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International Journal of Current Research
Vol. 9, Issue, 05, pp.49779-49783, May, 2017

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

PHOSPHATE SOLUBILISING RHIZOBACTERIA ASSOCIATED WITH *COFFEA ARABICA* L. IN COFFEE PLANTATIONS OF NORTH EASTERN GHATS, VISAKHAPATNAM DISTRICT, ANDHRA PRADESH, INDIA

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ARTICLE INFO

Article History:
Received 27th February, 2017
Received in revised form
13th March, 2017
Accepted 19th April, 2017
Published online 19th May, 2017

Key words:
Coffea arabica and
Phosphate solubilising
Rhizobacteria.

ABSTRACT

Phosphate solubilising rhizobacteria associated with *Coffea arabica* L. in coffee plantations of North Eastern Ghats, Visakhapatnam district, Andhra Pradesh, India, were investigated. The main purpose is to screen for potential microbial biofertilizers by assessing the isolated strains for phosphate solubilization efficiency and initial screening was performed on Pikovskaya's agar (PA). The present study could therefore be important with respect to screening of *Coffea arabica* associated rhizobacteria that possess direct plant growth promoting traits for extending the use of indigenous microbes as microbial biofertilizers.

Citation: Satyaprakash, M., Nikitha, T., Chaitanya, I. and Reddi, E. U. B. 2017. "Phosphate Solubilising rhizobacteria associated with *Coffea arabica* L. in coffee plantations of north eastern Ghats, Visakhapatnam District, Andhra Pradesh, India", *International Journal of Current Research*, 9, (05), 49779-49783.

INTRODUCTION

Coffea arabica L. is the most important world commodity, the principal source of revenue for the Andhra Pradesh Forest Development Corporation (APFDC) and local tribes of Visakhapatnam district greatly requires sustainable coffee production with healthier product quality to remain in the present competitive market. *Coffea arabica* is extensively cultivated in small and large scale plantations under a variety of shade trees in North Eastern Ghats, Visakhapatnam. While the use of mineral fertilizers is considered the quickest and surest way of boosting crop production, their cost and other environmental hazards. The current crisis in coffee prices in the world market due to over production (Albertin and Nair, 2004) and the progressive revival of interest in organically grown coffee, which is closely coupled with fears about environmental health and biodiversity (Van der Vossen, 2005), are additional problems to millions of coffee farmers in developing countries. These problems make it essential to look for alternative strategies that can ensure competitive coffee yields while protecting the health of soils.

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The current need for economically and ecologically acceptable fertilizer sources has prompted the search for a new approach to sustainable agriculture. Sustainable agriculture constitutes a major strategy to counteract the rapid decline in environmental quality by maintaining the long term ecological balance of ecosystems (Khan *et al*., 2006). The use of biofertilizers in agriculture has proven to be ecofriendly, productive and accessible option to continued application of soluble mineral fertilizers (Sheraz *et al*., 2010). Next to Nitrogen, Phosphorus (P) is the second most important macro nutrient required by the plants. Unlike for nitrogen there is no large atmospheric source that can be made biologically available for P availability (McVickar *et al*., 1963).Most natural ecosystems in tropical and subtropical areas are predominantly acidic and extremely P deficient (Khan *et al*., 2006) due to their strong fixation of P as insoluble phosphates of iron and aluminum. To alleviate P deficiency, chemical phosphatic fertilizers are widely used. However, a large proportion of the soluble forms of P fertilizers is precipitated in insoluble form soon after application and becomes unavailable to plants (Mahantesh and Patil, 2011). This in turn leads to a need for excessive and repeated application of soluble P fertilizers, which in addition to the economic restriction can pose a serious threat to groundwater. Bacteria in the rhizosphere can affect plant growth. The term 'Plant Growth-Promoting Rhizobacteria' (PGPR) is used to describe strains of naturally occurring root-colonizing soil bacteria that possess the capacity to stimulate plant growth and development via production and secretion of various regulatory chemicals in the vicinity of rhizosphere (Kloepper*et al*.,2003). PGPR can affect plant growth by a wide range of mechanisms such as solubilization of inorganic phosphate, production of phytohormones, siderophores and organic acids, lowering of plant ethylene levels, N_2 fixation and biocontrol of plant diseases (Muleta *et al*., 2007; Datta*et al*., 2011). The use of such beneficial bacteria as biofertilizers and biocontrol agents has currently attracted increased interest worldwide in attempts to achieve sustainability, particularly in agriculture, forestry and horticulture (Datta *et al*., 2011). Supplying P through biological means is a realistic alternative in order to lower the environmental risk and enhance the productivity (Vessey, 2003). Evidence is increasing that phosphate solubilizing bacteria(PSB) and fungi (PSF) play a vital role in conversion of insoluble P to bio available primary and secondary orthophosphate ions (Pal, 1998). Goldstein (1986) had demonstrated that various bacterial species are able to mobilize insoluble inorganic P compounds. The existence of soil microorganisms (bacteria, actinomycetes and some fungi) that solubilize soil precipitated or soil attached phosphate has been reported previously (Reyes *et al.,* 2001). Strains from bacterial genera *Pseudomonas*, *Bacillus*, *Rhizobium* and *Enterobacter* along with fungal species *Penicillium* and *Aspergillus* are the most powerful P solubilizers (Fankem*et al.,* 2006; Whitelaw, 2000).Very little is known about microorganisms associated with *Coffea arabica* in their functional characteristics towards plant growth promotion. The present study therefore aimed to screen coffee associated phosphate solubilizing rhizobacteria from our own collection and to evaluate their solubilizing efficiency in insoluble P in Pikovskaya's agar (PA).

