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Research Paper

Evaluation of Phosphate Solubilizing Activity and Indole Acetic Acid Production of Rhizobia

Microbiology

KEYWORDS : stress, biofertilizers, surface charge, metal tolerance

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ABSTRACT

Rhizobia are a group of soil bacteria that fix nitrogen symbiotically with legumes.They are gram negative, motile rod shaped bacteria. In this study, attempts have been made to evaluate the potential of Rhizobium to be used as phosphate solubilizing bacteria along with indole acetic acid production. Ajmer being a semi arid zone of Rajasthan was selected for the study of these phosphate solublizer and indole acetic acid producer isolated from root nodules of Trigonella foenumgraecum and Tephrosia purpurea .Twenty bacterial forms were isolated and screened . Phosphate solubilization was detected both quantitatively as well as qualitatively and effects of Tryptophan on production of Indole acetic acid and the optimal incubation period were also determined. IAA produced by the Rhizobium could be used as sprays for the substitute of growth hormones produced by plants .Sixteen strains were found positive for phosphate solubilization and fourteen were positive for Indole acetic acid production with 150 µg/ml of Tryptophan for 72 hrs of incubation period. Thus, the potential capacity of present Rhizobial isolate in phosphate solubilization and Indole acetic acid production can be used as biological fertilizers and would play significant role in achieving sustainable agriculture and also maintain the eco-friendly environment as a substitute of chemical fertilizers . Application of such strains in the form of biofertilizers to nutrient deficient soil could be used as promising mode for the enrichment of nutrient deficient soils.

Introduction

Phosphorus (P) is the second major nutrient after nitrogen (N) that acts as limiting factor in the plant growth (Gyaneshwar et al., 2002 and Fernandez et al., 2007). While the availability of P for the plant is less due to various chemical reaction marked especially in semi arid and arid region (Mehrvarz and Chaichi., 2008).This inadequate status of P adversely affects the growth of plant in semi arid and arid regions. Microorganisms can play significant role in affecting the accessibility of soil P to plant roots and increasing P mobilization in P deficient soil. The P deficiency can reduce plant growth and yield, especially in leguminous plant, where both the plants and their symbiotic bacteria are affected, and this may have a harmful effect on nodule formation and their development. (Robson et al.,1981). Large amount of phosphorus in the form of chemical fertilizer is not accessible to the plants after its application to the soil.(Arpana et al., 2002).Microorganism in the form of biofertilizers is being used for the purpose of phosphate solubilization since 1950.(Krasilinikov,1957).Rhizobia solubilize organic (Abd-Alla, 1994) as well as inorganic phosphates (Antoun et al., 1998) The utmost advantage of application of Rhizobia as phosphate solubilizing microorganism will be their dual nutritional benefits due to P mobilization as well asN2-fixation (Peix et al., 2001). Other than biological nitrogen fixation and phosphorus solubilization Rhizobium also plays role in synthesis of phytohormones such as indole acetic acid (IAA) and gibberellins (Deshwal et al., 2003 and Mehrvarz and Chaichi., 2008).Phytohormone are the substances that stimulate the plant growth at very low concentration. They are essential for regulation of all the physiological and development process during the life cycle of plant. The IAA played significant role in plant development as a regulator of several biological processes starting from cell division, elongation and differentiation to fruit maturation and senescence (Normanly et al., 1993). Indole acetic acid is considered as physiologically active auxin and L-Tryptophan metabolised by plant growth-promoting bacteria (PGPR) to produce Indole acetic acid (Ahmad et al., 2005; Gosh and Basu, 2006 and Mandal et al., 2007).Rhizobia can produce detectable amount of IAA in free living conditions as well as symbiotically in nodules of host (Ernstsen et al., 1987).

