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RESEARCH ARTICLE

A PRELIMINARY STUDY ON PHOSPHATE SOLUBILIZING BACTERIA (PSB) FROM TEA GARDEN SOIL OF ASSAM

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ABSTRACT

Phosphorus is a major component of plants and soil. Plants can absorb phosphorus as phosphate anion. A large number of microorganisms are present in the soil are known to solubilize and make the insoluble phosphorus in the available form to the plants. Bacterial colonies from tea garden soil were isolated on Pikovskaya's (PKV) agar medium, containing insoluble tri-calcium phosphate (TCP) as calcium source. The colonies showing clear halo zones around the bacterial growth were considered as phosphate solubilizers. Gram positive cocci bacterial colonies were identified which shows oxidase negative in their biochemical reactions. The pH of the medium was dropped because P-solubilizing activity of these strains. This finding reveals the availability of PSB in the tea garden soil which is acidic in nature. It may throw light in the PSBs prospect of application as biofertilizer in low fertile land of tea growing areas of Assam, where internationally valued organic tea are produced.

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INTRODUCTION

Phosphorus (P) is an essential macronutrient of plants. It is a major growth-limiting nutrient, and unlike the case for nitrogen, there is no large atmospheric source that can be made biologically available (Ezawa *et al.*, 2002). Phosphorus (P) is involved in almost all biochemical pathways as a component part of energy carrier compounds, ATP and ADP (Mishra, 2012). Microorganisms play an important role in soil phosphate cycle where they transform insoluble phosphorus into available form. P-solubilization ability of the microorganisms is considered as one of the most important traits associated with plant phosphate nutrition. It is generally accepted that the mechanism of mineral phosphate solubilization by PSB strains is associated with the release of low molecular weight organic acids (Goldstein, 1995; Kim *et al*., 1997a), which through their hydroxyl and carboxyl groups chelate the cations bound to phosphate, thereby converting it into soluble forms (Kpomblekou and Tabatabai, 1994). Therefore, Phosphate solubilizing bacteria (PSB) are being used as biofertilizer since 1950s. In acid soils, P adsorption is attributed to hydrolysis of Fe, Al, Mn etc. (Kannaiyan 2000). The low fertility of acidic soils is mainly due to the transformation of soluble forms of P into forms of poor solubility, particularly Fe-P and Al-P complexes, which can be regarded as unavailable to plants (Johnson and Loeppert, 2006; Rengel and Marschner, 2005). The presence of these complexes reduces the nutrient capacity of these soils for sustaining plant and microbial growth (Richardson, 2001). Release of phosphate by PSB from insoluble and fixed forms is an import aspect regarding P availability in soils. Information about the composition and diversity of the bacterial community characteristics in tea garden soil ecosystems is scarce (Xue *et al*., 2008).

Tea plants are grown in the acidic soils in Assam which are known for low fertility. In context with this, the objective of the study is framed to know about availability of Phosphate Solubilizing Bacteria (PSB) in tea garden soil (Matiapahar Tea Estate) and if present, to know their capability on transformation of soil phosphate into available form.

MATERIALS AND METHODS

Study Site

'Matiapahar Tea Estate', measuring about 200 acre tea garden land in Nagaon district of Assam, is identified as the study area to isolate the phosphate solubilizing bacteria.

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Sample Collection

Five numbers of soil samples were collected from four corners and the center of study site from 6-15 cm depth. Collected soil samples were stored in polythene bags aseptically and maintained at the laboratory.

Isolation of the strain

Prior to isolation, 100 gm of each five soil samples were mixed together in powdered form and used as source material. From this, 1.0 g soil were suspended in 9.0 ml of Milli Q water (pH 7.2) and serial dilutions (Aneja, 2002) were made (1:10, 1:100 and 1:1000). Pikovskaya's (PVK) agar medium (Pikovskaya, 1948) was used for inoculation where Tri-calcium Phosphate (TCP) was used as the phosphate source. The diluted samples were inoculated by pour plate and streak plate techniques (Aneja, 2002). The plates were incubated at 37°C for 48 hours. Colonies showing halo zones were picked and purified by 5 times subculture method on Pikovskaya's (PVK) agar medium for studying colony morphology (Goenadi *et al*., 2000). The pH of the media before and also after the growth of the cells was observed using a pH meter.

