International Journal of Advance Research in Science and Engineering
Vol. No.5, Issue No. 05 , May 2016
www.ijarse.com
IJARSE
ISSN 2319 - 8354

ISOLATION AND SCREENING OF PHOSPHATE SOLUBLIZING BACTERIA FROM SOIL OF DIFFERENT GARDENS

Ms. Shilpi Damor[1], Dr. Poonam Dhawan[2]

[1] Assistant Professor, Department of Zoology, [2] Associate Professor, Department of Botany, Poddar International College, Mansarovar Jaipur (India)

ABSTRACT

Phosphorus is one of the major plant nutrients required in optimum amount for proper plant growth. Without adequate P, the yield cannot reach the maximum economic level. The inoculation of P-solubilizing microorganisms is a promising technique because it can increase P availability in soils fertilized with rock phosphates. Thus, in the present work an attempt was made to isolate and screen potential phosphate solubilizing bacteria (PSB) which are able to solubilize phosphate and can be used as biofertilzer in future. Among the isolates PSB5 gives maximum solublization zone (16 mm) followed by PSB6 (12 mm).

Keywords: Phorphorus, PVK Media, Halo Zone, Biofertilzer.

I. INTRODUCTION

Phosphorus plays an indispensable biochemical role in photosynthesis, respiration, energy storage and transfer, cell division, cell enlargement and several other processes in the living plant. An adequate supply of phosphorus in the early stages of plant growth promotes physiological functions including early root formation, and is important for laying down the primordia for reproductive parts of plants. It is vital to seed formation and its content is higher in seeds than in any other part of the plant. It helps plants to survive winter rigors and also contributes to disease resistance in some plants (Balamurugan *et al*., 2010). In India, majority of the phosphorus is provided in the form of chemical fertilizers, abundant use of which decreases the fertility of soil after long period of time. In nature, wide range of microbial biosolubilization mechanisms exist which are necessary to maintain global cycle (Whitelaw, 2000). Many microorganisms are able to solubilize unavailable forms of phosphates by excreting organic acids. These microbes are present in the different forms and numbers in the soil (Kucey, 1983). A large number of bacteria including species of *Pseudomonas, Azospirillum, Azotobacter, Klebsiella, Enterobacter, Alcaligenes, Arthrobacter, Burkholderia, Bacillus, Rhizobium* and *Serratia* have reported to enhance plant growth with their different plant growth promoting activities including phosphate solubilization (Kumar *et al*., 2012). Phosphate solubilizing microbes can also produce phosphatase enzyme which can benefit sustainable organic farming systems especially in coastal ecosystems, and reduce the utilization of agrochemicals in agricultural fields (Widawati, 2011).

II. MATERIALS AND METHODS

2.1 Soil sampling

Soil samples were collected from various gardens of Jaipur for the isolation of phosphate solubilising bacteria. The samples were air dried under shade and used for isolation and identification of organisms.

2.2 Isolation of Phosphate Solubilizing Bacteria (PSB) from Soil

Soil samples were prepared by inoculating 1.0gm of each soil into 100ml of sterile distilled water. Homogenization of soil was carried out by keeping it on shaker for 30 minutes at 120 rpm at 27ºC temperature. After 30 minutes, samples were centrifuged at 10,000 rpm for 15 minutes. 10ml of supernatant was inoculated into 100ml of sterile Pikovaskaya's broth and kept it at 120 rpm at room temperature for 3 days for enrichment. After 3 days, serial dilution of the enriched medium was carried out and aliquots of serially diluted soil samples were subjected to spread on sterile Pikovaskaya's agar medium (Ponmurugan and Gopi, 2006). These plates were kept at 27±2ºC for 24 to 48 hrs. Morphologically distinct colonies were picked and purified by streaking on nutrient agar. Each culture was maintained at 4ºC.

