International Journal of Current Research in Life Sciences
Vol. 05. Issue. 06, pp. 563-566, June 2016

www.ijcrls.com

Full Length Research Article

POPULATION DYNAMICS OF *AZOSPIRILLUM*, *BACILLUS* AND *GLUCONACETOBACTER DIAZOTROPHICUS* IN THE RHIZOSPHERE SOIL OF SUGARCANE

***Prabudoss, V.,**

Department of Agriculture Microbiology, Annamalai University, Annamalainagar – 608002, Tamil Nadu, India

Accepted 17th May 2016; Published Online 30th June 2016

ABSTRACT

Beneficial bacterial organisms plays vital role in the life cycle of every plant, The PGPR organisms *Gluconacetobacter diazotrophicus*, *Azospirillum brasilense* and *Bacillus megaterium* associated with sugarcane were isolated from, five different locations namely Vilagam, Sivapuri, Vallampadugai, Erumpure and Killai, Cuddalore District, Tamil Nadu. In the present research two soil samples namely erumpure and killai showed zero percent population of *Gluconacetobacter diazotrophicus,* it shows the endphytic nature of *Gluconacetobacter diazotrophicus* in the absence of rich amount of organic matter content in soil.

Key words: Azospirillum, Bacillus, Gluconacetobacter diazotrophicus.

INTRODUCTION

The rhizosphere is the area in and around root zone and its adhered soil particles known to attract majority of PGPR and other bacterial organisms through root exudates, rhizodeposits and fraction of humus. These organic substances rich in nutrients, vitamins, minerals, carbohydrates, amino acids, organic acids, as well as other growth related compounds. Which are known to attract all kinds of microorganisms. The root exudates normally released in to the rhizosphere, and microorganisms were well known to be chemo attracted and move towards root exudates. If it may be a PGPR organisms it will colonize and multiply both in the rhizosphere, rhizoplane and in some cases some organisms enters into the plant parts as endophytes (Kloepper *et al*., 1989). PGPR are vital groups of bacterial organisms known for many beneficial characters *viz*., degradation of organic matter, nitrogen fixation, phosphorus solubilization, production of growth promoting substances (IAA, auxin, gibberellins, cytokinins and ethylene), production of siderophore, solubilization of zinc, iron and protecting the rhizosphere area by eliciting root metabolic activities and through the suppression and also eliminating phytopathogens. The well known PGPR include bacterial organisms belonging to the genera *Azospirillum, Azotobacter, Gluconacetobacter, Pseudomonas, Bacillus, Phosphobacteria,* etc. The colonization of plant growth promoting rhizobacteria in the rhizosphere soil may generally in the range of 10^7 – 10^9 CFU bacteria per gram of soil sample (Doberiener *et al*., 1988). Sugarcane is a long duration commercial sugar rich plant and standing in the farmer's field for about 10 months and producing large amount of biomass in the form of leaf litre and other matured roots which are added to soil and serves as organic matter and rhizosphere soil. Studying the population dynamics of beneficial organisms which are living in the rhizosphere of energy plant is a fruitful tool to know the soil richness, fertility and fitness of soil for the better growth, development and yield of crop plants. Due to long standing nature of sugarcane known to remove around 100:60:225 kg of N, P_2O_5 and K_2O respectively for the production of 60 to 100 tonnes of cane yield respectively for different varieties. If the adequate supply of these nutrients not ensured, the sugarcane growth and yield tends to decline even on most fertile soils. In addition, our universe mainly depends on sugarcane for sweetening agent namely crystallized cane sugars, in this view even though peoples using the sugars after processing and possibilities of having entered chemicals in the sugars are more (entrance of chemical in food chain). Hence, it is better to minimize the usage of chemical fertilizers and pesticides in sugarcane cultivation for better health and better environment. However, the continuous addition of inorganic chemical NPK fertilizers in large amount will leads to deteriorate soil health and resulting in drastic reduction in the population of many PGPR microorganisms as well as it causes reduction in the yield of sugarcane. Hence, there is an urgent need to preserve the soil fertility and plant environment by using balanced proportion of all required nutrients for sustainable crop production in sugarcane cultivation. The approach of integrated nutrient management through inoculation of PGPR organisms with graded levels of N, P and K fertilizers will go a long way to enhance the soil fertility as well as to obtain maximum yield in sugarcane by having minimized pollution or by having zero per cent pollution (Seldin *et al.,* 1984; Day, 1976b; Okon, *et al.,* 1988 and Sathyan and Thangaraju, 2003).

