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Solubilization of Tricalcium Phosphate and Production of IAA by Phosphate Solubilizing Bacteria Isolated from Tea Rhizosphere Soil

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Abstract

Rhizospheric soil from tea [*Camellia sinensis* L.] was screened for the presence of phosphate solubilizing bacterial populationin-vitro where eight isolates were able to solubilize tri calcium phosphate in Pikovskaya's agar. These isolates were also screened for phosphate solubilization in liquid medium. Phosphate solubilizing activities of these strains were associated with a drop in the pH of the medium. Furthermore, these 8 isolated strains were inoculated in specific media containing tryptophan to produce growth regulating substances indole acetic acid (IAA) under in-vitro conditions. Amount of phosphate solubilized ranged from 11.07±0.91-82.77±0.96mg/l and IAA production ranged from 11.23-28.78 mg/l. These bacterial strains may be further characterized and field tested for their use as effective growth promoters for hill crops.

Keywords: Tea, phosphate solubilizing bacteria, IAA

After nitrogen phosphorus (P) is the major plant growth-limiting nutrient despite being abundant in soils in both inorganic and organic forms. Even, the plant available free Pconcentration in fertile soil generally do not exceed 10 μM even at pH 6.5 where it is most soluble (Gyaneshwar *et al.* 2002). A greater part of soil P, approximately 95-99% is present as insoluble phosphates and hence cannot be utilized by the plants (Vassileva *et al.* 1998). Phosphate solubilizing bacteria (phosphorbacteriaor PSB) possess the ability to solubilize insoluble inorganic P and make it available to plants. The solubilization effect is generally due to the production of organic acids (Ponmurugan and Gopi, 2006) which lower the soil pH to bring about the dissolution of bound forms of P. Although it is not the only way by which P is solubilized (De Freitas *et al.* 1997; Kim *et al.* 1997).

Venkateswarlu *et al.* (1984) have reported that during thesolubilization of rock phosphate by fungi, the pH of theculture was lowered from 7 to 3. Several mechanisms likelowering of pH by acid production, ion chelation andexchange reactions in the growth environment have beenreported to play a role in P solubilization byphosphate solubilising micro-organisms (PSM) (Cunningham and Kuiack, 1992; Yadav *et al.* 1997).Although the mechanisms by which plant growthpromoting rhizobacteria (PGPR) promote plant growth arenot yet fully understood, many different

traits of thesebacteria are responsible for growth promotion activities (Cattelan *et al.* 1999). It includes the ability to produce or change theconcentration of the plant hormones like indoleacetic acid (IAA), gibberellic acid, cytokinins, and ethylene; fixing dinitrogen; suppress the growth of deleteriousmicroorganisms by production of siderophore, β-1, 3-glucanase, chitinases, antibiotics, and cyanide.

IAA produced by bacteria improves plant growth byincreasing the number of root hairs and lateral roots (Okon and Kapulnik 1986). Microbial biosynthesis of IAA in soil is enhanced bytryptophan from root exudates or decaying cells (Benizri *et al.* 1998; Frankenberger and Arshad 1991). Tea (*Camellia sinensis)* is regarded as an importantplantation crop of very high economic and commercialvalue in North-Eastern India. The studies on physic-chemicaland microbiological soil properties under teaplantation crop are scanty (Wilson and Clifford, 1992). The objective of this studywas to isolate PSM fromtea rhizosphere soils and examine their ability tosolubilize P and producing IAA inliquid cultures.

Materials and Methodology

Isolation of phosphate solubilizing bacteria

Tea (*Camellia sinensis* L.) rhizospheric soil from Darjeeling tea garden was collected and studied in the laboratory. 10 g each of soil samples was suspended in 90 ml of steriledistilled water and 10^{-1} dilution was obtained. Serialdilutions were prepared by mixing 1 ml of the suspensionmade into 9 ml sterile water blanks, until the 10^{-7} dilutionwas obtained. The Pikovskaya's (PKV) agar (Pikovskaya, 1948) (10 gglucose, 5 g tricalcium phosphate (TCP), 0.5 g ammoniumsulphate, 0.2 g potassium sulphate, 0.1 g magnesiumsulphate, 0.5 g yeast extract, trace amount of manganesesulphate and ferrous sulphate, 20 g agar, 1000 ml distilledwater) was used for isolation, enumeration andmaintenance of PSB. The serially diluted soil suspensionswere spread plated on Pikovskaya's agar plate's andincubated at 37°C for 7 days. Bacterial colonies causingclear zones around the colonies were selected as phosphatesolubilizers and further purified by replating on agarmedium supplemented with TCP. Eight phosphatesolubilizing bacterial strains thus screened were selectedfor further analysis. All the chemicals, reagents used in thiswork except otherwise stated were obtained from Hi-MediaLaboratories, Mumbai, India.

Quantification of P solubilization

The P solubilizing potential of PSB strainswas tested *in vitro* by estimating available P inthe Pikovskaya's broth amended with known amount of TCP as a substrate. A control without any inoculation wasalso maintained. The organisms were allowed to grow for 7days at 30°C and centrifuged at 10,000 rpm for 10 min in acooling centrifuge Sample preparation was done by using a research centrifuge **(**Model: Sigma 2-16 PK, Germany**).** Solublephosphate was determined in supernatant following thestandard protocol (Fiskeand Subbarow, 1925).

Measurement of pH and titrable acidity

A change in pH of the medium due to the growth of PSBwas measured with a pH meter (Model: Systronics-335) after 5 days ofincubation. In order to study the titrable acidity of culturemedium, 5days old cultures were centrifuged at 1000 rpmfor 10 min. 10 ml culture filtrate was taken in a 50 ml conical flask, 1% phenolphthalein solution was added to the aliquot and titrated a with 0.1 N NaOH solution. The end point of titration was determined as pink color. The result was expressed as µeq NaOH /ml spent media.