Study Area

The study area is located between co-ordinates of N 17° 51.675, E 82° 21.196 and Altitude: 2824 feet and N 17° 48.248, E 82° 29.712 Altitude: 2834 feet of North Eastern Ghats, Visakhapatnam district, Andhra Pradesh, India at 980 meters above the mean sea level.

The climate in this region is tropical climate with 23.4 °C mean annual temperatures. Maximum temperature values exceed 39°C, and minimums are around 30°C in summer and this is the coldest place in Andhra Pradesh, with night temperatures dip as low as 0°C during December – January. When compared with winter, the summers have much more rainfall. The climate here is classified as Aw by the Koppen-Geiger system. In a year, the average rainfall is 1231 mm. The study area comprises of tropical semi-evergreen forests, which are moist deciduous forests, mixed with evergreen elements. The North Eastern Ghats is one of the most unique landscapes on Earth and is comprised of complex geological formations and deposits. Soils found in this study area are black and red soils on hills and ranges. Vegetation includes coffee plantation, understory of evergreen trees and an emergent canopy of taller deciduous trees. The most common plant genera are *Michelia champaca, Artocarpus lakoocha, Dillenia pentagyna, Bridelia tomentosa, Xylia xylocarpa, Psychortia fulva, Leea crispa and Boehmeria platyphylla.* The common climbers in these forests are *Ampelocissus latifolia, Bauhinia vauhli* and *Smilax zeylanica.*

MATERIAL AND METHODS

Collection of sample

Soil samples were collected at a depth of 0-15cm from the rhizosphere. During the soil sample collection, surface was cleaned by removing the litter and top soil layer. All samples were placed in polythene bags, brought to the laboratory in ice boxes, and stored at 4°C in refrigerator for further analysis.

Microbial count

Microbial population was estimated by plate count method (Ravina *et al.,* 1992; Vincent, 1970). Ten grams of soil was suspended in 100 ml sterile distilled water in Erlenmeyer flask and mixed thoroughly for 30 minutes using a mechanical shaker at 110 rpm. Then 1ml of aliquot transferred with sterile pipettes to 9 ml sterile distilled water in test tube. This suspension was stirred for 10seconds. A subsequent serial dilution was prepared as above to 10^{-7}. From each serial dilution, 0.2 ml of aliquot was transferred to sterile petriplate, over poured, and dispersed swirling with agar media.

