Int J Pharm Bio Sci 2015 Oct; 6(4): (B) 561 - 565

Research Article **Biotechnology**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

EFFECT OF COCONUT HUSK ON PHOSHATE SOLUBILIZING BACTERIA AND ITS CARRIER BASED INOCULANTS FOR GROWTH OF LEGUMES

S.ANBUSELVI* AND GUDDU KUMAR

Department of Industrial Biotechnology, Bharath University, Chennai-73.

ABSTRACT

Bio fertilizers are also known as microbial inoculants or bio inoculants. It is a product of selected, beneficial and live microorganism which help to improve plant growth and productivity or, mainly through supply of plant nutrients. Phosphate solubilizing organisms play an important role in growth of the plants. The bio inoculants improve the shelf live and survivability of microbes in soil and stimulate the growth of the plants. The present study was to extract the phosphate solubilizing bacteria from coconut husk and its formulation in growth of pulses.

KEYWORDS: **PSB, Coconut husk, Biofertilizer, Bioinoculants**

S.ANBUSELVI
Department of Industrial Biotechnology, Bharath University, Chennai-73

INTRODUCTION

Plants can absorb phosphate only in soluble form. The transformation of insoluble phosphate into soluble from is carried out by a number of microbes present in the soil. A large number of soil microbes can dissolve inorganic phosphate present in the soil and convert them available to the plant[1]. Phosphorus (P) is sequestered by adsorption to the soil surface and precipitation reaction with soil cations, particularly iron, aluminums and calcium. A large amount of Phosphate fertilizer has been used to increase plant growth, which is likely to cause negative impact in respect to both environment and economy[2].. Phosphate Solubilising bacteria helps to enhance the solubilization of insoluble phosphate compounds through the release of organic acids and phosphates enzymes. In plants phosphorus increase the strength of cereal straw, promotes flower formation and fruit production, stimulated root development and also essential for seed formation. Phosphate enriched fertilizers may improve the quality of fruits, vegetable and grain crops and increase their resistance to diseases and adverse conditions. It is essential for the development of meristematic tissues, in stimulation of early root growth and plant maturity[3]. High concentration of PSM is accumulated in the rhizosphere, and they are metabolically more active than from other sources. Bacilli are very common in soil, where spirally are very rare in natural environment. The PSB are found to be variation in forms and population in different soils. Population of PSB depends on different soil properties (physical and chemical properties, organic matter, and P content) and cultural activities[4]. Phosphate solubilization takes place through various microbial processes including organic acid production and proton extrusion. The PSB dissolves the soil phosphate through production of low molecular weight organic acids mainly gluconic and keto gluconic acids .This leads to lower the pH of rhizophere is lowered through biotical production proton / bicarbonate release.[5] Use of PSM based biofertilizers can increase crop yields up to 70 % combined inoculation of arbuscular mycorrhizae and PSB shows better uptake of both native phosphate in the soil as well as in the phosphatic rock. Higher crop yields result from solubilization of fixed soil phosphate and applied phosphate by PSB. Microorganisms with phosphate solubilizing potential increase availability of soluble phosphate and stimulate the plant growth by improving biological nitrogen fixation[6]. Bio-fertilizers manufactured in India presently are carrier based; in general, it showed short shelf life, poor, quality, high contamination and unpredictable field performance. Death of the organisms in the inoculated seed is one of the important factors contributing the failure of inoculation response in field's condition. In order to reduce the problems, PSB based inoculants are given in the form of biofertilizers to improve the yield of the food grains. The present study deals with the isolation of phosphate solubilising bacteria from the fresh coconut husk and formulation of PSB based biofertilizer with coconut waste.

MATERIALS AND METHODS

Fresh coconut husk, rhizosphere and soil of coconut tree were collected. Samples were air dried powdered and physicochemical properties like pH, moisture, bulk density, water holding capacity and NPK content of the soil were analyzed. 10gm of soil sample was dissolved in 100ml distilled water sterilized water and mix the sample well and considered the diluted the soil sample in sterilized distilled water up to 10^{-7} dilution (each test tube containing 9ml of sterilized distilled water) then 10^{-5} 10^{-6} 10^{-7} dilutions taken for spread plate technique. Sterilized nutrient agar prepared and poured into petridishes after solidification of the medium 0.1MLsample was poured into agar medium plate by using L-Rod spread the sample evenly over the agar surface and then incubated at 37^{0} C for 24 hours.