Morphological and biochemical identification of bacteria

PSB isolates grown on Pikovskaya's agar medium were characterized for colony morphology (Prescott, 2002), Gram staining (Gram, 1884) and biochemical analysis. Morphology of colony as well as microorganism were observed under microscope and discussed in the light of relevant literatures (Prescott, 2002). Gram Staining method was done to know whether the bacteria is gram positive or gram negative (Gram, 1884). Isolates were also tested for oxidase following the procedure of Kovacs (1956). To the Pikovskaya Agar media plates, overnight cultures of the test isolates were spotted and plates were incubated for 24 h at 37ºC. After incubation, two to three drops of tetramethyl-phenylenediamine dihydrochloride was added to the surface of the growth of each test organism to record change of colour.

Figure 1. Bacterial growth observed in PVK media

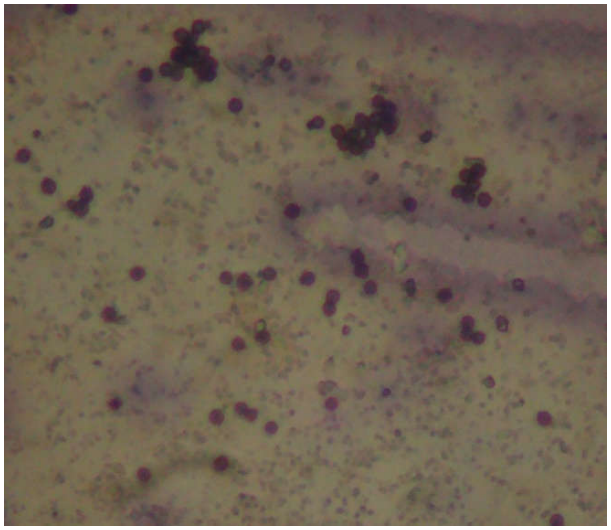

Figure 2. Gram positive bacterial colony observed under microscope

RESULTS

Growth of bacterial cells was observed in the PVK agar plates (Figures 1 and 2).

The cells were visualized under a light microscope. The bacteria were identified as Gram positive Cocci (Figure 2).The pH of the media was found to be considerably dropped to 4.3 from an initial value of 7.0 due to the release of organic acids. Isolates were able to solubilize tri-calcium phosphate (TCP) in Pikovskaya's agar medium as zone of clearance were observed due to Phosphate solubilization in both pour plates (Figure 1) and streak plates. No color change from white to purple or maroon was observed in oxidase test. Hence, the bacteria were tested negative for oxidase.

DISCUSSION

The use of PSB as biofertilizer may convert insoluble phosphorus to soluble phosphorus and make it available to the plant. (Rathour *et al.*, 2015). Thereby, PSB as bioinoculant or biofertilizer enhanced the crop yield. In 1948, Gerretsen reported increased yield in Oats accompanied with high phosphorus uptake followed by Pikovskaya (1948). There are a good number of records in India regarding the increase yield of various crops due to PSB inoculation e.g. Sharma and Singh (1971) in maize; Kundu and Gaur (1980a, 1980b) in cotton and wheat; Sarkar *et al*., (2014) in wheat; Kathiresan *et al*., (1995) in sugarcane; Pandiyarajan (1995) in onion bulb; Ravikumar *et al*. (2013) in rice etc. Although microbial inoculants are in use for improving soil fertility during the last century, however, a meager work has been reported on P solubilization compared to nitrogen fixation (Mohammadi, 2012). India is importing nearly 95% of its P required against demand as the country does not have any phosphorus reserve resulting drainage of foreign exchange. On the other hand, PSB are ubiquitous and present in all types of soils. This particular investigation was done to isolate soil PSB from tea garden area and result showed presence of the PSB in agar plates. It is also indicated by the drop of pH due to production of organic acid.

Relevant finding is also reported by Song *et al*. (2008). The bacteria were identified as Gram positive Cocci. Therefore, this experimental finding has an importance in isolating PSB from tea growing area and it opens a window for further studies on crop yield as well as use of bioinoculant or biofertilizer in low fertility acidic soil. It will also enhance the knowledge on native microorganisms and to address the issues related to organic tea production that has great demand in international market.

Conclusion

Now-a-days, maintenance of soil productivity and ecological sustainability is the global requirement in agriculture. Judicious use of chemical fertilizers by the marginal farmers is still standing as a challenging task in context to soil health and fertility. Use of native microbial strains as bioinoculant or biofertilizer can facilitate the process of sustainability of natural resource.

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