***Corresponding author: Prabudoss, V.,**
Department of Agriculture Microbiology, Annamalai University, Annamalainagar – 608002, Tamil Nadu, India.

Table 1. Physico – chemical properties of rhizosphere soil of sugarcane

Sl. No.	Location	pH	Soil type	Soil organic carbon (%)	EC (dSm^{-1})	Available 'N' ($kg\ ha^{-1}$)	Available 'P' ($kg\ ha^{-1}$)	Available K ($kg\ ha^{-1}$)
1	Vilagam	7.5	Clay	0.50	1.5	116.00	15.00	160.0
2	Sivapuri	7.6	Clay loam	0.56	1.7	116.00	15.00	160.0
3	Vallampadugai	7.5	Clay loam	0.56	1.6	122.00	18.00	180.0
4	Erumpur	7.9	Clay	0.48	1.8	112.00	10.50	150.0
5	Killai	8.1	Sandy clay	0.40	2.0	110.0	10.00	140.0

Based on above views its quite essential to know the population dynamics of PGPR organisms in the rhizosphere of sugarcane.

MATERIALS AND METHODS

Collection of rhizosphere soil samples and physico - chemical properties: In each location, the rhizosphere soil samples were collected from sugarcane roots and surrounding soil from different locations of Cuddalore district *viz*., Vilagam, Vallampadugai, Sivapuri, Erumpur and Killai and were elevated to an intensive of 15 – 20 cm. Hence, after the collection of soil samples were transferred to polythene bags for further isolation.

Soil reaction (pH): The pH of the soil was measured in 1:2.5 soil: water suspension by Elicomodel LT-10T pH meter (Jackson, 1973).

Estimation of soil organic carbon: The soil organic carbon content of, the soil sample was estimated by following the method of Walkley and Black (1947).

Estimation of Electrical conductivity (EC): The EC of the soil sample was estimated by using electrical conductivity bridge (Jackson, 1973).

Estimation of soil nitrogen (Subbiah and Asija, 1956): The available nitrogen content of the soil sample was estimated by alkaline permanganate method.

Estimation of soil Available phosphorus (Olsen *et al.*, 1954): The available phosphorus content of the soil sample was estimated by Olsen's method.

Estimation of soil available potassium (Jackson, 1973): The available potassium content of the soil sample was estimated by the neutral ammonium acetate method.

Enumeration and Isolation of PGPR organisms: The PGPR organisms namely *Gluconacetobacter diazotrophicus*, *Azospirillum brasilense* and *Bacillus megaterium* were enumerated either by MPN (Most Probable Number) method or serially dilution method. The reference strains were collected from TNAU (*Gluconacetobacter diazotrophicus* PAL5, *Azospirillum brasilense* – ATCC-29145 and *Bacillus megaterium* MTCC – 10127). After that the case of *G. diazotrophicus* and form from positive tubes were purified the microbial cultures isolated by enrichment and serial dilution technique. The purified cultures were identified, characterized and confirmed for authentification through biochemical tests and molecular technique.

The authentificated cultures were screened for their efficiency and the efficient strains were maintained as slant culture and used for the further research works.

Isolation of *G. diazotrophicus*: *G. diazotrophicus* cultures were isolated from the sugarcane rhizosphere soil samples by following the methodology of Cavalcante and Dobereiner (1988). One gram of the sugarcane soil samples were collect from which 1g was serially diluted upto 10^{-5} then from the dilution 10^{-5} 1 ml aliquot was transfer to various enrichment media *viz*., semisolid diluted can juice medium, semisolid LGI medium and semisolid acetic LGI medium supplemented with yeast extract (20 mg l^{-1}) the tubes were incubated at room temperature without disturbance until the formation of sub surface pellicles.