2.3 Screening of Potential Phosphate Solubilizing Bacteria

Each of the isolates was screened for their ability to solubilize calcium phosphate present in the Pikovaskaya's medium (Pikovaskya, 1948; Gupta *et al*., 1994). A loopful of pure culture was placed on the center of the same agar plates and incubated for 27±2ºC for 5 days. The solubilization zone was determined by subtracting the diameter of bacteria colony from the diameter of total zone.

2.4 Qualitative Estimation of soluble phosphate in Pikovaskaya's broth by measuring pH change

The isolates showing zone of solubilziation on Pikovaskaya's agar were further examined for their ability to release phosphate in broth media. Thus, 1.0ml of each isolates culture (18hrs old) was inoculated separately into 100ml of sterile Pikovaskaya's broth in the 250ml Erlenmeyer flasks. Each flask was incubated at 28±2°C at 120 rpm for 5 days. Simultaneously, the uninoculated control was also kept under similar conditions. To estimate the amount of phosphate released by the isolates, 10ml of each sample was withdrawn at regular intervals of 24 hrs and was examined for pH. The cultures were harvested by centrifugation at 10,000 rpm for 15 minutes. pH of thesupernatant was measured by pH electrode.

III. RESULTS

3.1 Isolation and Screening of phosphate solubilizing bacteria

Wide number of bacterial isolates was found from soil of different gardens (Table 1). Each of the isolates was purified successfully on nutrient agar media and maintained at 4ºC. On the basis of zone of solubilization in Pikovaskaya's agar medium, total 06 bacterial strains were screened for further studies. These data are represented in the table 2. Other isolates which shows hazy zone or without zone were not selected for further studies

TABLE 1: Description of bacterial isolates isolated from different areas.

Sr. No	Sample Numbers	Locations of rhizosphere soil	Number of Isolates
1	Sample 1	Garden soil of Poddar college	07
2	Sample 2	Society's garden soil	07
3	Sample 3	House garden soil	09

TABLE 2: Bacterial isolates showing zone of solubilization in Pikovaskaya's medium.

Sr. No.	Isolates Code	Zone of Solubilization (mm)
1	PSB1	6.0
2	PSB2	4.0
3	PSB3	8.0
4	PSB4	6.0
5	PSB5	18.0
6	PSB6	12.0

Sharp zone of solubilization was found among six isolates, ranging from 3.0 to 18.0 mm. Figure 1 shows maximum zone of solubilization with PSB5 (18mm) and PSB 6 (12 mm) within 48hrs. Other isolates showed the least solubilization zone. It was also observed that increase in the incubation time, increases the zone size of each isolates.

FIGURE 1: Bacterial isolates PSB5 and PSB6 shows maxiumum zone of solubilization on Pikovaskaya's Agar Medium.

3.2 Solubilization of phosphate in Pikovaskaya's broth

Measurement of the pH was also carried out every day. It was gradually decreased with the increased incubation time. After 72 hrs, pH was decreased between 5.0-4.0.

IV. DISCUSSION

The need of increased agricultural production has resulted in enhanced application of chemical fertilizers in an effort to maximize returns. One impact of this is the accumulation of large amount of insoluble phosphates in the soil due to the chemical fixation. Ever since Gerretson (1948) demonstrated the ability of microorganism to convert fixed phosphate in the soil in to available form, extensive research has gone into isolation of efficient organisms, understanding the biochemical basis and more recently molecular basis of this phenomenon to identify the microbes (Gerreston, 1948). Present study was aimed to screen soil bacteria which can solubilize complex form of metal compounds. In our study, total 48 isolates was found. On the basis of ability to grow and formation of halo zone on Pikovaskaya's agar medium, only 06 strains were selected for further studies. The role of phosphate solubilizing bacteria in making phosphorus available for plant is well known (Vikram *et al.*, 2007). Generally, the ability of solubilization depends upon the production of organic acids (Park *et al*., 2010). The ability of each PSB to quantitatively dissolve phosphate and thus change the pH of the liquid media was also measured upto 5 days. Decrease pH indicates that the isolates have ability to produce organic acids in the different concentration range (Jeon et al., 2003; Perez *et al*., 2007; Whitelaw *et al*., 1999; Ramachandran *et al*., 2007). Thus, among six isolates it might be possible that PSB5 and PSB6 producing more organic acids or having an effective phosphatase enzyme by which it solubilize maximum amount of phosphate. After 48 to 72 hrs because of high acidic pH, inhibition of bacteria was observed which finally affect the solubilization efficiency of the isolates. Thus, in the present study PSB5 and PSB6 found to be more efficient bacterial strains to solubilize the phosphate.

