ISSN 2319 – 7730 EISSN 2319 – 7749

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Indian Journal of Science

Biofertilizer effect on growth of sugarcane (*Saccharum officinarum L.*) cultivated in Barak Valley, Assam

Chakraborty D[1*], Sharma GD[2], Deb B[3]

1. Research scholar, Microbiology Laboratory, Department of Life Sciences, Assam University, Silchar- 788011, Assam, India
2. Vice Chancellor, Bilaspur University, Chhattisgarh, India
3. Assistant Professor , Department of Botany, G.C College, Silchar-788004, Assam, India

***Corresponding author:** Research scholar, Microbiology Laboratory, Department of Life Sciences, Assam University, Silchar- 788011, Assam, India
Mob. +91-9435079602, E-mail- chakrabortydebamita09@gmail.com

Received 16 February; accepted 18 March; published online 01 April; printed 16 April 2013

ABSTRACT

The aim of this study is to evaluate the effect of *Azotobacter vinelandii, Paenibacillus polymyxa and Pseudomonas fluorescens* isolated from rhizosphere of field-grown sugarcane in Barak Valley, Assam, as nitrogen biofertilizer on sugarcane plant growth under pot experiment condition. Four types of treatment i.e control with no inoculums (T0), *Azotobacter vinelandii* (T1), *Paenibacillus polymyxa* (T2), *Pseudomonas fluorescens* (T3) were used as biofertilizer. The difference in the growth among different treatment is found to be statistically significant at 5% significance level.

Key Words: Sugarcane, biofertilizer, nitrogen, diazotrophs, nitrogenase activity

1. INTRODUCTION

Diazotroph: Diazotrophs are bacteria and archaea that fix atmospheric nitrogen gas into a more usable form such as ammonia. Examples of organisms that do this are rhizobia and *Frankia* (in symbiosis) and *Azospirillum*. All diazotrophs contain iron-molybdenum or -vanadium nitrogenase systems.

Biological nitrogen fixation: Biological nitrogen fixation (BNF) is the term used for a process in which nitrogen gas (N_2) from the atmosphere is incorporated into the tissue of certain plants. Only a selected t group of plants is able to obtain N this way, with the help of soil microorganisms.

Nutrient requirement of sugarcane crop is very high. An estimated N requirement of sugarcane is about 150 Kg N ha^{-1}. Biofertilizer application provides equivalent output to 30-40 kg/ha N chemical fertilizer (Chauhan, et al., 2010). Evidence for biological nitrogen fixation in sugarcane (*Saccharum spp.*) was reported in Brazilian sugarcane varieties (Baldani, et al., 2002). Studies on long-term N-balance and 15N isotope dilution technique (Urquiaga, et al., 1992) have shown that some sugarcane varieties may actually obtain up to 70% of their N requirements by biological nitrogen fixation. Both rhizospheric and endophytic diazotrophs seems to participate in this process (Baldani, et al., 1997). The use of these diazotrophs as biofertilizer could promote sugarcane yield equivalent or greater as promoted by the recommended chemical N fertilizers (Muthukumarasamy, et al., 1999). Three different types of nitrogen fixing bacteria *viz*, *Azotobacter vinelandii*, *Paenibacillus polymyxa* and *Pseudomonas fluorescens* were isolated from rhizosphere of field-grown sugarcane in Barak Valley, Assam. Nitrogenase activity of *Azotobacter vinelandii, Paenibacillus polymyxa* and *Pseudomonas fluorescence* was found to be 403.05, 209.15 and 107.80 (nM C_2H_2/h /mg protein) respectively (Chakraborty et al., 2012).

2. SCOPE OF THE STUDY

The experiment was undertaken to evaluate the efficacy of the native diazotrophic strains isolated from the sugarcane agro-ecosystems of South Assam, India in sugarcane growth promotion.

2.1. Materials

The materials used include: Sugarcane sets, *Azotobacter vinelandii*, *Paenibacillus polymyxa* ,*Pseudomonas fluorescens* ,charcoal, steel tape, pots , pipette, weighting balance, conical flasks.