Isolation of PSB

Pikovskaya's agar medium was found to be as selective media for the isolation of phosphate solubilising bacteria. The composition of pikovskaya medium was maintained in[11]. The sterilized pikovskaya medium was prepared

and poured into Petri dishes .After solidification of the medium, 0.1ml sample was poured into agar medium plate by using L-Rod spread the sample evenly over the agar surface and then incubated at 37^0 C for 24 hours[7].

Detection of PSB

0.1 ml of PSB were isolated from each sample was subjected into pikovskaya agar medium containing insoluble tri calcium phosphate and incubated at 27-30ºC for 7 days. Insoluble tri calcium phosphate is present in the medium used for halo zone formation at 37^oC in two weeks. The morphological features of isolated bacteria via shape, size elevation, surface margins, surface texture and color were observed [8] and characterized by gram staining.

Solubilisation Index

10 ml of each PSB culture was preserved in sterile distilled water. This was placed in pikovskaya agar medium at 28ºC for seven days. Solubilisation index was measured by Edi-premono [9].

Physical parameters of soil

The pH, moisture, bulk density, specific gravity and water holding capacity of the soil, rhizoshere and coconut husk were analyzed. The nitrogen, phosphorus and nitrogen content of the samples were qualitatively assessed by soil testing kit.

Preparation of liquid inoculants

3The pikovskaya's broth incubated with water in a 250ml conical flask. It was allowed to multiply by incubating at 32ºC in an incubator cum shaker at 100 rpm for 72 hours. The broth containing approximately $25x10^{11}$ cfu/ ml was used as a starter culture for the production of liquid inoculants. This can be used as nutrient enhancer for growth of plants.

Table 1
Physicochemical properties of samples for isolation of PSB

S.No	Types of samples	pH	Bulk density (g/cm^3)	Specific gravity (g/cm^3)	Moisture content (%)	Water holding capacity (%)	N	P	K
1	Rhizosphere	6.8	0.0117	0.19	67	50	H	M	H
2	fertile soil	7.5	0.0119	0.02	30	23	L	L	H
3	Coconut husk	7.7	0.128	0.08	45	76	M	M	M
4	Non fertile soil	7.0	0.0105	0.01	10	18	L	L	L

H=High; M=Medium; L=Low

Table 2
Phosphate solubilizing efficiency on Bacillus and Pseudomonas

S.NO	Carriers	5 days of incubation	10 days of incubation
1	Coal dust+*Pseudomonas*	2 mm	5mm
2	Coconut husk+ *Pseudomonas*	3mm	7mm
3	*Pseudomonas* liquid inoculant	8mm	12.6 mm
4	Coal dust+ *Bacillus*	1.5 mm	3mm
5	Coconut husk+ *Bacillus*	2.3mm	6.8 mm
6	*Bacillus* liquid inoculant	7.4 mm	11.5 mm

