Asian Journal of Plant Sciences

ISSN 1682-3974

science alert

ANSInet
an open access publisher
http://ansinet.com

OPEN ACCESS Asian Journal of Plant Sciences

ISSN 1682-3974
DOI: 10.3923/ajps.2020.8.13

Research Article
Phosphate Solubilisation and Indole Acetic Acid Production by Bacteria Isolated from Root System of *Dipterocarpus alatus*

[1]Kiriya Sungthongwises, [2]Anan Wongcharoen and [3]Chutinan Choosai

[1]Agronomy Section, Faculty of Agriculture, Khon Kaen University, 40002 Khon Kaen, Thailand
[2,3]Department of Entomology and Plant Pathology, Faculty of Agriculture, Khon Kaen University, 40002 Khon Kaen, Thailand

Abstract

Background and Objectives: Phosphate solubilizing bacteria could potentially benefit sustainable organic farming systems and reduce the utilization of agrochemicals in agricultural fields. PSB isolation have been termed important prior to the use of biofertilizer in crop productions. This study was conducted to isolate and determine the potential of PSB from the root system of *D. alatus* in Northeast Thailand. In order to understand the impact of the ecosystem services of *D. alatus* in a land use system, we must address the absence of knowledge on the influence of soil fertility on biological processes within the soil. **Materials and Methods:** PSB isolated from soil in different provinces of Northeast Thailand, where soils are mainly sandy and retain a P deficiency. PSB isolates were tested using different P sources [Tri-calcium phosphate ($Ca_3(PO_4)_2$) and ferric phosphate ($FePO_4$)] on specific culture media [National Botanical Research Institute Phosphate Growth Medium, (NBRIP)] under controlled conditions. **Results:** Our results indicated that PSB isolated from Amnat Charoen, Khon Kaen and Mukdahan provinces solubilized higher amounts of $FePO_4$. The highest solubilisation activity was achieved for $FePO_4$, which is the main form of insoluble phosphate in acidic sandy soil. Indole acetic acid (IAA) production was also observed. These results provide essential information on the influence of soil fertility upon *D. alatus* ecosystem services. **Conclusion:** *Nguyenibacter vanlangensis* was the most abundant and solubilized a wide range of $FePO_4$ concentrations better than $Ca_3(PO_4)_2$ and also produced indole-3-acetic acid.

Key words: Auxin, bacteria, ecosystem, isolation, orthophosphate, phosphorus, Yang

Citation: Kiriya Sungthongwises, Anan Wongcharoen and Chutinan Choosai, 2020. Phosphate solubilisation and indole acetic acid production by bacteria isolated from root system of *Dipterocarpus alatus*. Asian J. Plant Sci., 19: 8-13.

Corresponding Author: Kiriya Sungthongwises, Agronomy Section, Faculty of Agriculture, Khon Kaen University, 40002 Khon Kaen, Thailand
Tel: 00 66 090-3241635

Copyright: © 2020 Kiriya Sungthongwises *et al.* This is an open access article distributed under the terms of the creative commons attribution License, which permits unrestricted use, distribution and reproduction in any medium, provided the original author and source are credited.

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

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

INTRODUCTION

Soil microorganisms play a significant role in the food chain and geochemical cycling of carbon, nitrogen, sulphur and phosphorus[1-3]. Phosphate solubilizing bacteria (PSB) such as *Pseudomonas*, *Bacillus*, *Rhizobium*, *Burkholderia*, *Achromobacter*, *Agrobacterium*, *Micrococcus*, *Aerobacter*, *Flavobacterium* and *Erwinia* have been found in the rhizosphere, especially in fertile soils of forests and organic farms[4-6]. Phosphate solubilizing bacteria (PSB) play a vital role in supplying phosphorus to plants[6,7], which improves and maintains the fertility of farmlands[8]. PSB are capable of producing organic acids (acetate, formate, lactate, oxalate, malate and citrate), amino acids, vitamins and growth promoting substances (IAA/indole-3-acetic acid and gibberellic acid) that stimulate the growth of the plants[7,9,10]. The mineralization of organic phosphate has been reported to be mediated through the production of phosphatase enzymes (phosphomonoesterase, phosphodiesterase, triphosphomonoesterase and phosphoramidase) that hydrolyse[2-13] organic-P into $H_2PO_4^-$ and HPO_4. Research on PSB could potentially benefit sustainable organic farming systems, especially in Northeast Thailand and reduce the utilization of agrochemicals in agricultural fields. Because isolation of PSB have been termed important prior to the use of PSB as a biofertilizer in crop productions[14], this study was conducted to determine the potential of PSB isolated from the root system of *D. alatus* in different provinces of Northeast Thailand.

