Journal of Biotechnology and Crop Science
1(1): 33-37, 2012

Identification of phosphate solubilizing bacteria in different antagonistic bacterial strains

Anand Kumar, RC Ram, Rajesh Singh

Received: 18 March 2012 Revised Accepted: 10 April 2012

ABSTRACT

Mushroom cultivation represents, one of the economically viable processes for bioconversion of agricultural and industrial wastes into protein rich food making it a potent weapon against malnutrition in developing countries. Strains were identified *Pseudomonas, fluorescence*, according to the characteristics of its morphology and homology analysis (BLAST) of its 16S-r DNA sequencing. In the present investigation collect 54 strains of bacteria from different rhizospheric soil. In selective media identified all strains of bacteria were found *Actinomycetes,* fluorescence and phasphate solublizing. In the Phasphate solublizing bacteria some strain of bacteria (P_3, P_4), were found which produces black colour in the nutrient broth medium 13-15 days after incubation period. This black colour was found due to secretion of some chemicals by bacteria. The experiment helped in isolating different strains fluorescence, actinomycetes and phosphate solublizing bacteria in rhizospheric soil from selected plants of different regions to examine the antagonistic agent against *Pseudomonas agarici* and to characterize the antagonistic effect in biochemical.

Key Word: *Antagonestic agent, Blast, Mushroom, Phosphate solublizing bacteria, 16s-r DNA*

INTRODUCTION

Phosphate solubilizing bacteria (PSB) are the bacteria that possess the capability to change the insoluble form of phosphorus into soluble one. Phosphorus is one the most essential element for plant growth second only to nitrogen in requirement for plants. Phosphorus plays a significant role in physiological and biochemical plant activities. Most of the essential plant nutrients remaining insoluble form in soil (ABD-ALLA, M.H. 1994). Approximately 95– 99% of soil phosphorous is present in the form of insoluble phosphates and cannot be utilized by the plants (Vincent 1982). A greater portion of inorganic phosphates applied to soil as fertilizer is rapidly immobilized after application therefore, it becomes unavailable to plant. Thus, the insoluble and fixed form of phosphorous is released in order to increase the plants, allowing a Sustainable use of phosphate is fertilizers. PSB isolated from soils produce IAA, GA3 and cytokinin like substance, which ultimately enhance the plant metabolism. Soil phosphorous availability (Arpana *et al* 2002). Seed or soil inoculation with phosphate solubilizing bacteria known to improve solubilization of fixed soil phosphorus and applied phosphates resulting in higher crop yields (Yadav 1997). The best suitable pH for phosphorous uptake by plants is 6.5 this was indicated by (Malakooti 2000). PSB have been used to improve rock P value because they convert insoluble rock P into soluble forms available for plant growth (Nahas 1990). This conversion is through acidification, chelation and exchange reactions (Gerke L 1992). Solubilization of inorganic insoluble phosphate by microorganisms leads to the production of organic acids and chelating oxo acids from sugars. Several mechanisms like lowering of pH by acid production, ion chelation and exchange reactions in the growth environment have been reported to play a role in phosphate solubilization by phosphate solubilizing microorganisms (Halder 1991). PSMs

Anand Kumar (✉)
School of Biochemical Engineering IIIT BHU Varanasi
Email: anand9411@gmail.com

RC Ram
Mycology and Plant Pathology, IAS, BHU, Varanasi-221005

Rajesh Singh
Genetics and Plant Breeding, IAS, BHU, Varanasi-221005

play an important role in supplementing phosphorus to the production of IAA varies greatly among different crops and is also influenced by culture conditions, growth stage and availability of substrate (S). Bio-fertilizers (phosphate solubilizing bacteria) are, considered among the most effective plant assistants to supply phosphorus at a favorable level. These fertilizers are produced on the basis of selection of beneficial soil microorganisms which have the highest efficiency to enhance plant growth by providing nutrients in a readily absorbable form. Application of inoculants provided from these microorganisms enhances an abundant population of active and effective microorganisms to the root activity zone which increases plant ability to uptake more nutrients (Mehrvarz 2008). The growth of phosphate solubilizing bacteria depends on cultural activities and different soil properties such as physical and chemical properties, organic matter, and soil phosphorus content (Kim 1989).