2.2. Methodology

Three different types of nitrogen fixing bacteria *viz, Azotobacter vinelandii*, *Paenibacillus polymyxa* and *Pseudomonas fluorescens* with nitrogenase activity of 403.05, 209.15, 107.80 (nM C_2H_2/h /mg protein) respectively were previously isolated from rhizosphere of field-grown sugarcane in Barak Valley, Assam using Burk's media as described by Chakraborty, et al (2012). Pure cultures of the strains were maintained in nutrient broth containing 50% glycerol at -80^{o}C.

2.3. Experimental design

The experiment was laid out in pots with four different treatments *viz.* control with no inoculum (T0), *Azotobacter vinelandii (*T1*), Paenibacillus polymyxa* (T2), *Pseudomonas fluorescens* (T3). Each treatment was replicated 10 times. The

Experiment
Research method for testing different assumptions (hypotheses) by trial and error under conditions constructed and controlled by the researcher. During the experiment, one or more conditions (called independent variables) are allowed to change in an organized manner and the effects of these changes on associated conditions (called dependent variables) is measured, recorded, validated, and analyzed for arriving at a conclusion.

experimental units were arranged in completely randomized design.

Rhizosphere: It is the soil zone that surrounds and is influenced by the roots of plants. Within the rhizosphere, roots secrete a slimy lubricating substance, called mucigel that cause the particles of soil to adhere to the roots, assisting in the uptake of water, and encourages the growth of nitrogen-fixing bacteria and other beneficial microorganisms.

2.4. Inoculation of Sugarcane in Pot Experiment

For inoculation of plants, selected bacterial strains were grown in 100 ml of LB liquid medium in a water bath (25°C; 150 rpm) for overnight The cells in active growth stage were harvested by centrifugation at 10,000 rpm for 10 minutes and the pellets were resuspended in sterile distilled water to attain a concentration of 10^8 cfu ml^{-1} .Then sterilized charcoal powder was mixed with the aqueous suspension of the diazotroph strains to prepare carrier based inoculants for sugarcane plants grown in pots. Sets with two buds were used in this experiment. The sets treated with the slurry of sterilized charcoal powder, devoid of diazotroph strain were used as control.

2.5. Growth observation

The plant height was measured from tip of the top most leaf to the base of the stem by using steel tape (5 m length) after 90 days.

2.6. Statistical Analysis

The data for each treatment were subjected to a variance analysis .When analysis of variance showed significant treatment effects; the treatment means were differentiated by a Tukey's honest significant difference (HSD) at $P = 0.05$.

3. RESULTS

Table 1 Effect of nitrogen fixing bacteria on sugarcane plant height

Treatment	Height (cm) after 90 days
T0	55.5 ^{d}
T1	224.9 ^{ab}
T2	209.7 ^{abc}
T3	199.6 ^{abc}

Means followed by the same letters in the columns is not significantly different at P ≤ 0.05

The results pertaining to the bioinoculation effect of diazotrophs were presented in Table 1. Differences between inoculated treatments and the controls were visible in all experiments. In all assays, T1 *(Azotobacter vinelandii)* performed best and followed by T2 *(Paenibacillus polymyxa*). The difference in the growth among different treatment is statistically significant at 5% significance level.

Biofertilizer: A biofertilizer (also *bio-fertilizer*) is a substance which contains living microorganisms which, when applied to seed, plant surfaces, or soil, colonizes the rhizosphere or the interior of the plant and promotes growth by increasing the supply or availability of primary nutrients to the host plant.

4. DISCUSSION

A possible factor for the increased yield of the inoculated sugarcane plants over the uninoculated controls may be that N_2-fixation contributes N whenever the plants require it, as suggested by Bashan, et al. (1989) for *Azospirillum* inoculation. Improvement in height due to use of diazotrophs may be due to the ability of the inoculants to produce some biological active compounds such as gibberellins and vitamins which can stimulate plant growth directly (Martinezteledo et al., 1988). Muhammad, et al (2011) also observed beneficial effects of biofertilizer inoculation on sugarcane grown in pots. Increase in sugarcane yield by the inoculation of endodiazotrophs has been reported by Oliverira *et al.* (2003). Muthukumarsamy, et al (1999) have observed in a field trial that plants co-cultivated with endophytes accumulate more N in comparison to control fertilized with commercial N fertilizers. Cong, et al. (2009) also found that inoculants (*Pseudomonas* sp. and *Bacillus* sp.) significantly improved the efficiency of N uptake and could save up to 43 kg of N fertilizer ha^{-1} together with increasing rice yield by 270 kg ha^{-1}. Many other scientists also reported the beneficial effects of diazotrophs in enhancing nutrient uptake by non-legume plants such as rice (Biswas, et al., 2000).