MATERIALS AND METHODS

Study area: The experiment was conducted in Amnat Charoen, Khon Kaen, Mahasarakham, Mukdahan, Roi Et and Yasothon provinces of Northeast Thailand from October, 2017 to September, 2018.

Sampling site and soil sample collection: *Dipterocarpus alatus* trees that were studied were located in Amnat Charoen, Khon Kaen, Maha Sarakham, Mukdahan, Roi Et and Yasothon provinces of Northeast Thailand, which are characterized by a tropical climate with acidic sandy soils. Soil samples were collected at a depth of 0-10 cm from root system of *D. alatus*, with 10 replicates (n = 10) and were preserved in plastic bags at 4°C for chemical and physical analysis along with PSB isolation.

Isolation of phosphate solubilizing bacteria by enrichment culture: To extract the PSB, 5 g of each soil sample was transferred to the National Botanical Research Institute's Phosphate growth medium (NBRIP)[15]. This liquid growth medium contained 10 g glucose, 5 g $MgCl_2 \cdot 6H_2O$, 0.25 g $MgSO_4 \cdot 7H_2O$, 0.2 g KCl and 0.1 g $(NH_4)_2SO_4$ L^{-1}. A modified NBRIP media, containing either $FePO_4$ or $Ca_3(PO_4)_2$ as the sole source of insoluble P, was also used in the initial screening step. The pH of the agar medium was adjusted to 7.0. The sources of insoluble P were autoclaved separately and other sterile ingredients were aseptically mixed in after autoclaving. Erlenmeyer flasks containing 50 mL of the medium with inoculants were incubated for 3 days at 30°C in an incubator shaker at medium speed (150 cycles min^{-1}). During the next 3 days, 5 mL of the medium incubated with inoculants were again transferred into 50 mL Erlenmeyer flasks with new liquid medium at 30°C in an incubator shaker at medium speed. At the end of each 3 days cycle in the NBRIP growth liquid media, aliquots of each dilution were spread over the NBRIP medium and further incubated at 30°C for 7 days. After 6 PSB isolations, colonies were selected from the plates based on the appearance of a clear halo and the clones were further purified on a minimal medium, based on each insoluble phosphate form. Once purified, each isolate was stored in glycerol stock at -20°C.

Mineral phosphate solubilisation: The phosphate solubilizing (PS) activity of each isolate was determined through 4 replicates by the molybdenum-blue method[16]. The isolates were grown in NBRIP liquid media containing different insoluble forms of phosphate [$Ca_3(PO_4)_2$ and $FePO_4$] for 3 days at 30°C in an incubator shaker at medium speed (150 cycles min^{-1}). The solubilisation efficiencies were determined by reactions with ammonium molybdate for phosphorus compounds, such as ammonium phosphomolybdate and reduced to molybdenum blue with a compound ascorbic acid. Later, the isolates were incubated for 30 min at room temperature for colour development. Lastly, the absorption of light within a 580 nm wavelength was measured with a Shimadzu UV-120-01 spectrophotometer. The concentration of PS activity was compared to a standard curve of KH_2PO_4 at concentrations ranging from 0-0.9 mL.

Indole acetic acid production (IAA): PSB strains that were selected based on their ability to solubilize P were analysed for IAA production[16]. The selective bacterial strains were grown in 50 mL of Luria-Bertani medium (LB), containing 10 g L^{-1} Tryptone, 5 g L^{-1} NaCl and 5 g L^{-1} yeast extract at 30°C for 2 days. The 5 mL of PSB solutions were tested through their reaction with 1 mL of Tris-TMRT reagent, containing 10 g L^{-1} D-mannitol, 0.2 g L^{-1} yeast extract,

0.2 g L^{-1} $CaCl_2 \cdot 2H_2O$, 0.2 g L^{-1} $MgSO_4 \cdot 7H_2O$, 1.21 g L^{-1} Tris-base and 0.061 g L^{-1} L-Tryptophan, at 28°C for 10 days. The 2 mL mixtures were synthesized with 0.01 M $FeCl_3$ in 35% $HClO_4$ for 30 min at 25°C in a dark setting. The positively isolated mixtures presented a red colour indicating indole acetic acid production. To determine the volume of IAA production, the PSB centrifugation (14,000 rpm), at which time the suspended supernatant was reacted with 0.01 of M $FeCl_3$ in 35% $HClO_4$ for 30 min, at 25°C, in dark conditions. We measured the absorbance at 660 nm to calculate the concentration of indole acetic acid produced by the bacteria, which was compared to a standard curve of IAA at concentrations ranging from 0-150 mg mL^{-1}.