MATERIALS AND METHODS

Collection of Rhizospheric bacteria: 27 strains of bacteria collected from the soil samples of Varanasi, Mirzapur, Allahabad, Bareilly, Lucknow region. Strains of bacteria characterized by growing on the selective media as actinomycetes for actinomycetes bacterial, Pikovaskya for Phosphate solublizing bacteria and king's B media for fluorescence bacteria. After growing on selective media 12 strains were found actinomycetes, 9 strains fluorescence and the rest were phosphate solublizing bacteria.

Collection of strains: Strains of bacteria from rhizospheric soil isolated by a serial dilution method from eastern and western region of Uttar Pradesh.

Isolation and Characterization of Phosphate Solubilizing Bacteria (Qualitative estimation): Bacteria representative of the predominant morphological types present on the plates were selected at random and purified on minimal medium(Nautiyal 1999). National Botanical Research Institute's phosphate growth medium (NBRIP) and Pikovskaya (Pikovskaya 1948) medium (PVK), was developed for isolation and identification of phosphate solubilizing microorganisms.The composition of the media was shown in the Table 1. Phosphate solubilisation test was conducted qualitatively by plating the bacteria in Pikovaskaya agar media and NBRIP media. PSB was grown on Pikovskaya agar media (Gaur 1981) diluted in distilled water. The pH of the media was adjusted to 7.0 before autoclaving. Bacterial strains were tested by plate assay using PVK and NBRIP. Four strains per plate were stabbed in triplicate using sterile toothpicks. The halo and colony diameters were measured after 14 days of the incubation of plates at 28ºC. Colonies of PSB were detected by clear zones of solubilization around them. The isolates were identified following Bergey's manual for bacteriology methods systematic (Krieg 1984).

Antagonistic Screening of rhizospheric Bacteria against Pathogenic bacteria: Diffusion were applied for screening of antagonistic bacteria against pathogen. Poured the 25 ml sterilized medium in sterilized petri plate. Incubate the plate for semi solidification for 30 minutes. Make a well in the center and 4 well around the center, drop the pathogenic broth culture in the central well and rhizospheric broth culture in another two well. Incubate for 24 hours.

RESULTS AND DISCUSSION

Pathogenic bacteria isolated from diseased mushroom, the growth rate was very fast as within 12 hrs fill the whole petriplate, secrete some yellowish substances around the colony. Then follow Kotch postulate and reinfect the pure mushroom and consequently diseased occurred on mushroom. During gram's staining it was found gram's positive.

Phosphate solublization: Phosphate solublization determine by biochemical test. P_1, P_2, P_3, P_4, P_5 strains of bacteria isolated from Brassica of Allahabad were found phosphate solublizing bacteria.P_6,P_7,P_8,P_9 isolated from wheat Mirzapur also found phosphate solublizing bacteria. P_9,P_{10},P_{11},P_{12},P_{13},P_{14} strains of bacteria isolated from maize in Varanasi were found

Table 1 Rhizospheric Bacteria showing phosphate solublizing activity, *Actenomycetes* and fluorescent activity.