Materials and Method

Rhizobium bacteria isolated from root nodules of Trigonella foenumgraecum and Tephrosia purpurea were screened for phosphate solubilization and indole acetic acid production

Qualitative phosphate solubilization

The 20 isolates of Rhizobia were spot inoculated on Pikovskayas agar plates (Glucose 10.0 gram, Tri calcium phosphate – 5.0 gm, Ammonium sulphate-0.50 gm, Potassium chloride-0.20 gm, Magnesium sulphate - 0.10 gm, Manganese Sulphate – 0.0001 gm, Ferrous Sulphate- 0.0001 gm , Yeast extract- 0.50 gm & Agar-25gms in 01 liter of distilled water.) The above said plates were incubated for 5-10 days. After incubation phosphate solubilization was detected by the formation of clear zone around colony formed by the isolates.

Quantitative phosphate solubilization

Phospho-molybedate blue colour method of Murphy & Riley, 1962 with some modification was used for determination of available phosphorous. Pikovskayas's broth (150ml) (adjusted to PH 7) with sucrose and Tricalcium phosphate (0.3g /100ml) was poured in 250ml flasks. The flasks were autoclaved at 121OC for 20 minutes. In each autoclaved flask, loop full of rhizobial strain were inoculated and kept at rotary shaker for 12 days. The suspension was then centrifuged (2000g, for 25 min,) to remove bacterial cells and other insoluble materials. The available phosphorous (P) was determined using spectrophotometer at 882nm and calibrated with standard KH2PO4 curve. The available phosphorous (P) was measured in the form of solublization of tri-calcium phosphate.

Determination of Indole Acetic

To study the effect of incubation period on IAA production, the YEM broth containing L-tryptophan was inoculated with Rhizobium strains and thereafter it was incubated at Ph 7 on a rotatory shaker at 200 rpm. The amount of IAA produced was estimated for 96 hrs using Salkowsky's reagent.

For optimization of concentration of L-tryptophan for maximum production of IAA by the Rhizobium strains, all the isolates were inoculated in flasks containing 100 ml of YEM broth supplemented with different concentrations of L-tryptophan ranging from 50-200 µg/ml. The inoculated flasks were incubated on rotatory shaker at 200 rpm for 7 hrs.which was used to determine IAA using Salkowsky's reagent

Estimation of IAA

For the estimation of IAA mannitol broth supplemented with 150µg/ml of tryptophan was incubated with rhizobium strain for 72 hrs. Then the cultures were centrifuged at 4000 rpm for 25 min and the cell free supernatant was used for IAA production. To the 10 ml of supernatant, 2 ml of Salkowsky's reagent

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was added and incubated for 30 min under darkness. The amount of IAA produced was estimated calorimetrically at 540 nm as described by Gorden and Weber, 1951.

Result and discussion

Phosphate solubilization

Studies carried out by earlier workers confirmed that the number of phosphate solubilizing bacteria showed promising role in dissolution of insoluble phosphate(Belimov et al., 1995 and Das and Mukherjee, 2000).Phosphate solubilizing microorganisms can improve the availability of P for plants by mineralizing organic phosphorus compound and by transformation of inorganic phosphorus into more usuable forms (Baryosef et al., 1999).In the present study ,it was observed that most of the rhizobial isolate have the availability to solubilize phosphate except the Strain No.RTF 4, RTF 9.RTP3.RTP10. Other than the four strains, remaining all 16 strains formed colonies with clear zones on agar plates indicating their ability to solubilize phosphate in range of 42 µg/ml to 81 µg /ml as shown in Figure1. Maximum solubilization was observed in RTF 7 i.e.81µg/ml. it was found that during the present study, most of the rhizobium posses the ability to solubilize the phosphorus along with N2 fixation, therefore these rhizobium could be used as multi strain inoculums for the production of biofertilizer as reported earlier by Gull et al., 2004; Hafez et al., 2002 and Bashan and Holguin , 1997