PCR amplification of 16S rRNA and sequencing: The gene-encoding 16S rRNA was amplified from selected strains by the polymerase chain reaction (PCR) using bacterial universal primers 20F (5 -GAG TTT GAT CCT GGC TCA G-3′) and 1500R (5 -GTT ACC TTG TTA CGA CTT-3′)[17]. The PCR mix consisted of 2.0 µM of each primer, 10 µL of 10X tag buffer, 2.5 U of Taq DNA polymerase, 2.0 mM $MgCl_2$ and 0.2 mM dNTP at pH 8.8 that contained $(NH_4)_2SO_4$, which was comprised of 750 mM Tris-HCl, 200 mM $(NH_4)_2SO_4$ and 0.1% Tween 20. The following cycle conditions were carried out: an initial denaturation step at 94°C for 3 min, followed by 25 cycles of 94°C for 1 min, annealing at 50°C for 1 min and elongation at 72°C for 2 min, followed by a final amplification step at 72°C for 3 min (Geneaid Biotech Ltd., Taiwan). The PCR products were purified from agarose gels with the PCR Clean-up Gel Extraction Kit (Macherey-Nagel, Germany) and sequenced. The nucleotide sequences were compared using the BLASTn program[18] and the closest match of known phylogenetic affiliation was used to assign the isolated strains to specific taxonomic groups.

Statistical analysis: An analysis of variance was conducted on data obtained from each parameter in each treatment. All analysis were carried out using Statistic version 8.0. The least significant differences (LSD) were calculated at $p<0.05$. Duncan's multiple-range test was employed to test the significant differences between treatments.

RESULTS AND DISCUSSION

Soil chemical and physical analysis: The results of the analysis of measured chemical properties within the soil in all sites are shown in Table 1. A pH of 5.4 represented the successful growth of specific microorganisms, like bacteria. Soil pH, total N, available P, exchangeable K Ca and Mg, organic matter (OM) as well as the electrical conductivity (EC) were not significantly different, however, soil moisture, soil density, total P and cation exchange capacity (CEC) demonstrated a significant difference. The relevant chemical properties of soil were: (i) Soil reaction (pH), (ii) Organic matter content and (iii) Fertility status. Microorganisms, such as bacteria, fungi, actinomycetes, algae and protozoa help in improving soil structure, aeration, water permeability and soil nutrient availability. Soil pH may decrease in summer, due to the activities of the microorganisms. Acidity or alkalinity of soil influences the biological activity in soil and the availability of certain minerals, such as phosphates. Sound soil moisture improves nutrient uptake and its fertility status can be further improved through proper soil management[19].

Table 1: Soil properties in different provinces of Northeast Thailand

	Root rhizosphere of *Dipterocarpus alatus* in different provinces						
Soil properties	Amnat Charoen	Khon Kaen	Maha Sarakham	Mukdahan	Roi Et	Yasothon	F-test
pH (1:1 H_2O)	6.13	5.72	6.22	5.78	6.21	6.34	ns
Total N (%) (micro-Kjeldahl)	0.06	0.07	0.07	0.07	0.05	0.06	ns
Total P (mg kg^{-1})	547.61^{a}	365.30^{ab}	348.94^{ab}	252.06^{b}	233.93^{b}	270.18^{b}	*
Available P (mg kg^{-1}) (Bray II method)	14.15	14.62	28.43	13.00	16.89	36.00	ns
Exchangeable K (mg kg^{-1})	150.51	199.39	120.33	155.34	75.54	160.25	ns
Exchangeable Ca (mg kg^{-1})	456.32	639.91	816.74	416.24	342.77	454.47	ns
Exchangeable Mg (mg kg^{-1})	134.86	154.47	128.30	146.86	54.89	111.44	ns
Exchangeable Na (mg kg^{-1})	25.40^{ab}	17.97^{ab}	47.05^{a}	6.63^{b}	23.08^{ab}	8.63^{b}	*
OM (%)	1.37	1.77	1.46	1.47	1.14	1.35	ns
EC (mS cm^{-1})	0.06	0.11	0.14	0.05	0.07	0.06	ns
CEC (C mol kg^{-1})	5.28^{ab}	6.40^{a}	6.00^{a}	4.92^{ab}	3.49^{b}	4.92^{ab}	*
Moisture (%)	5.75^{ab}	10.07^{a}	7.24^{ab}	9.07^{a}	3.78^{ab}	2.40^{b}	*
Density (g cm^{-2})	1.11^{b}	1.42^{a}	1.29^{ab}	1.34^{ab}	1.35^{ab}	1.40^{ab}	*