EXPERIMENTAL RESULTS

Physico - chemical properties of sugarcane rhizosphere soil
In the present investigation the rhizosphere soil samples of sugarcane were collected from five different locations from Cuddalore district namely Vilagam, Vallampadugai, Sivapuri, Erumpur and Killai and are analysed for phsico- chemical properties and the values obtained after analysis were recorded and presented in Table 1. The collected soil samples were utilized for the isolation of PGPR organisms namely *Azospirillum brasilense*, *Gluconacetobacter diazotrophicus* and phosphobacteria (*Bacillus megaterium*). The rhizosphere soil sample of sugarcane from the above locations showed the pH in the range of 7.5 to 8.1 and EC ranged from 1.5 $dSm^{-1}$to 2.0 dSm^{-1} for the sample collected from five different locations. A slight variation was observed on the organic carbon contents of the soil samples from sugarcane rhizosphere. The soil available nitrogen content ranged from 110 kg ha^{-1} to 122 kg ha^{-1} and categorized as low, in the available phosphorous ranged from 10.00 kg ha^{-1} to 18.00 kg ha^{-1} and categorized as medium, whereas the soil available potassium ranged from 140 kg ha^{-1} to 180.00 kg ha^{-1} and categorized as high. Among the sugarcane rhizosphere soil samples analysed for N, P and k contents, the rhizosphere soil sample from Vallampadugai soil respectively recorded 122.00, 18.00 and 180 for N, P and K. The soil samples of vilagam, sivapuri and vallampadugai showed slightly over values to neutral pH, whereas erumpur and killai showed alkali pH . In the present investigation there is an evident for the correlation of population between nutrient content and pH, the soil from vallampadugai, vilagam showed nearly neutral pH and recorded moderate to high content of nutrient and PGPR population.

Occurrence of plant growth promoting rhizobacteria in the rhizosphere soil samples of sugarcane: The plant growth promoting bacteria were enumerated from the rhizosphere soils of sugarcane grown in 5 different locations namely Vilagam, Sivapuri, Vallampadugai, Erumpur and Killai from

Cuddalore District, Tamilnadu, India. Based on morphological, biochemical and molecular basis they are identified of as *Gluconacetobacter*, *Azospirillum* and *Bacillus.* The population were also enumerated and the plant growth promoting rhizobacterial populations were found to be highest in Vallampadugai and recorded (8.6 x 10^4 cfu g^{-1}) for *Azospirillum*, the population density (4.0 x 10^4 cfu g^{-1}) for *Gluconacetobacter* and (8.00 x 10^4 cfu g^{-1}) for *Bacillus* respectively. The minimum populations of PGPR organisms were recorded in Killai and showed the population load of (3.6 x 10^4 cfu g^{-1}) for *Azospirillum* (3.00 x 10^4 cfu g^{-1}) for *Bacillus* and there is no *G. diazotrophicus* population encounted in the same Killai sugarcane rhizosphere soil. It is an evident for the inhibition of alkali pH on the population of *G. diazotrophicus*. The values recorded for the enumeration of PGPR organisms were presented in Table – 2.

Table 2. Enumeration of plant growth promoting rhizobacteria (PGPR) from the rhizosphere soils of sugarcane

Sl. No.	Locations	Populations (1x 10^4 cfu g^{-1} on oven dry weight)		
		Gluconacetobacter	*Azospirillum*	*Bacillus*
1	Vilagam	2.0	7.6	4.0
2	Sivapuri	2.0	4.6	4.6
3	Vallampadugai	4.0	8.6	8.0
4	Erumpur	0.0	4.6	3.6
5	Killai	0.0	3.6	3.0
	SEd	0.74	0.96	0.86
	CD (P=0.05)	2.07	2.69	2.44