5. CONCLUSION

From the study it can be concluded that application of diazotrophs as biofertilizer can influence growth of sugarcane. Thus the study indicated the suitability of *Azotobacter vinelandii, Paenibacillus polymyxa* and *Pseudomonas fluorescens* as candidate for biofertilizer production for sugarcane growth promotion.

SUMMARY OF RESEARCH

1. This work, within the limit of available resource, has provided useful information about the growth promoting effect of native diazotrophs isolated from rhizosphere of field grown sugarcane in Barak Valley, Assm.
2. It has availed scientists the opportunity to research more on the application of *Azotobacter vinelandii, Paenibacillus polymyxa* and *Pseudomonas fluorescens* as biofertilizer.

FUTURE ISSUES

Azotobacter vinelandii, Paenibacillus polymyxa and *Pseudomonas fluorescens* can be processed for the commercial production of biofertilizer in large scale.

DISCLOSURE STATEMENT

There is no financial support for this research work from any funding agency.

ACKNOWLEDGMENT

Thanks are due to authorities of Assam University, Silchar and G.C College, Silchar for providing laboratory facilities to carry out the research work.

REFERENCE

1. Baldani J, Reis VM, Baldani LD, Döbereiner JA. Brief story of nitrogen fixation in sugarcane — reasons for success in Brazil. *Functional Plant Biology.,* 2002, 29(4), 417 – 423
2. Baldani JI, Caruso L, Baldani VLD, Goi SR, Döbereiner J. Recent advances in BNF with non-legume plants. *Soil Biol. Biochem*., 1997, 29, 911–922
3. Bashan Y, Levanony H, Mitiku G. Changes in proton efflux of intact wheat roots induced by *Azospirillum brasilense*. *Can J Microbiol*., 1989, 35, 691–697
4. Biswas JC, Ladha JK, Dazzo FB, Yanni YG, Rolfe BG. Rhizobial inoculation influences seedlings vigor and yield of rice. *Agronomy Journal.,* 2000, 92, 880-886
5. Chakraborty D, Sharma GD, Deb B. Diversity of Free Living Nitrogen Fixing Bacteria in Sugarcane Rhizosphere of Barak Valley, Assam. *J. pure applied Microbiol*., 2012, 6(3), 1351-1355

6. Chauhan H, Sharma A, Siani SK. Response of sugarcane to endophytic bacterial inoculation. *Indian Journal of Sugarcane Technology, 2010,* 25(1&2),1-4
7. Cong PT, Dung TD, Hien TM, Hien NT, Choudhury ATMA, Kecskes ML ,Kennedy IR. Inoculant plant growth promoting microorganisms enhance utilization of urea-N and grain yield of paddy rice in southern Vietnam. *European Journal of Soil Biology* ., 2009, 56, 52-61
8. Martinez-Teledo MV, Rubiadela T, Moreno J, Gonzalezlopaez J. Root exudates of zea mays and production of auxins, gibberellins and cytokines by *Azotobacter chroococcum. Plant and Soil* ., 1988, 111,149-152
9. Muhammad AA, Rasool M, Sajjad MM. Nitrogen Fixation and Indole Acetic Acid Production Potential of Bacteria Isolated from Rhizosphere of Sugarcane (*Saccharum officinarum* L.). *Advances in Biological Research*., 2011, 5(6), 348-355
10. Muthukumarasamy R, Revathi G, Lakshminarasimhan C. Influence of N fertilisation on the isolation of *Acetobacter diazotrophicus* and *Herbaspirillum* spp. from Indian sugarcane varieties. *Biol. Fert. Soils.,* 1999, 29, 157–164
11. Oliverira AM, Canuto EL, Reis VM, Baldani JI. Response of micro propagated sugarcane varieties to inoculation with endophytic diazotrophic bacteria. *Brazilian Journal of Microbiology .,* 2003, 34, 59–61
12. Urquiaga S, Cruz KHS, Boddey RM. Contribution of nitrogen fixation to sugarcane: Nitrogen-15 and nitrogen balance estimates. *Soil Sci. Soc. Am. J*., 1992, 256, 105–114