OM: Organic matter, EC: Electrical conductivity, CEC: Cation exchange capacity, ns: Not significantly different, *: Significant difference at $p \leq 0.05$, different letters indicate a significant difference at $p \leq 0.05$

Table 2: Phosphate solubilizing effectiveness of tested bacteria from root

	Solubilized phosphate (ppm)	
PSB-isolates	$Ca_3(PO_4)_2$**	$FePO_4$
A1	0.00[c]	1.34
A2	1.05[b]	2.12
K1	1.96[a]	1.95
M1	1.88[ab]	1.88
M2	0.00[c]	1.95
M3	0.01[c]	1.99

PSB: Phosphate solubilizing bacteria, **Significant difference at $p \leq 0.01$, different letters indicate a significant difference at $p \leq 0.05$

Table 3: Indole acetic acid production properties of PSB isolates from root rhizosphere of *Dipterocarpus alatus*

PSB-Isolates	IAA production (mg L^{-1})
A1	102.56[b]
A2	260.79[a]
K1	236.28[ab]
M1	171.17[ab]
M2	237.78[ab]
M3	300.42[a]
F-test	*
CV (%)	37.70

PSB: Phosphate solubilizing bacteria, IAA: Indole acetic acid, **Significant difference at $p \leq 0.01$, different letters indicate a significant difference at $p \leq 0.01$

PSB Isolation: Populations of PSB observed from different collected soil samples around the root rhizosphere of *D. alatus* in Northeast Thailand grown on various media are shown in Table 2. Only 6 isolates showed the formation of halozones around the growing colonies (Fig. 1). The phosphates tested ($Ca_3(PO_4)_2$ and $FePO_4$) were clearly dissolved by growing colonies. $FePO_4$ are the main form of insoluble phosphate in acidic sandy soil[7]. This preliminary observation suggests the existence of bacterial isolates that exhibited different degrees of PS efficiency in the soil samples collected. Plant growth promoting bacteria (PGPB) found in the agricultural rhizosphere of plants play a key role in soil P dynamics and catalyse the hydrolysis of organic phosphate esters into orthophosphate anions, through phosphatases[20]. Phosphatase efficiency is related to the microbial fauna, soil temperature, humidity, pH, carbon and nitrogen sources[21], in addition to the associated bacterial communities[22]. Other significant factors include the physiological state of the plant and the type of rooting.

Phosphate solubilisation efficiency: The isolates were differentiated based upon morphological observation and biochemical characterization (Table 2). For the 6 purified isolates, our results showed that free-living bacteria play a key role in soil P dynamics, which catalyse the hydrolysis of inorganic phosphate into orthophosphate anions while using phosphatases to improve P availability. The four isolates that

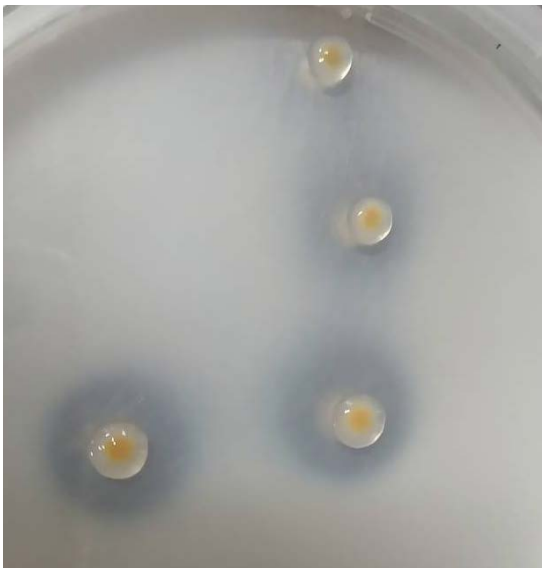

Fig. 1: PSB colonies

solubilized $Ca_3(PO_4)_2$ and the 6 isolates that solubilized $FePO_4$ are able to solubilize the main forms of insoluble phosphates in acidic sandy soils[23]. After evaluating their P solubilisation capacities, we concluded that each of the 6 isolates solubilized $FePO_4$ better than $Ca_3 (PO_4)_2$, especially the isolates found in the Amnat Charoen, Khon Kaen and Mukdahan provinces.

