizobacteria isolated from wild dipterocarp samplings growing naturally on medium-strongly acidic tropical peat soil. Journal of Bioscience, Biotechnology and Biochemistry, 74: 2202-2208.
3. Babalola OO, 2010. Beneficial bacteria of agriculture importance. Biotechnological Letters, 32: 1559-1570.
4. Babalola OO and BR Glick, 2012. Indigenous African agriculture and plant associated microbes: Current practice and future transgenic prospects. Scientific Research Essays, 7: 2431-2439.
5. Chen YP, PD Rekha, AB Arun, FT Shen, WA Lai, and CC Young, 2006. Phosphate solubilizing bacteria from subtropical soil and their tricalcium phosphate solubilizing abilities. Applied Soil Ecology, 34: 33-41.
6. Edi PM, AM Moawad, and PLG Vlek, 1996. Effect of phosphate-solubilizing Pseudomonas putida on the growth of maize and its survival in the rhizosphere. Indonesia Journal of Crop Science, 11: 13-23.

7. Figueiredo MVB, L Seldin, FF Araujo and RLM Mariano, 2010. Plant growth promoting rhizobacteria: Fundamentals and applications. Plant Growth and Health Promoting Bacteria, Microbiology Monographs, 18: 21-43.
8. Goldstein AH, 1995. Recent progress in understanding the molecular genetics and biochemistry of calcium phosphate sulubilization by gram negative bacteria. Biological, Agriculture and Horticulture, 12: 185-193.
9. Hashidoko Y, M Tada, M Osaki and S Tahara, 2002. Soft gel medium solidified with gellab gum for preliminary screening for root-associating, free-living nitrogen-fixing bacteria inhabiting the rhizoplane of plants. Bioscience, Biotechnology and Biochemistry, 66: 2259-2263.
10. Idris SE, DJ Iglesias, M Talon and R Borriss, 2007. Tryptophan-dependent production of Indole-3-Acetic Acid (IAA) affects level of plant growth promotion by Bacillus amyloliquefaciens FZB42. Molecular Plant-Microbe Interactions, 20: 619-626.
11. Kamnev AA and D Lelie, 2000. Chemical and biological parameters as tools to evaluate and improve heavy metal phytoremediation. Bioscience Report, 20: 239-258.
12. Khan AG, 2005. Role of soil microbes in the rhizosphere of plants growing on trace metal contaminated soils in phytoremediation. Journal of Trace Elements in Medicine and Biology, 18: 355-364.
13. Khan MS, A Zaidi, PA Wani and M Oves, 2009. Role of plant growth promoting rhizobacteria in the remediation of metal contaminated soils. Environmental Chemistry Letters, 7: 1-19.
14. Kloepper JW, J Leong, M Teintze and MN Schroth, 1980. Enhanced plant growth by siderophores produced by plant growthpromotingrhizobacteria. Nature, 286: 885- 886.
15. Nautiyal CS, 1999. An efficient microbiological growth medium for screening phosphate solubilizing microorganisms. FEMS Microbiology Letters, 170: 265-270
16. Nautiyal CS and S Mehta, 2001. An Efficient Method for Qualitative Screening of Phosphate-Solubilizing Bacteria. Microbiology, 43: 51-56.
17. Patel HA, RK Patel, SM Khristi, K Parikh and G Rajendran, 2012. Isolation and characterization of bacterial endophytes from Lycopersiconesculentum plant and their plant growth promoting characteristics. Nepal Journal of Biotechnology, 2: 37-52.
18. Rashid M, S Khalil, N Ayub, S Alam and F Latif, 2004. Organic acids production and phosphate solubilization by phosphate solubilizing microorganisms (PSM) under in vitro conditions. Pakistan Journal of Biological Science, 7: 187-196.
19. Rodriguez H and R Fraga, 1999. Phosphate solubilizing bacteria and their role in plant growth promotion, Biotechnology Advances, 17: 319-339.
20. Shanab RI, JS Angle, TA Delorme, RLChaney, P Berkum, H Moawad, K Ghanem and HA Ghozlan, 2003. Rhizobacterial effects on nickel extraction from soil and uptake by Alyssum murale. New Phytologist, 158: 219-224.
21. Trivedi P and A Pandey, 2008. Recovery of plant growth-promoting rhizobacteria from sodium alginate beads after 3 years following storage at 4°C. Journal of Industrial Microbiology and Biotechnology, 35: 205-208.
22. Vazquez P, G Holguin, M Puente, AE Cortes and Y Bashan, 2000. Phosphate solubilizing microorganisms associated with the rhizosphere of mangroves in a semi-arid coastal lagoon. Biology and Fertility of Soils, 30: 460-468.
23. Verma JP, J Yadav, KN Tiwari, Lavakush and V Singh, 2010. Impact of plant growth promoting rhizobacteria on crop production. International Journal of Agricultural Research, 5: 954-983.
24. Wheelis M, 2008. Principles of Modern Microbiology, Jones and Bartlett Publishers, Inc., Sudbury, MA.
25. Zaidi A, MS Khan, M Ahemad and M Oves, 2009. Plant growth promotion by phosphate solubilizing bacteria. Acta Microbiologica et Immunologica Hungarica, 56: 263-284.