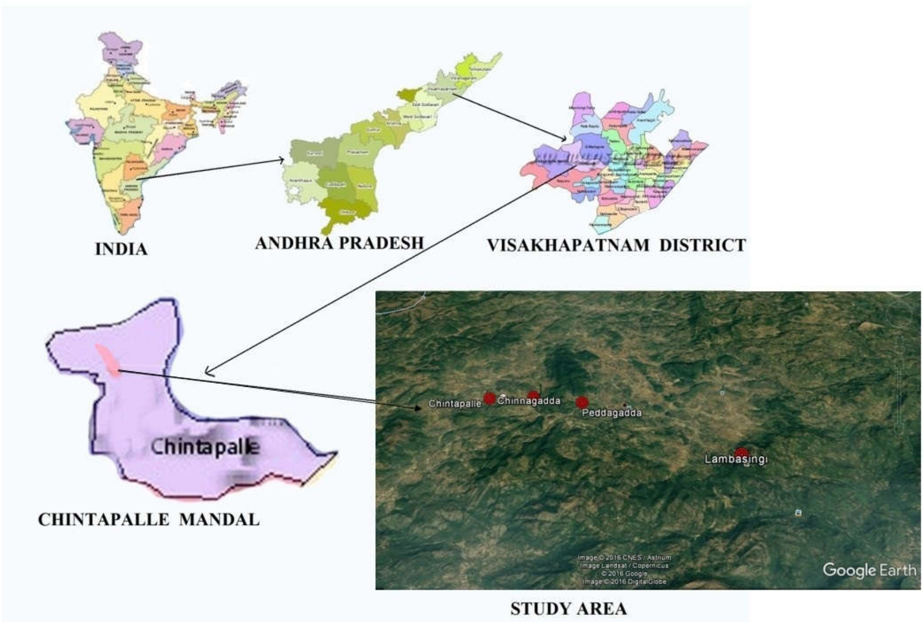


Fig. 1. Location map of the study area

Isolation and identification of the Phosphate Solubilizing Bacteria

A known quantity (1gm) of soil sample was suspended in a known volume of sterile water (10 ml) and serial dilutions of the suspension made in sterile water blanks. Appropriate dilutions were plated on phosphate-containing solid media (Pikovskaya's medium- modified by Sundara Rao and Sinha, 1963) for obtaining phosphate solubilizers. The isolates were identified following Bergey's manual for bacteriology methods systematic (Krieg and Holt, 1984).

Screening for p-solubilising capacity

PSB isolates obtained were re-tested by plate assay for phosphate solubilization in PA medium. These bacteria were stabbed in triplicate using sterile toothpicks. The halo zone around the colony was presumptive confirmation of phosphate solubilization and was measured after 96 hrs of incubation at 30º C. Halo size was calculated by subtracting colony diameter from the total zone of colony and halo zone as described by Sharma *et al.,*(2007).

RESULTS

Microbial population was estimated by plate assay method using Plate count agar and PA. Highest number of microbial colonies was counted from the soil sample collected at Lambasingi followed by site Peddagadda, Chintapalli and Chinnagadda. Highest number of PSB colonies were collected from the sample collected at Lambasingi followed by site Chintapalli, Peddagadda and Chinnagadda. There were fluctuation in total and phosphate solubilizing bacterial count in P solubilizing rhizobacteria. Highest number of total microbial count i.e., 88.3 x 10^5 cfu/gm is observed at Lambasingi and lowest i.e., 70.7 x 10^5 cfu/gm at Chinnagadda and highest number of PSB count i.e., 21 x 10^5 cfu/gm was observed at Lambasingi and lowest i.e., 13 x 10^5 cfu/gm at Chinnagadda. Totally, 15 PSB obtained from four sites of North Eastern Ghats, Visakhapatnam district, Andhra Pradesh,

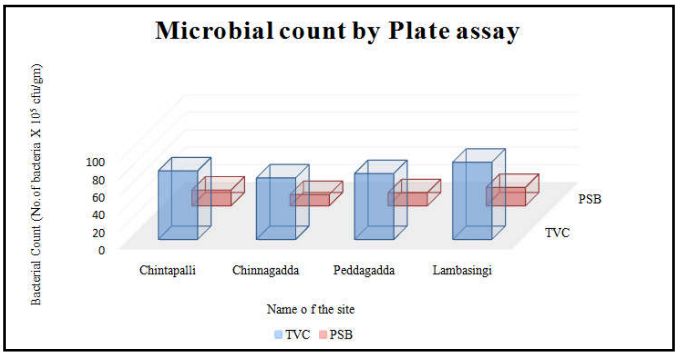
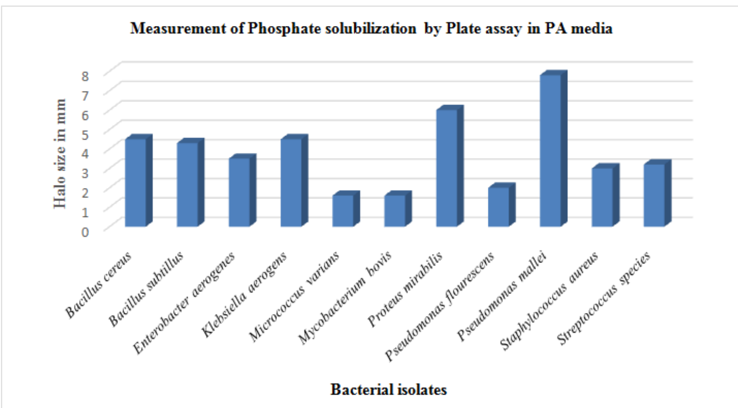


Figure 2. Enumeration of Total Viable Count (TVC) and Phosphate Solubilizing Bacteria (PSB) based on altitude

Table 1. List of isolated phosphate solubilizing bacteria from four sites of North Eastern Ghats, Visakhapatnam