IAA production of PSB isolates: The microorganisms isolated from the root rhizosphere of *D. alatus* in Northeast Thailand had the ability to produce IAA as secondary metabolites. IAA is generally considered the most important native auxin on root growth in seedlings, especially root elongation. This group of bacteria is known as plant growth promoting bacteria (PGPB). IAA production contributes to plant growth through phytostabilization and by increasing the root and shoot biomasses[24], which are involved in nutrient uptake[25]. In this study, the plant growth promoting effects of selected PSB were evaluated through the analysis of IAA production. IAA production was observed in PSB isolates A1, A2, K1, M1, M2 and M3, which indicated that these strains could utilize L-tryptophan as a precursor for growth. Phosphate solubilizing bacteria M3 and A2 achieved the highest IAA production (300.42 and 260.79 mg L^{-1}, respectively, Table 3). IAA production in bacteria varied among different species and strains and was also influenced by culture condition, growth stage and substrate availability[26]. Additionally, the production of IAA was greater in YMD, than in LB media and the YMD media with tryptophan proved more suitable for IAA production, compared to YMD without tryptophan[27].

Identification of PSB isolates: Nucleotide sequencing of PCR-amplified 16S rRNA genes and sequence comparisons with available data in the GenBank using the BLAST algorithm[18] allowed us to identify the majority of the PSB isolates (Table 2). Based on a sequence identification of 99.92% or greater[26], they were all isolates similar to species of the *Nguyenibacter* genus. The *Nguyenibacter vanlangensis* strain seems to be more able to solubilize $FePO_4$ and produce IAA.

CONCLUSION

Phosphate solubilizing bacteria (PSB) were found in different root rhizospheres of *D. alatus* trees, indicating that their wide distribution. *Nguyenibacter vanlangensis* was the most abundant and solubilized a wide range of $FePO_4$ concentrations better than $Ca_3(PO_4)_2$ and also produced indole-3-acetic acid.

SIGNIFICANCE STATEMENT

This study discovers *N. vanlangensis* under root rhizospheres of *D. alatus* trees that can be beneficial for solubilized $FePO_4$ and also produced indole-3-acetic acid. This study will help the researcher to uncover the diversity of phosphate solubilizing bacteria in different root rhizospheres that many researchers were not able to explore. Thus, a new agricultural utility bacteria and possibly function may be arrived at.

ACKNOWLEDGMENTS

This study was fully supported by the Research and Technology Transfer Affairs of Khon Kaen University. We wish to express our sincere gratitude to the Research Institute of Molecular Genetics, in the Northeast Agriculture Research and Development Centre in the Faculty of Agriculture at Khon Kaen University, Thailand.