Strain	Soil sample	Region	Phosphate solublizatio	Actinomycetes
A1	Rose	Bareilly	-Ve	-Ve
A2	Rose	Barielly	-Ve	-Ve
A3	Rose	Maize	-Ve	-Ve
A4	Rose	Maize	-Ve	-Ve
A5	Rose	Lucknow	-Ve	-Ve
A6	Rose	Lucknow	-Ve	-Ve
A7	Rose	Lucknow	-Ve	-Ve
A8	Wheat	Mirzapur	-Ve	-Ve
A9	Wheat	Mirzapur	-Ve	-Ve
A10	Wheat	Mirzapur	-Ve	-Ve
A11	Maize	Varanasi	-Ve	-Ve
A12	Maize	Varanasi	-Ve	-Ve
A13	Maize	Varanasi	-Ve	-Ve
A14	Parthenium	Allahabad	-Ve	-Ve
A15	Parthenium	Allahabad	-Ve	-Ve
A16	Parthenium	Allahabad	-Ve	-Ve
A17	Parthenium	Allahabad	-Ve	-Ve
A18	Parthenium	Allahabad	-Ve	-Ve
K1	Rose	BHU	-Ve	-Ve
K2	Rose	BHU	-Ve	-Ve
K3	Rose	BHU	-Ve	-Ve
K4	Rose	BHU	-Ve	-Ve
K5	Rose	BHU	-Ve	-Ve
K6	Rose	BHU	-Ve	-Ve
K7	Rose	BHU	-Ve	-Ve
K8	Rose	BHU	-Ve	-Ve
K9	Rose	BHU	-Ve	-Ve
K10	Kamini	Mirzapur	-Ve	-Ve
K11	Kamini	Mirzapur	-Ve	-Ve
K12	Kamini	Mirzapur	-Ve	-Ve
K13	Kamini	Mirzapur	-Ve	-Ve
K14	Kamini	Mirzapur	-Ve	-Ve
K15	Kamini	Mirzapur	-Ve	-Ve
K16	Brassica	Bareilly	-Ve	-Ve
K17	Brassica	Bareilly	-Ve	-Ve
K18	Brassica	Bareilly	-Ve	-Ve
K19	Brassica	Bareilly	-Ve	-Ve
P1-P5	Brassica	Allahabad	+Ve	-Ve
P2	Brassica	Allahabad	+Ve	-Ve
P3	Brassica	Allahabad	+Ve	-Ve
P4	Brassica	Allahabad	+Ve	-Ve
P5	Brassica	Allahabad	+Ve	-Ve
P6-P9	Wheat	Mirzapur	+Ve	-Ve
P8	Wheat	Mirzapur	+Ve	-Ve
P9	Wheat	Mirzapur	+Ve	-Ve
P10	Wheat	Mirzapur	+Ve	-Ve
P11	Maize	Varanasi	+Ve	-Ve
P12	Maize	Varanasi	+Ve	-Ve
P13	Maize	Varanasi	+Ve	-Ve
P14	Maize	Varanasi	+Ve	-Ve
P15-P18	Wheat	Bareilly	-Ve	+Ve
P16	Wheat	Bareilly	-Ve	+Ve
P17	Wheat	Bareilly	-Ve	+Ve
P18	Wheat	Bareilly	-Ve	+Ve

phosphate solublizing bacteria. P_{15},P_{16},P_{17} strains isolated from wheat of Bareilly were found strong phosphate solublizing bacteria. Out of 54 Samples, only one isolate exhibiting halozone was found showing the capability of P solubilization was obtained from graveyard sample of 10^{-6} dilution

(Yahya 1999). This Isolate had the morphological features like colorless colonies which did not produce pigment, cells were gram negative, rod shaped and on the basis of biochemical reactions it was found to be *Pseudomonas fluorescens*. Upon gram staining, it was found as gram negative characteristics. Isolate produced slimy, white colonies with irregular margins, cells were gram positive and on the basis of biochemical reactions this isolate was identified as *Bacillus megaterium*.

Actinomycetes and fluorescence: A1, A2,A3, A4,A5,A6,A7 strains of bacteria that isolated from the Bareily and Lucknow were found *Actinomycetes*. Some strains A_8, A_9, A_{10} isolated from wheat of Mirzapur were also having *Actinomycetes*. The strains of Varanasi (A_{11}, A_{12} and A_{13}) that isolated from maize were *Actinomycetes*. Strains of Allahabad (A_{14}, A_{15}, A_{16}, A_{17} and A_{18}) that isolated from *Parthenium* were having *Actinomycetes*.