Figure 1
Detection of phosphate solubilizing bacteria

RESULTS AND DISCUSSION

The physicochemical characteristics of rhizosphere, soil and coconut husk were analyzed. Coconut showed high amount of water holding capacity and good translocation of nutrients to the plants.(anbuselvi). The physicochemical properties of samples were determined in Table 1. The phosphate solubilizing bacteria were isolated from samples using pikovskaya medium. Out of three samples the rhizosphere of coconut tree and coconut husk exhibit halozone formation (Fig1). This was due to phosphate solubilization by PSB and fungi with production of organic and inorganic acids. PSB showed high production of acids with hydroxyl and carbonyl groups. This was lead to chelate the cations and decrease the pH in the basic soil. The soil produced low molecular organic acids of gluconic and ketogluconic acids[10].The bacterial isolate from rhizosphere of coconut tree had colorless colonies which belonged to gram negative, rod shaped and on the basis of biochemical reactions it was found to be *Pseudomonas*. The isolate extracted from coconut husk showed slimy white colonies with irregular margins. Cells were gram positive and on the basis of biochemical reactions, identified as *Bacillus*. The pikovskaya medium is a selection medium for Phosphate Solubilizing Microbes which contain tricalcium phosphate[11]. The isolated PSM were used as bioinoculants for the growth of green gram plants. The phosphate solubilizing efficiency of PSM can be checked by using different formulations such as carrier based coal dust, coconut husk and liquid formulations on the production of clear zones. (Table2). Pseudomonas was maintained in all carriers tested up to 2 months of storage period without reduction. Liquid inoculants of pseudomonas showed a maximum population of $26.5x10^7$. Cfu/ml and Bacillus had microbial population of $24.35x10^6$ cfu/ml. Thus bioinoculants were used as biofertilizer for growth of plants. Alternate to peat, coconut husk and coal dust were used as carriers. The carrier material used for inoculant preparation have high organic matter, water holding capacity and natural pH for the survival of the organism[12]. .According to Kandasamy and Prasad also recommended that carrier mediated as well as liquid inoculants showed better seed germination, root and shoot growth etc., Pseudomonas spp have improved the plant growth and seedling vigour via high production of aminoacids and indole acetic acid[13,14]. The rapid growth was observed in coconut husk treated plants and minimal growth was found in untreated and coal dust treated plants. The nitrogen and phosphorus content of the soil after plantation were gradually increased and maintained the nutrients in soil.

CONCLUSION

Microorganisms with plant growth promoters have been used to produce inoculants coconut

husk material are able to support good growth and survival of bacteria in soil. The agronomic use of agrowaste as substrates causes changes in the soil affecting the physicochemical characteristics and microbial activity in rhizosphere. The breakdown of such materials to simple sugars which provide energy sources for heterotrophic microorganisms such as p-solubilizing and nitrogen fixing bacteria. From this study, coconut husk waste will be a good carrier material for the inoculants of phosphate solubilizing bacteria.

REFERENCES

1. Chabot RH, Antoun and Cescas MP, Stimulation of growth of maize and lettuce by inorganic phosphate solubilising microorganisms, Canadian. J. Micro.Biol,39: 941-947(1993).
2. Bisen PS and Verma K, In Handbook of Microbiology, CBS Publishers and distributors, 130-138(2012).
3. Goldstein AH, Bacterial phosphate solubilisation, Historical perspective and future prospects, Am.J.Alt.Agri,1,:57-65(1996).
4. Guar AC, Phosphate solubilising microbes as biofertilizers,Omega scientific publishers, New Delhi,76,(1990).
5. Nahas E, Factors determining rock phosphate solubilisation by microorganisms isolated from soil, World .J.Microbiol.Biotechnol.,12:,18-23(1996).
6. Kannaiyans S, Kumar K and Govindarajan, Role of phosphate solubilising microbes in farming, Scientific pub,Jodhpur,35-38(2004).
7. Pikovskaya RI, Mobilization of phosphorus in soil in connection with vital activity of some microbial species. Microbiologiya, 17:362-370(1948).
8. Lal L, Role of phosphate solubilising bacteria in agriculture, In,Agritech.pub.Academy, Udaipur,224(2002).
9. Edi;Premona MA, Moawa and PLG Vleck, Effect of phosphate solubilising bacteria on the growth of maize and its survival in rhizosphere, Indonesian J of crop science,11:13-23(1996).
10. Sonam Sharma, Vijay kumar and Ram babu Tripathy, Isolation of phosphate solubilizing microorganism from soil, J.Microbiol.Biotech.Res, 11(2):90-95 (2011).
11. Sharma K, Lab manual of Microbiology, Isolation, purification and identification of bacteria, Ane books publishers, 41(2005).
12. Tilak KVBR, Subba Rao, NS, Carriers for legume inoculants, Fer.News, 23:25-28(1978).
13. Kandasamy R and Prasad NN, Lignite as a carrier of rhzobia, Current Science, 40:496(1971).
14. Mugilan I, Gayathri P, Elumalai EK and R Elango, Studies on improve survivability and shelf life of carrier using liquid inoculantion of *Pseudomonas striata*, Inter.J.Pharmaceutical and biological Arch.,2(4):1271-1275(2011).