REFERENCES

1. Kummerer, K., 2004. Resistance in the environment. J. Antimicrob. Chemother., 54: 311-320.
2. Das, S., P.S. Lyla and S.A. Khan, 2007. Biogeochemical processes in the continental slope of Bay of Bengal: I. Bacterial solubilization of inorganic phosphate. Rev. Biol. Trop., 55: 1-9.
3. Bahig, A.E., E.A. Aly, A.A. Khaled and K.A. Amel, 2008. Isolation, characterization and application of bacterial population from agricultural soil at Sohag province, Egypt. Malays. J. Microbiol., 4: 42-50.
4. Raghu, K. and I.C. MacRae, 1966. Occurrence of phosphate-dissolving micro-organisms in the rhizosphere of rice plants and in submerged soils. J. Applied Bacteriol., 29: 582-586.
5. Widawati, S. and Suliasih, 2006. Augmentation of potential Phosphate Solubilizing Bacteria (PSB) stimulate growth of green mustard (*Brasica caventis* Oed.) in marginal soil. Biodiversitas: J. Biol. Divers., 7: 10-14.
6. Widawati, S. and Suliasih, 2009. The role of phosphate solubilizing bacteria and free living nitrogen fixing bacteria on the growth and adaptation of *Gmelina arborea* Roxb. grown on degraded land. Proceedings of the International Seminar to Protect Diversity of Bioresources in the Tropical Area, November 25-26, 2009, Research Center for Biology LIPI, Cibinong, Indonesia.
7. Gyaneshwar, P., G.N. Kumar, L.J. Parekh and P.S. Poole, 2002. Role of soil microorganisms in improving P nutrition of plants. Plant Soil, 245: 83-93.
8. Nautiyal, C.S., S. Bhadauria, P. Kumar, H. Lal, R. Mondal and D. Verma, 2000. Stress induced phosphate solubilization in bacteria isolated from alkaline soils. FEMS Microbiol. Lett., 182: 291-296.
9. Richardson, A.E., 2001. Prospects for using soil microorganisms to improve the acquisition of phosphorus by plants. Aust. J. Plant Physiol., 28: 897-906.
10. Ponmurugan, P. and C. Gopi, 2006. *In vitro* production of growth regulators and phosphatase activity by phosphate solubilizing bacteria. Afr. J. Biotechnol., 5: 348-350.
11. Pang, P.C.K. and H. Kolenko, 1986. Phosphomonoesterase activity in forest soils. Soil Biol. Biochem., 18: 35-39.
12. Mearyard, B., 1999. Phosphate enzymes from plants. J. Biol. Educ., 33: 109-112.
13. Lal, L., 2002. Phosphatic Biofertilizers. Agrotech Publication Academy, Udaipur, India, Pages: 224.
14. Mohammadi, K., A. Ghalavand, M. Aghaalikhani, G.R. Heidari and Y. Sohrabi, 2011. Introducing a sustainable soil fertility system for chickpea (*Cicer arietinum* L.). Afr. J. Biotechnol., 10: 6011-6020.
15. Nautiyal, C.S., 1999. An efficient microbiological growth medium for screening phosphate solubilizing microorganisms. FEMS Microbiol. Lett., 170: 265-270.
16. Nuntagij, A., M. Abe, T. Uchiumi, Y. Seki, N. Boonkerd and S. Higashi, 1997. Characterization of *Bradyrhizobium* strains isolated from soybean cultivation in Thailand. J. Gen. Applied Microbiol., 43: 183-187.
17. Brosius, J., T.J. Dull, D.D. Sleeter and H.F. Noller, 1981. Gene organization and primary structure of a ribosomal RNA operon from *Escherichia coli*. J. Mol. Biol., 148: 107-127.

18. Altschul, S.F., T.L. Madden, A.A. Schaffer, J. Zhang, Z. Zhang, W. Miller and D.J. Lipman, 1997. Gapped BLAST and PSI-BLAST: A new generation of protein database search programs. Nucl. Acids Res., 25: 3389-3402.
19. Priyadarshan, P.M., 2011. Biology of Hevea Rubber. CABI, London, UK., ISBN-13: 9781845937133, Pages: 234.
20. Saengsanga, T., 2015. Biosynthesis of indole-3-acetic acid (IAA) of nitrogen fixing bacteria isolated from rubber tree *Hevea brasiliensis* Mull-Arg. Proceedings of the 7th National Science Research Conference, March 30-31, 2015, Naresuan University, Phitsanulok, Thailand.
21. Mujahid, T.S., S.A. Subhan, A. Wahab, J. Masnoon, N. Ahmed and T. Abbas, 2015. Effects of different physical and chemical parameters on phosphate solubilization activity of plant growth promoting bacteria isolated from indigenous soil. J. Pharm. Nutr. Sci., 5: 64-70.
22. Zahran, H.H., 1999. *Rhizobium*-legume symbiosis and nitrogen fixation under severe conditions and in an arid climate. Microbiol. Mol. Biol. Rev., 63: 968-989.
23. Sungthongwises, K., 2012. Phosphate-solubilizing bacteria: An alternative to increase availability of phosphorus for crop production. J. Acad. Serv. Khon Kaen Univ., 3-4: 15-24.
24. Liphadzi, M.S., M.B. Kirkham and G.M. Paulsen, 2006. Auxin-enhanced root growth for phytoremediation of sewage-sludge amended soil. Environ. Technol., 27: 695-704.
25. Datta, C. and P.S. Basu, 2000. Indole acetic acid production by a rhizobium species from root nodules of a leguminous shrub, cajanus cajan. Microbiol. Res., 155: 123-127.
26. Van Waasbergen, L.G., 2004. What Makes a Bacterial Species? When Molecular Sequence Data Are Used, Is rRNA Enough? In: Microbial Evolution: Gene Establishment, Survival and Exchange, Miller, R.V. and M.J. Day (Eds.). Chapter 21, ASM Press, Washington, DC., USA., ISBN-13: 9781555812713, pp: 339-356.
27. Mohite, B., 2013. Isolation and characterization of Indole Acetic Acid (IAA) producing bacteria from rhizospheric soil and its effect on plant growth. J. Soil Sci. Plant Nutr., 13: 638-649.