PUBLISHED ON 29TH FEB 2012



ENHANCED GROWTH OF *VIGNA ACONITIFOLIA* MEDIATED BY *PSEUDOMONAS* SPP., AN EFFICIENT PHOSPHATE SOLUBILIZ ER A.P. PATHAK *, AMIT KULKARNI , A.G. SARDAR AND S. MOHAN KARUPPAYIL

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ABSTRACT:

Fifteen phosphate solubilising bacteria (PSB) were isolated from rhizosphere of various crop plants. Efficiency of phosphate solubilisation was determined to select most efficient phosphate solubiliser amongst these isolates. Highest phosphate solubilisation was observed by isolate PSB1 and PSB2. Morphological and biochemical characterisation of isolates was carried out and both were identified as *Pseudomonas spp1*. Effect of PSB 1 was determined on percent seed germination, development of radical, plumule and foliage growth of Vigna *aconitifolia*. 20 % rise in seed germination and three fold enhancements in overall growth were observed in *Vigna aconitifolia* plants, treated with our inoculum.

KEY WORDS: PSB, Vigna aconitifolia, PGPR, Bioinoculum.

INTRODUCTION:

Phosphorus is one of the major essential macronutrient for plants are applied to soli in the form of phosphate fertilizers. However a large portion of soluble inorganic phosphate applied to the soil as chemical fertilizer is immobilized rapidly and becomes unavailable to plant. (Chen et al 2006). Fortunately a considerable number of bacterial species are able to solubilize insoluble inorganic phosphate in to soluble form that plants can absorb. Mostly these organisms are associated with plant rhizoshpere and called as rhizobacteria. These are also called as plant growth promoting bacteria that include few genera such as Pseudomonas, Erwinia, Berkholderia, Rizobium, Bacillus etc. Organic acids like gluconic and 2-ketogluconic synthesized by these soil microorganism results in solubilization of immobilized phosphorus. Insoluble phosphorus can also be solubilized by enzyme phosphatase secreted by PGPRs. In recent years a number of studies have been conducted to isolate PGPRS from soil and different habitats. In our investigation we report the isolation, selection and characterization of efficient phosphate solubilizer (PSB-1) and we also report its effect on germination of *Vigna aconitifolia* seeds and its foliage growth.

MATERIALS AND METHODS:

Sample collection, isolation and screening

Soil samples were collected from university garden from ten different sites. All samples were mixed in equal proportion and used for isolation of Phosphate solubilizing bacteria. The serially diluted soil samples were plated on Pikovskya's medium (Pikovskaya, R.I 1948) and incubated at 30°C for three days. Typical colonies with clear zone were selected for further investigation. Out of many being a most efficient PSB1 was selected as a bioinoculum candidate for pot experiments and germination experiments. PSB1 was identified using morphological and biochemical characterization methods before use.

Fifty seeds of *Vigna aconitifolia* were soaked in freshly prepared bioinoculum for six hours and rate of germination were compared with seeds of blank set soaked in distilled water. In pot trials sterile soil was used to fill up pots and after sowing *Vigna aconitifolia* seeds (Fifty seeds per pot) bioinoculum was spread thrice in a week. Blank set of pots was treated with distilled water. Foliage growth, number of leaves appeared were counted and recorded.

RESULTS AND DISCUSSION:

The temperature of soil sample was 30°C and pH of soil sample was 7.2. Collected soil samples were serially diluted and used for isolation of efficient phosphate solubilizer. After incubation of 72 hours Pikovsky's plates showed countable number of colonies for 10⁻³, 10⁻⁴ and 10⁻⁵ dilutions. Fourty percent of colonies showed zone of clearesence and 15 out of that were selected and isolated. Most efficient phosphate solubilization was carried out by PSB-1 and hence it was selected for further investigation and characterized. PSB1 is gram negative nonspore forming short rod catalase and indole production was not recorded. Glucose, lactose, Xylose and arabinose were utilized as carbon source with acid and gas formation by PSB 1. Amylase and gelatinase production was not recorded. The characteristics of isolate PSB1 are listed in Table 1. PSB 1 was identified as *Pseudomonas spp.* by comparing its characteristics with reference strain given in Berguys manual of systematic bacteriology. (Peter H.A. et al 1985)

Bioinoculum was prepared freshly by inoculating PSB1 in saline (50 cfu/ml) and used.

Effect of bioinoculum on seed germination was observed after 24 hours. Number of seeds germinated were counted and compared with blank. 20% rise in germination rate was recorded in bioinoculum treated seeds against blank. Effect of bioinoculum on foliage growth was also studied it showed three fold rise in foliage growth as compared to blank. Effect of bioinoculum on seed germination and foliage growth is recorded in table No.2.

Table 1: Biochemical characterization of PSB1

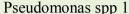
Characteristics	PSB1	
Morphology	Rods	
Gram nature	negative	
Motility	motile	
Colony pigmentation	White	
Temp. optimum ⁰ C	30^{0} C	
PH Optimum	7.2	
Utilization of sugar		
Glucose	+	
Fructose	+	
Xylose	+	
Arabinose	+	
Sucrose	+	
Lactose	+	
Maltose	-	
Indol production test	-	
Vogues-Proskauer test	-	
Methyl Red test	+	
Catalase	-	
Amylase	-	
Gelatinase	-	
Identified as	Pseudomonas Spp	

Table 2: Effect of PSB 1 on germination of Vigna aconitifolia (after 24 hours)

Seeds treated with	Seeds germinated out of 50	
Bioinoculum (PSB1)	41.6±2.88	
D/w	30±0.0	

Table 3: Effect of PSB1 on Foliage growth of Vigna aconitifolia (after 7days)

Seeds treated with	Root	Shoot	Number of Leaves
Bioinoculum (PSB1)	5cm	6cm	2each
D/w	1.5cm	1.5cm	1each





Effect of PSB 1 on Seed germination

CONCLUSION:

The present report provides adequate information about the PSB1 isolated by us as well as its effect on germination rate and foliage growth of *Vigna aconitifolia*. The study reflects potential of PSB1 as bioinoculum with reference to phosphate solubilization.

AKNOWLEDGEMENT:

Hon. Vice Chancellor Dr. S. B. Nimse is gratefully acknowledged for providing infrastructure facility.

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