S.No	Site name	GPS readings	Isolated phosphate solubilizing bacteria
1	Chintapalli	N 17º 51.675 E 82º 21.196 Altitude: 2824 ft	*Bacillus cereus; B. subtillus; Enterobacter aerogenes; Mycobacterium bovis; Klebsiella aerogens; Pseudomonas flourescens*; *P. mallei; Staphylococcus aureus*
2	Chinnagadda	N 17º 51.738 E 82º 22.736 Altitude: 2580ft	*Bacillus subtillus; Corynebacterium kutscheri; C. xerosis; Streptococcus* species
3	Peddagadda	N 17º 51.408 E 82º 24.560 Altitude: 2560ft	*Bacillus megaterium; B. cereus; B. subtillus; Proteus vulgaris*; *Staphylococcus aureus*; *Micrococcus varians, Proteus mirabilis*
4	Lambasingi	N 17º 48.248 E 82º 29.712 Altitude: 2834 ft	*Bacillus cereus; B. subtillus; Pseudomonas flourescens; Staphylococcus aureus; Enterobacter aerogens; Streptococcus* species



India. Among them, *Bacillus* species is the dominant in four sampling sites followed by *Staphylococcus* species in three sampling points, *Pseudomonas* species and *Corynebacterium* species in two sampling points.

Measurement of Phosphate solubilization: PSB isolates obtained were re-tested by plate assay for phosphate solubilization in PA medium. On measuring of halo size of Phosphate solubilization *Pseudomonas fluorescens, Proteus mirabilis, Bacillus cereus, Bacillus subtilis, Enterobacter aerogens, Mycobacterium bovis, Pseudomonas mallei*and *Staphylococcus aureus* and *Staphylococcus aureus* showed phosphate solubilization activity.

DISCUSSION

PSB were found in majority of soils (Chhonker and Taraedar, 1984).Evidence for naturally occurring phosphate solubilizing microorganism dates back to 1903 (Khan *et al*., 2007).The PSB population was higher in soils under mild and moist climates than in dry ones (Subbarao, 1982).Phosphate solubilising rhizobacteria associated with the rhizosphere of *Coffea arabica* at North Eastern Ghats, Visakhapatnam district, Andhra Pradesh, India. This study is the first report on coffee associated beneficial rhizobacteria at North Eastern Ghats, Visakhapatnam district coffee plantations. Different bacterial genera and within genera different bacterial species have been reported to have P solubilizing capacity. Strains from bacterial genera *Pseudomonas*, *Bacillus*, *Rhizobium* and *Enterobacter* (Whitelaw, 2000), *Bacillus megaterium*, *B. circulans*, *B.subtilis*, *B. polymyxa, B. sircalmous, Pseudomonas striata*, and *Enterobacter* are the most powerful P solubilizers (Subbarao, 1988). *Acetobacter*sp. (Joseph and Jisha, 2009), *Corynebacterium*sp. (Gupta *et al*., 1998), *Azotobacterchroococcum* (Kumar and Narula, 1999), *Burkholderia* sp., *Gluconacetobacter*sp., *Enterobacter* sp. (Chung *et al*., 2005; Kim *et al*., 2003), *Micrococcus* sp. (Goldstein, 2001), *Pseudomonas, Bacillus, Micrococcus, Flavobacterium* (Rodriguezand Fraga, 1999) are among the frequently reported PSB. Colonization of soil by non-indigenous microorganism depends both on its interaction with indigenous flora associated with plants and its ability to utilize diverse substrates in the soil (Miethling *et al.,* 2000). The most dominant phosphate solubilizing bacteria in found were aerobic and of which, some are spore forming bacteria. Identification of this group showed that *Bacillus* sp. was the most predominant PSB and it is found in all the sites of soils tested. *Pseudomonas mallei* showed high solubilization followed *Proteus mirabilis*. Other PSB involved were *Klebsiella aerogenes*, *Bacillus cereus, B.subtillus, Enterobacter aerogens, Streptococcus* species, *Staphylococcus aureus, Pseudomonas flourescens, Mycobacterium bovis, Micrococcus varians, Proteus vulgaris* and *B.megaterium.*

Conclusion

The current study identified the presence of many potent PSB in the rhizosphere of coffee plants calls for thorough and continuous studies of their field applicability as inoculants, after adequate formulation, in establishing a potentially important adjuvant for agricultural practices, particularly on sites where P is a main constraint. Phosphate solubilizing microorganisms promise a better alternative to this problem. It is eco-friendly and cost effective agro technology to improve crop production. Hence, there is an urgent need to improve better research in this field for developing this technology and to minimize the use of chemical fertilizers and make use of biofertilizers in large scale in agronomic practices to obtain better results. Inoculations with potent indigenous microorganisms are in accord with contemporary views on the possible future role of plant growth-promoting and soil-supporting bacteria in enhancing plant yields.

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