DISCUSSON

Occurrence of PGPR on the rhizosphere of sugarcane

The occurrence of plant growth promoting rhizobacteria was directly influenced by soil neutral pH and moderate level of nitrogen, phosphorous and potassium. Among the soil physico chemical properties like EC and the increase or decreases in pH level from.7.0 is negatively correlated and causes reduction in the PGPR population recorded. The soil type was found to have no influence on the rhizosphere population of plant growth promoting bacteria (An *et al*., 2001; Ma *et al.,* 2001). In the present research organic matter content also decides the population of PGPR organisms among Thirteen isolates from PGPR 'N' fixing organisms namely *Azospirillum,* and *Gluconacetobacter.* Apart from *Azospirillum, Gluconacetobacter* and *Bacillus* were also obtained from sugarcane rhizosphere soils collected from different locations of Cuddalore District, Tamilnadu and based on morphological and physiological characters. These isolates further studied for species characterization and finally best isolates are again identified by molecular techniques from the 13 isolates of PGPR and among which 5 from *Azospirillum,* five from *Bacillus* and three isolates belonged to *Gluconacetobacter diazotrophicus*. The *Azospirillum* population ranged from (3.66 x10^4cfu g^{-1}) to (8.60 x10^4cfu g^{-1}) of oven dry soil, *Gluconacetobacter* population ranged from (2.00 x10^4cfu g^{-1}) to (4.0 x10^4cfu g^{-1}) and phosphate solubilizing *Bacillus megaterium* ranged from (3.00 x10^4cfu g^{-1}) to (8.00 x10^4cfu g^{-1}) per gram of oven dry rhizosphere soil of sugarcane. The sampling locations differed significantly in supporting the microbial load of different types of root associated growth promoting rhizobacteria (PGPR). The sugarcane rhizosphere soil collected from Vallampadugai of Cuddalore District support highest population of *Azospirillum*, *Gluconacetobacter* and *Bacillus* in the present investigation. In the present research, five soil samples were collected and used for the isolation of PGPR organisms out of five soil samples only three rhizosphere soil samples only three samples showed *Gluconacetobacter diazotrophicus* and remaining two samples failed to show population of *Gluconacetobacter diazotrophicus* and the isolates were designated as PGPRG1, PGPRG2 and PGPRG3. These results shows poor survival percentage of *Gluconacetobacter diazotrophicus* than *Azospirillum brasilense* and *B. megaterium* and apart from its presence in soil already enormous evidence are stated the presence of *Gluconacetobacter diazotrophicus* in the internal parts of sugarcane as endophytes. The endophyte *Gluconacetobacter diazotrophicus* population in many sugarcane varieties in several parts and region of Brazil and its numbers varied in the range of 10^3 to 10^7 in rhizosphere soil and plant parts, in majority of cases population of *G. diazotrophicus* only based on the presence of organic matter and partially degraded trashes (Dobereiner *et al*., 1988). Recently, a new hypothesis that the sugarcane has the discriminatory ability to choose the compatible endophyte from several possible reasons in which one has been proposed, it explains that the sugarcane produces two different pools of glycoprotiens containing a heterofructon as glycocidic moiety, high molecular mass amid molecular mass glycoproteins. *Fluorescent* labeled glycoproteins is able to bind *G. diazotrophicus* (Legaz *et al*., 2000). Since, the propagation of the endophytic nitrogen fixing bacteria is from stem cuttings in to the developing sugarcane plant (Patriquin *et al*., 1980). The present population level of *G. diazotrophicus* in the study in accordance with findings of Debereiner *et al*. (1988). The present study revealed that the universal occurrence of plant growth promoting bacteria with mederal to very good load as influenced by soil environmental conditions, root exudates and rhizodeposits in the rhizosphere of sugarcane rhizosphere samples collected from five different locations namely Vilagam, Vallampadugai, Sivapuri, Erumpure and Killai of Tamilnadu where it is grown as monocrop for the past five years. Geetha (2003) and Karthikeyan *et al*. (2007a) isolated *Azospirillum* from the rhizosphere of *O. sanctum, Phyllanthus amarrus, W. sonifera, aloe vera, and C. roseus*. Mishra *et al*. (2011) isolated *Bacillus* and *Pseudomonas*. Prabudoss (2011) isolated PGPR *Azospirillum* and phosphobacteria from the rhizosphere of Crossandra and studied their N fixing and P solubilizing efficiency. Bharathiraja and Tholkappian (2010) observed the growth enhancing efficiency of PGPR organisms namely *Azospirillum* and phosphobacteria on the yield of marigold and Crossandra. (Muralikrishnan and Muthukaruppan 1998; Pazhaniraj and Prabudoss, 2014) observed effective influence of PGPR organisms *Azospirillum*, *Pseudomonas*, *Bacillus* and *Gluconacetobacter* on the growth development and yield of sugarcane – *Saccharum officinarum*. Sakthivel and Karthikeyan (2012) isolated the twenty isolates of *Azospirillum*, *Azotobacter, Pseudomonas* and *Bacillus* from the rhizosphere soil of *Coleus forskohlii*. They also reported the pronounced fluctuation in the PGPR upon growth of host plants.

Conclusion

Microbes needs optimum content of organic matter in order to have their growth, if soil rich in organic matter content it known to influence and horbour even endophytes in

rhizosphere soil. Hence it is quite essential to improve organic matter content in order to boost the population of beneficial organisms in the rhizosphere soil.

REFERENCES

An, Q.L., X.J. Yang, Y.M. Dong, L.J. Feng, B.J. Kuang and J.D. Li. 2001. Using conofocal laser scanning microscope to visualize the infection of rice roots by GFP-labelled *Klebsiella oxytoca* SA_2, an endophytic diazotroph. Acta Bot. Sinica, 43: 558-564.

Day, J. M. and J.dobereiner. 1976. Physiological aspects of nitrogen fixation by a *Spirillum* from digitaria roots. Soil Boil. Biochem., 8: 45-50.