The most dominant phosphate solubilizing bacteria found were aerobic and of which, some are spore forming bacteria. Identification of this group showed that *Bacillus sp*, was the most predominant PSB was found in all of soils tested, followed by *B. cereus* and *B. subtilis* were in soil samples numbers 2, 3,5,12,13 and 15 respectively as shown in the Table 1. Other PSB involved were *Klebsiella sp, K Aerogenes, K Pneumoniae, Proteus sp, P. mirabilis, P. vulgaris, P. inconstans, Enterobacter sp., E aerogens* and *Pseudomonas sp*, Sporeformers were well known to resist adverse conditions such as high temperature and dryness this was observed by Taha *et al* (Taha 1969). Thus the important PSB can overcome such unfavorable conditions.

The present investigation was carried out to study the occurrence of PSB. The isolated microbes were identified, screened and characterized. Most of the bacteria were isolated from soil samples with pH values close to 7 and 8. There is a close relationship between the phosphate solubilizing activity and low pH levels in the growth medium. This suggests that phosphate solubilization could be the result of organic acids released from bacterial metabolism, as reported in literature.

REFERENCES

ABD-ALLA MH (1994) Phosphatases and the utilization of organic phosphorus by Rhizobium leguminosarum biovar viceae. Lett Appl Microbiol 18: 294-296.

Arpana N, Kumar SD, Prasad TN (2002) Effect of seed inoculation, fertility and irrigation on uptake of major nutrients and soil fertility status after harvest of late sown lentil. Journal of Applied Biology 12(1/2): 23-26.

Nautiyal CS (1999) An efficient microbiological growth medium for screening phosphate-solubilizing microorganisms. FEMS Microbiology Letters 170: 265-270.

Gaur AC (1981) Phospho microorganism and various transformation incompost technology, Project Field Document no 13 Rome FAO.

Gerke L (1992) Phosphate, aluminum, and iron in the soil solution of three different soils in relation to varying concentrations of citric acid. Z. Pfl.-Ernähr. Bodenkde 155: 17–22.

Halder AK, Mishra AK, Chakarbarthy PK (1991) Solubilization of inorganic phosphate by Bradyrhizobium. Ind J ExpBiol 29: 28-31.

KIM KY, Jordan D, Mcdonald GA (1989) Effect of phosphatesolubilizing bacteria (PSB) and VAM on tomato growth and soil microbial activities. Biology of fertility Soils 26: 79-87.

Krieg NR, HOLT JG (1984) Bergey's Manual of Systematic Bacteriology. Vol 1. Baltimore: Williams and Wilkins.

Mehrvarz S, Chaichi MR (2008) Effect of phosphate solubilizing microorganisms and phosphorus chemical fertilizer on forage and grain quality of barely (*Hordeum vulgare* L.). American-Eurasian J Agric Environ Sci 3(6): 855-860.

Malakooti MJ, Nafisi M, Malakooti MJ (2000) sustainable agriculture cultivars and yield increment by optimum fertilizer utilization in Iran. 2nd edition. Agricultural Extension Publications Iran.

Nahas E, Banzatto DA, Assis LC (1990) Fluorapatite solubilization by *Aspergillus niger* in vinasse medium. Soil Biol Biochem 22: 1097–1101

Pikovskaya RI (1948) Mobilization of phosphorus in soil in connection with the vital activity of some microbial species. Mikrobiologiya 17: 362-370.

Vincent JM (1982) Enumeration and determination of growth. In Vincent, J.M. (Ed.). Nitrogen Fixation in Legumes. Sidney: Academic Press.

Yadav KS, Dadarwal KR (1997) Phosphate solubilization and mobilization through soil microorganisms. In Biotechnological approaches in soil microorganisms for sustainable crop production (Dadarwal, K.R., Ed.). Scientific Publishers Jodhpur pp 293-308.