Quantification of IAA Production

The production of IAA was determined according to the method of Bano and Mussaraat (Bano and Mussarat 2003). The testedbacterial strains was grown in LC medium in the presenceof tryptophan (100mg/l) and incubated at 30°C. The IAAproduction by bacterial strains was measured after 5 daysof incubation at 30°C. A 2 ml culture was removed fromeach tube and centrifuged at 10,000 rpm for 15 min in acooling centrifuge (Model: Sigma 2-16 PK, Germany), 1 ml ofsupernatant fluid was transferred to fresh tube to which100 ìl of 10 mM orthophosphoric acid and 2 ml of reagentconsisting of 1 ml of 0.5 % $FeCl_3$ in 50 ml of 35% $HClO_4$ were added sequentially. The absorbance of the developedpink color was read at 530 nm after 25 min in a Systronics make digital Spectrophotometer (Model: Systronics-167). IAA concentration in the culture wasdetermined by using a calibration curve of pure IAA as astandard.

Results

Out of 49 bacterial strains isolated from thetea rhizosphere, only 8 isolates showed the clearzones around the bacterial colonies indicating PSBs. Those PSB strains were designated asTPB-1, TPB-2, TPB-3, TPB-5, TPB-7, TPB-8, TPB-9, and TPB-10. Table 1 summarizes the values of P (mg/l) solubilizedin liquid culture and the pH of the corresponding mediaafter five days of

incubation. It clearly appears that inmedia amended with TCP, the values of solubilized P obtained withall the isolates were significantly higher from those ofcontrol, showing that the tested isolates have effectivelyconverted the inorganic insoluble phosphate into solubleform. Also, a decrease of pH values was observed in thetested isolates compared to control. Overall, TPB-5 (82.77±0.96 mg/l) was the most efficient P-solubilizer while strain TPB-2 showed the least P-solubilization (11.07±0.91mg/l). The solubilization ofTCP in the liquid medium by different strains wasaccompanied by a substantial drop in pH up to3.95from aninitial pH of 6.72 after five days of incubation.

Despite the reduction in pH of the medium, an increase intitrable acidity was also observed which might be due tosecretion of organic acids by PSB (Lal, 2002). The results ofproduction of growth promoting substance IAA indicated thatall the isolates of PSB were able to produce IAA. The strain TPB-2 produced highest amount of IAA (28.78 mg/l) followed by the TPB-3 (24.76 mg/l) while isolate TPB-5produced the least (11.23 mg/l) (Table 2).

Discussion

The results that were obtained in this study focused on the existence of PSB inrhizospheric soils of tea plants. Baby *et al*. (2001) carried outan investigation on microbial dynamics in the rhizosphereof tea plants and reported that there was a significantdifference on the population level of PSB in differentclones/seedlings of tea. Further, they also reported thatthe population of nitrogen fixing *Azospirillum* and PSB were higher in young tea fields than older fields.

In general, Ca-phosphate solubilization seems to belinked with pH decrease of the medium but this pH decreasewas not strictly proportional to the amount of thephosphate solubilized. These findings were supported byother reports (Illmer and Schinner, 1992a) that despite thehigh culture filtrate pH, high P solubilization can be observed in mediumoccasionally.

Table 1. Soluble phosphate, pH and titratable acidityof PKV broth inoculated with PSB strains after 5 days of incubation at 30°C

PSB strains	pH of the culture filtrate	Titratable acidity (µeq NaOH/ml)	Soluble phosphate (mg/l) of culture filtrate#
TPB-1	4.08	21.57	60.90±0.77
TPB-2	5.31	7.67	11.07±0.91
TPB-3	4.13	6.43	16.77±1.55
TPB-5	5.24	12.03	82.77±0.96
TPB-7	4.13	29.08	46.08±0.48
TPB-8	4.11	25.93	62.96±0.18
TPB-9	3.95	4.66	22.44±1.25
TPB-10	4.45	17.37	13.53±0.89
Control	6.72	4.45	5.40±0.45

Results are expressed as mean ± SD of three differentindependent readings.

Table 2. IAA production by PSB strains after 5 daysof incubation at 30°C

PSB strains	IAA (mg/l)
TPB-1	15.54
TPB-2	28.78
TPB-3	24.76
TPB-5	11.23
TPB-7	15.67
TPB-8	18.75
TPB-9	12.59
TPB-10	11.25

This could be attributed to the chelation of organic acids with Ca^{2+} ion intricalcium phosphate.

Similarly, it has been reported that pH had no effecton P-solubilization (Whitelaw *et al.* 1999; Narsian *et al.* 1995; Salih *et al.* 1989; Asea *et al.* 1988). Similar observationswere reported with *P. aurantiogriseum* (Illmerand Schinner, 1992b), and *P.radicum* (Whitelaw *et al.* 1999). The pH drop in PSM liquid cultures have beenreported in several researches which supports the pHchange in present study (Bar-Yosef *et al.* 1999; Cattelan *et al.* 1999; Motsara *et al.* 1995; Illmer *et al.* 1995). The amount of IAA produced by some isolates washigher than that have been reported by De Freitas *et al.* (1997) which ranged from 2.31 to 9.43 mg/l and was lowerthan that have been reported by Ponmurugan and Gopi (2006) which ranged from 34.02 to 45.31 mg/ml.

Conclusion

In conclusion, results of this study have shown thatseveral naturally occurring PSB isolates from tearhizospheres of Darjeeling hills are capable of producingplant growth promoting substance IAA, capable of solubilizing inorganic phosphates thereby decreasing the pH of the medium. Further studies are required to use these PSB isolates as bio-inoculums for the betterproductivity of crops for foodsecurity.

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