Dobereiner, J. 1988. Isolation and identification of root associated diazotrophs. Plant Soil, 110: 207-212.

Dobereiner, J., V.M. Reis and A.C. Lazarini. 1988. New N_2 fixing bacteria in association with cereals and sugarcane. In: Nitrogen Fixation: Hundred years after. (eds.) H. Bothe, F.J. de Bruijn and W.E. Newton, Gustav Fischer, Stuttgart, Germany, pp.712-722.

Geetha, S. 2003. Studies on the mycotrophy of certain medicinal herbs and its antimicrobial property. M.Sc.(Ag.) Thesis, Annamalai Univ., Annamalai Nagar.

Jackson, J. 1973. The Remedial options for metal contaminated sites. Curr. Opin. Biotechnol., 5: 285- 290.

Karthikeyan, a. S. Palanivel. S. Parvathy and r. Bhakya raj, 2007a Hairy root induction from hypocotyl segments of groundnut (Arachis hypogaea L.) African Journal of Biotechnology 6 : 1817-1820.

Kloepper, J. W., R. Lifshitz and R. M. Zahlotouicz. 1989. Free - living bacterial inocula for enhancing crop productivity. Trends in Biotechnology, 7: 39 - 44.

Lopez de Victoria, G. and Lovell,C.R. 2000. Chemotaxis of *Azospirillum* species to aromatic compounds. Appl. Environ. Microbiol., 59(9):2951-2955.

Ma, W., K. Zalec and B.R. Glick. 2001. Biological activity and colonization pattern of the bioluminescence labeled plant growth promoting bacterium *Kluyvera ascorbata* SUD 165/26. FEMS Microbiol. Ecol., 35: 137-144.

Mishra,R.K., Om Prakash, A.K. Tiwari, A.Pandy, M. Alan and A. Dikshit 2011. Culture filtrate antibiosis of plant growth promoting rhizobacteria PGPB against phytopathogens infecting medieated and aromatic plants. Int.J. RES. Biol., Sci., 1 (4): 45-51

Muralikrishnan, V. and Muthukaruppan, S.M. 1998. Effect of inoculation of *acetobacter diazotrophicus* with different levels of nitrogen on the yield of sugarcane variety CoC 92061. Madras Agric. J., 85: 556-557.

Okon,Y.,kapulnik,Y. and sarig,S. 1988. Fields inoculation studies with *Azospirillum* Inisrael. In biological nitrogen fixation: recent developments (ed) subbaral,N.S. oxford and IBH publishing company, new Delhi, pp 175-195.

Olsen, S.R., C.V. Cole, F.S. Watenabe and L. Peon. 1954. Estimation of available phosphorus in soils by extraction with sodium bicarbonate. U.S.D.A. Cere, U.S. root printing office. Washington D.C., Pp. 40-45.

Patriquin, D.G., L.A. Graciolli and A.P. Ruschel. 1980. Nitrogenase activity of sugarcane propagated from stem cutting in sterile vermiculite. Soil Biol. Biochem., 12: 413-417.

Pazhaniraja. P and Prabudoss. V 2014 Zinc Solubilizing Nature of *Gluconacetobacter diazotrophicus* Indian Streams Research Journal Volume-4, Issue-5, June-2014.

Prabudoss, V. and D. Stella. 2010. Growth enhancing association of *Gluconacetobacter diazotrophicus* and AM fungi in sugarcane. J. Curr. Res., 4: 140-141.

Sakthivel and Karthikeyan. 2012. Isolation and characterization of plant growth promoting (PGPR) from the rhizosphere of *Coleus forskohlii* . Int. J.Recent. Sci. Res. # (5): 288-296.

Sathyan, R. and M. Thangaraju. 2003. Isolation and characterization of endophytic nitrogen fixing bacterium *Acetobacter diazotrophicus* from the tissues of sweet potato *(Ipomoeabatatus).* Madras Agric. J., (In Press).

Seldin,L., Van Essas, J.D. and Penido, E.G.C. 1984. *Bacillus azotofixans* sp. nov., nitrogen fixing species from brazillian soil and grass roots. Int. J. Syst. Bacteriol., 34: 451-456.

Subbiah, B.V and G.L. Asjia. 1956. A rapid procedure for estimation of available nitrogen in soils. Current Science, 25: 259-260.

Walkely, A., and I.A. Black ,1947. Chronic and titration method for determination of soil organic matter. Soil Sci, 63: 251.

****