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Research Article



PGPB – Plant Growth Promoting Rhizobacteria and their influence on the growth and yield of sugarcane – *Saccharum officinarum*. L

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Abstract

PGPB – Plant growth promoting bacterial organisms are vital group of beneficial organisms found in the rhizosphere, rhizoplane and sometimes in plant parts as endophytes. In the present research PGPB organisms namely *Azospirillum*, *Gluconacetobacter* and *Bacillus* were enumerated from the rhizosphere of sugarcane and the results clearly showed the dominance of *Azospirillum* and *Bacillus* in the rhizosphere where as poor population of *Gluconacetobacter* was also noticed in the present study. In the pot culture experiment treatment T₉ . 50 % NPK and PGPB consortium recorded significant values in growth parameters and yield parameters compared with other treatments except the treatments constitute either 100% NPK or 100% NPK + consortium or 100% NPK with single inoculation of PGPB organisms, in addition the best treatment T₉ recorded on par values with T₁₄-75%NPK +PGPB consortium.

Keywords: PGPB, *Azospirillum*, *Gluconacetobacter* and *Bacillus*, rhizosphere of sugarcane

Introduction

PGPB organisms are the group of bacterial organisms which are multibeneficial to crop plants constituting nitrogen fixing, phosphate and zinc solubilizing. Siderophore producing, growth promoting substances producing and biocontrolling bacterial organisms are now used in agriculture as multibeneficial plant growth promoting organisms.

Sugarcane is being long duration crop needs much amount of nutrients inputs for their usual and normal growth, development and yield, if any of required nutrients has not supplied in needed amount then the yield will tend to decline, in this junction farmers are using huge quantum of chemical fertilizers. Hence without knowing the proper knowledge on pollution, farmers who are cultivating sugarcane crop by polluting the soil by way of adding enormous amount of NPK fertilizers, in addition farmers are also using pesticides which causes several and severe alteration in the physical, chemical and biological properties of soil, water and air and leads to cause injurious effects

on all types of living organisms starting from the minute living things i.e. microorganisms to the superior living things in the world i.e. human beings. Naturally these kinds of toxic chemical substances enters into the food chains and by which it causes alterations in the metabolic process of individual cells and leads to poor immune system in human beings, ultimately sensitive to several varieties of bacterial, fungal and viral infections. In some cases these toxins of pesticides directly causes lethal effect on living things.

Hence the scientific word must find a suitable ecofriendly technology for the cultivation of long duration crop like sugarcane.

The present research aimed to utilize multibeneficial bacterial organisms namely PGPB as an alternative and substitute for the hazardous chemical fertilizers and pesticides.

Materials and Methods

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 collected and from which 1g was seriously diluted upto 10^{-5} then from the dilution 10^{-5} 1 ml aliquot was transferred to various enrichment media viz., semisolid diluted cane juice medium, semisolid LGI medium and semisolid acetic LGI medium supplemented with yeast extract (20mg l^{-1}) the tubes were incubated at room temperature without disturbance until the formation of sub surface pellicles.

Isolation of *Azospirillum*

Ten fold serial dilution of each soil sample, ranging from 10^{-1} to 10^{-4} were made in mineral salts solution of Day and Dobereiner (1976). One ml of each dilution was inoculated in a set of five tubes containing 9 ml of nitrogen free semisolid malate medium (Day and Dobereiner, 1976). At least three consecutive dilutions were inoculated and tubes were incubated for three days at $30 \pm 2^\circ\text{C}$ finally tubes showing sub – surface, thin pellicle growth were identified as positive tubes for *Azospirillum*. The MPN counts of *Azospirillum* were calculated on the basis of positive tubes by referring the MPN table (Cochran, 1950).

Isolation of *Bacillus*

10 g of sugarcane rhizosphere soil samples placed in a conical flask containing 100 ml sterile water blank and vigorously shaken. The rhizosphere soil suspension

serially diluted to 10^{-6} . One ml aliquots of 10^{-5} and 10^{-6} dilutions were transferred to sterile petriplates and poured with apatite medium (Katznelson and Bose, 1959). After mixing the medium with aliquots in the plate by rotating clock and anticlockwise, the medium was allowed to solidify and plates were incubated at room temperature for 3 to 5 days. The number of colonies was counted and the population was estimated.

The colonies showing clear zone around were picked, streaked over nutrient agar medium and purified on Pikovskaya's agar. The purified culture was screened for phosphate solubilization in liquid medium and screened for phosphate activity. The identified best isolate was maintained in agar slants for further use.

Results and Discussion

In the present research about five places from Cuddalore District were selected and surveyed for the occurrence of PGPB organism namely *G. diazotrophicus*, *A. brasilense* and *B. megaterium*, among the places vallampadugai recorded significant population for all the PGPB organisms. In comparison *G. diazotrophicus* population was very meager when compared to other PGPB organisms and even in two rhizosphere soil samples the population was zero. While analysing the population and nutrient content there was some correlation that is, if the soil shows low N content and rich in organic matter showed some amount of *G. diazotrophicus* population and other PGPB organisms also and while seeing other parameters, the soil pH is more than the population was less in all PGPB organisms. The present findings are in accordance with Pazhaniraja and Prabudoss 2014

Table 1 Enumeration of plant growth promoting rhizobacteria (PGPB) from the rhizosphere soils of sugarcane

Sl. No.	Locations	Populations (1×10^4 cfu g^{-1} on oven dry weight)		
		<i>Gluconacetobacter</i>	<i>Azospirillum</i>	<i>Bacillus</i>
1	Vilagam	2.00	7.6	4.00
2	Sivapuri	2.0	4.6	4.6
3	Vallampadugai	4.0	8.6	8.00
4	Erumpur	0.00	4.6	3.6
5	Killai	0.00	3.6	3.00

Effect of microbial consortium with graded levels of NPK on the growth and yield of sugarcane variety CoC24 under pot culture

The pot culture experiments showed the efficiency of PGPB organisms on boosting the growth, development and yield of sugarcane. The treatment T₉ *Gluconacetobacter*, *Azospirillum* and *Bacillus* along with graded levels of NPK i.e. 50% N, P and K recorded much significant values in yield parameters compared with control and T₉ recorded Milleable

canes (104.0) 000 ha⁻¹, Individual cane weight (kg) (1.50) and Cane yield t ha⁻¹(118.0) respectively milleable cane individual cane weight and cane yield.