V. CONCLUSION

Our work suggested that many of the bacteria had P solubilizing properties suggesting the importance of preliminary screening *in vitro* for a wide range of bacteria to characterize their potent P-solubilizing or mineralizing trait.

REFERENCES

[1] Balamurugan *et al*. (2010). Isolation and characterization of phosphate solubilising bacteria in tea (*Camellia sinensis*) *J. Biosci. Res*., **1(4)**, 285-293.

[2] Gerretson, F. (1948). The influence of microorganisms on the phosphate intake by the plant. *Plant soil*. **1**, 51-81.

[3] Gupta, R., Rekha, S., Aparna and Kuhad, R.(1994). A modified plate assay for screening phosphate solubilizing microorganims. *J. Gen. Appl. Microbiol.* **40**, 255-260.

[4] Jeon, J., Lee, S., Kim, H., Ahn, T. and Song, H. (2003). Plant growth promotion in soil by some inoculated microorganisms. *J. Microbiol*. **41(4)**, 271-276.

[5] Kucey, R. M. N. (1983). Phosphate solubilizing bacteria and fungi in various cultivated and virgin Alberta soils. *Can. J. Soil. Sci*. **63**, 671-678.

[6] Kumar, A., Kumar, A., Devi, S., Patil, S., Chandani, P. and Nagi, S. (2012) Isolation, screening and characterization of bacteria from rhizospheric soils from different plant growth promotion activities: as in vitro study. *Recent research in science and technology.* **4(1)**, 01-05.

[7] Park, J., Bolen, N., Megharaj, M. and Naidu, R. (2010). Isolation of phosphate solubilizing bacteria and characterization of their effects on lead immobilization. *J. Hazard. Mater*. **185(2-3)**, 829-836.

[8] Perez, E., Sulbaran, M., Ball, M. and Yarzabal, L. (2007). Isolation and characterization of mineral phosphate solulibilizing bacteria naturally colonizing a limonitic crust in the south-eastern Venezuela region. *Soil Biol.Biochem*. **39**, 2905-2914

[9] Pikovaskya, R. (1948). Mobilization of phosphorus in soil in connection with vital activity of some microbial species. *Mikrobiologiya*. **17**, 362-370.

[10] Ponmurugan, P. and Gopi, C. (2006).Distribution pattern and screening of phosphate solubilizing bacteria isolated from different food and forage crops. *Journal of Agronomy*. **5(4)**, 600-604.

[11] Ramachandran, K., Srinivasan, V., Hamza, S. and Anadaraj, M. (2007). Phosphate solubilizing bacteria isolated from the rhizosphere soil and its growth promotion on black pepper (*Piper nigrum* L.) cutting. *Plant Soil Sci*. **102**, 325-331.

[12] Vikram, A., Alagawadi, A., Amzehzarghani H. and Krishnaraj, P. (2007). Factors related to the occurrence of phosphate solubilizing bacteria and their isolation invertisols. *Int. J. Agri. Res*. **2(7)**, 571-580.

[13] Whitelaw, M. (2000). Growth promotion of plants inoculated with phosphate solubilizing fungi. *Adv. Agron*. **69**, 99-151.

[14] Whitelaw, M., Harden, T. and Heylar, K. (1999). Phosphate solubilization in solution culture by the soil fungus *Penicillium radicum*. *Soil Biol. Biochem*. **31**, 655-665.

[15] Widawati, S. (2011). Diversity and phosphate solubilization by bacteria isolated from laki island coastal ecosystem. *Biodiversitas*. **12(01)**, 17-21.