Indole acetic acid

Calorimetric measurement showed that fourteen Rhizobium strains produced indole acetic acid when inoculated with 50-200ug/ml of Tryptophan in broth. The amount of IAA produced varied from strain to strain and relatively more amount was observed in Rhizobium strains numbered as RTF 1, RTF3, RTP 6, RTP7 after 72 h of incubation as shown in Figure 2. Among these four strains, maximum amount of IAA was produced by RTP 6 i.e. 368 µg/ml after 72 hrs. Significant amount of IAA was detected within 24 hrs of incubation which reached its maximal value at 72 hrs and then decreased. Similar to the present study many researchers observed similar findings (Sarwar et al., 1992; Calamar and De Klerk, 2002; Sridevi and Mallaiah, 2007).The decrease in IAA concentration might be due to the action of IAA degrading enzymes for example IAA oxidases and peroxidases (Datta and Basu, 2000). For the maximum growth and IAA production was observed in all the fourteen strains at 150 µg/ml of tryptophan as shown in Figure 3. Maximum IAA production was observed in RTP 6. RTP 6 synthesized 258 µg/ml of IAA at 150 µg/ml of tryptophan. The decrease in IAA concentration at higher concentration of tryptophan might be due to dominance of catabolism over auxin production. Many earlier researchers have reported similar results in agreement to the present study who have demonstrated that rhizobia are capable of producing auxin and auxin biosynthesis increased many folds in Tryptophan supplemented medium (Bhattacharyya and Basu, 1997; Roy and Basu, 2004; Gosh and Basu, 2006; De and Basu, 2007; Sridevi and Mallaiah ,2008; Etesami et al., 2009).

Conclusion

Twenty bacterial isolates were studied, out of which Sixteen strains were found positive for phosphate solubilization and fourteen were positive for indole acetic acid production with 150 µg/ml of tryptophan for 72 hrs. of incubation .Nutrient deficiency is regular feature of semi arid region. Thus, the present study can play role in improvement of agricultural fields as the symbioses between Rhizobium and legumes are not only cheaper but also more effective agronomic practice for ensuring an adequate supply of N along with P. Indole acetic acid as phytohormone aids in plant growth and development. Thus, the potential capacity of present rhizobial isolate in phosphate solubilization and indole acetic acid production can be used as biological fertilizers and would play significant role in achieving sustainable agriculture.

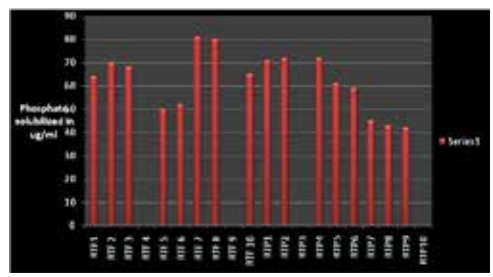

Figure 1 Phosphate solubilization patterns of rhizobial strains

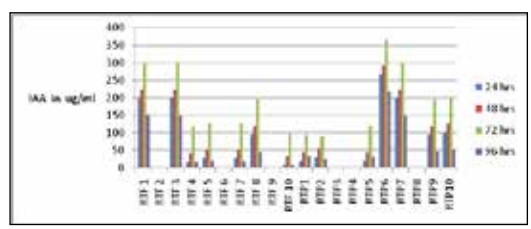

Figure 2 IAA production at different incubation period

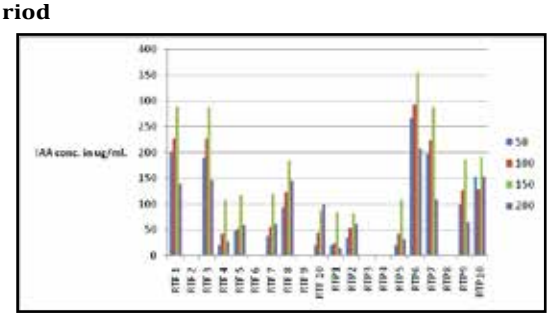

Figure 3 IAA Concentration at different concentration of tryptophan

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