The best treatment T₉ recorded values on par values which are constitute 100 per cent NPK either with single or consortium inoculated treatments with T₁₄ - (75% NPK + T₁+T₂+T₃) and treatments T₁₅, T₁₆, T₁₇, T₁₈ and T₁₉. And recorded on par with one another and values were presented in table-2.

Table 2Effect of microbial consortium with graded levels of NPK on the growth and yield of sugarcane variety CoC24 under pot culture study

Sl.No.	Treatments	Milleable canes (000 ha ⁻¹)	Individual cane weight (kg)	Cane yield (t ha ⁻¹)
1	T ₀ – Absolute control			
2	T ₁ – <i>A. brasilense</i>	58.8	0.70	54.60
3	T ₂ – <i>G. diazotrophicus</i>	62.0	0.86	60.00
4	T ₃ – <i>B. megaterium</i>	58.6	0.69	54.20
5	T ₄ - T ₁ +T ₂ +T ₃	63.0	0.89	62.00
6	T ₅ – 50% NPK alone	88.0	0.98	90.00
7	T ₆ - 50% NPK + <i>A. brasilense</i>	89.0	1.00	110.0
8	T ₇ - 50% NPK+ <i>G. diazotrophicus</i>	90.0	1.10	112.0
9	T ₈ - 50% NPK + <i>B. megaterium</i>	89.0	1.00	110.0
10	T ₉ - 50% NPK + T ₁ +T ₂ +T ₃	104.0	1.50	118.0
11	T ₁₀ – 75% NPK alone	100.0	1.30	112.0
12	T ₁₁ - 75% NPK + T ₁ – <i>A. brasilense</i>	101.0	1.30	112.0
13	T ₁₂ - 75% NPK + T ₂ – <i>G. diazotrophicus</i>	100.0	1.20	112.0
14	T ₁₃ – 75% NPK + T ₃ – <i>B. megaterium</i>	98.00	1.00	112.0
15	T ₁₄ - 75% NPK + T ₁ +T ₂ +T ₃	104.0	1.50	118.0
16	T ₁₅ – 100% NPK alone	103.0	1.48	116.0
17	T ₁₆ - 100% NPK + T ₁ – <i>A. brasilense</i>	103.0	1.49	116.0
18	T ₁₇ - 100% NPK + T ₂ – <i>G. diazotrophicus</i>	103.0	1.49	116.0
19	T ₁₈ - 100% NPK + T ₃ – <i>B. megaterium</i>	102.0	1.48	116.0
20	T ₁₉ -100% NPK+ T ₁ +T ₂ +T ₃	104.0	1.49	118.0
	SE	0.38	0.02	0.48
	CD	0.99	0.09	1.2

In the present investigation about 13 isolates of bacterial organisms were obtained from soil then they are screened for PGPB nature and from the screening studies the best bacterial PGPB organisms were selected and used for field experiments in the form of individual inoculation and combined inoculation i.e. in the form of consortium along with graded levels of N, P and K fertilizers. In the pot culture experiment all possibilities by using PGPB organisms with graded

levels of N, P and K were tried in the form of 20 different treatments.

Natural characters and adaptations of microbes are interesting features that decides the ecological balance of its population in the environment as well as environmental variations. In the open environment microbes can able to have its regular growth and activities for that they have the interesting regulatory system. Many microbes in the soil able to fix

the nitrogen from the atmosphere through a biological process called biological nitrogen fixation by the involvement of a vital enzyme namely nitrogenase. Hence the diazotrophs to regulate its nitrogen requirements in the similar way microbes is soil regulates other nutritional requirement also only in few cases microbes fails to synthesis some compounds like vitamins and growth promoting substances.

The present results showed the efficiency of PGPB organisms on boosting or enhancing the growth of sugarcane plants. Interestingly the combined inoculation of PGPB which constitute addition *Azospirillum*, *Bacillus megaterium* and *Gluconacetobacter* records moderate value in growth parameter whereas the same PGPB organisms along with 50% NPK treatment namely T₁₉ recorded significant values on growth parameters compared with all other 19 treatments included T₂₀ which constitute 100% addition of NPK fertilizers. Hence the present findings play a vital role in the reduction of usage of chemical fertilizers in the cultivation of sugarcane, without having much pollution on environment. The present findings in accordance with the findings of Cavalcante and Dobereiner 1988; Boddy *et al.*, 2001.

All the above studies are correlated with PGPB nature of bacterial organisms. Nitrogen and phosphorus are the essential nutrients which are largely influence the growth of microbes, plant, animals and human beings in addition growth promoting substances are vital factor which limits the growth of crop plants.

Hence based on above view and studies clearly showed the plant growth promoting bacteria efficiency of *Azospirillum brasilense*, *Gluconacetobacter diazotrophicus* and *Bacillus megaterium* and further an interest has stimulated to develop a consortium by using all the plant growth promoting bacteria organisms to ascertain their PGPB natural on sugarcane crop in natural environmental condition namely in field.

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