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Isolation and Characterization of Diazotrophic Bacteria and Its Effects on Growth and Yield of Sugarcane

K.M. Alam[1]*, S. Islam[1], M.S. Islam[2], M.A. Haque[1], A.S. Mitu[1], M.S. Rahman[3] and G.M.A. Hossain[1]

[2] Station Incharge, BSRI Sub-station, Joypurhat

[1]Soils and Nutrition Division, [3]Agronomy and Farming Systems Division

Bangladesh Sugarcrop Research Institute, Ishurdi-6620, Pabna, Bangladesh

ABSTRACT

The study was conducted both in the laboratory and field at Bangladesh Sugarcrop Research Institute during 2013-2014 cropping year. Two diazotrophic bacterial strains were isolated from the surface sterilized stems of sugarcane. The study was aimed to isolate endophytic nitrogen-fixing bacteria and to assess its performances on the growth and yield of sugarcane. The two species were characterized based on 16S rRNA gene sequences named as *Pantoea agglomerance* and *Klebsiella pneumoniae*. Nitrogen fixing ability was verified by the acetylene reduction assay that was found 6.98 to 7.60 nmole C_2H_4/ml/h. The highest indole acetic acid production was found in *Klebsiella pneumoniae*, which was 62.0µg/ml. A field experiment was carried out with six treatments viz., T_1 = No fertilizers and bacterial strains, T_2 = Recommended Dose of Fertilizers (RFD), T_3 = 50% N of RFD + *Klebsiella pneumoniae*, T_4 = 50% N of RFD + *Pantoea agglomerans*, T_5 = 75% N of RFD + *Klebsiella pneumoniae*, T_6 = 75% N of RFD + *Pantoea agglomerans* and two sugarcane varieties Isd 37 and Isd 39. The variety Isd 37 showed the highest germination, Brix per cent and yield in sugarcane. The highest pol per cent was found in T_5 (10.84%) where 75 % N of RFD + *Klebsiella pneumoniae* was applied. Considering the interaction effect, it was found that V_1T_5 produced the highest yield of cane (99.26 tha^{-1}) where 75 % N of RFD + *Klebsiella pneumoniae* was applied in the variety Isd 37. Thus it revealed that N_{124} $P_{55}K_{120}S_{30}Zn_{2.5}Mg_{10}B_{1.5}$ + *Klebsiella pneumoniae* was found the best treatment combination of nitrogen-fixing bacteria and inorganic fertilizer for higher sugarcane yield.

Key words: Sugarcane, diazotrophs, yield, nitrogen fixation

INTRODUCTION

Sugarcane is a long duration and exhaustive sugar crop, providing over 76% of the sugar for human consumption. An estimated N requirement of sugarcane in Bangladesh is about 165 Kg N ha^{-1}. The use of elevated doses of fertilizer may have negative and unpredictable effects on the environment, and contribute to the contamination of soil, water and natural areas. Such impacts pose a serious threat to human and animal health. In addition, developing countries have to face the demand of high costs for such technology and chemical utilization. An interesting option for decreasing the use of chemical fertilizers could be the exploitation of nitrogen fixing bacteria. Several nitrogen fixing bacteria such as *Enterobacter cloacae*, *Bacillus*

* Corresponding author: K.M. Alam, Senior Scientific Officer
e-mail: mohiulalam74@yahoo.com

polymyxa, *Klebsiella pneumoniae*, *Azotobacter vinelandii*, *Azospirillum spp. Herbaspirillum seropedicae* and *Acetobacter diazotrophicus* have been isolated from different internal or external parts of sugarcane plants (Baldani *et al.*, 1987; Oliveira *et al.*, 2003). The bacteria's ability to provide its host sugarcane, a monocot, with large amounts of fixed nitrogen without the formation of nodules led to the recognition of its importance. These bacteria may provide a natural and harmless means to improve the growth and yield of crops, thereby minimizing the use of agrochemicals. Presence of symbiotic or free living N_2 fixing bacteria has been reported in non legumes too like sugarcane. It is reported that several Brazilian sugarcane varieties are capable of obtaining over 60% of their nitrogen (>150 kg N ha^{-1} $year^{-1}$) from BNF through free-living and associative bacteria in sugarcane (Boddey *et al.*, 1995). It is necessary to replenish the soil with plant nutrients to increase crop productivity along with maintenance of soil health. This could only be possible by the use of bacterial supplements either partially or in integrated way of nutrient management. The use of these microbes as biofertilizer would support sugarcane yield equivalent or greater as supported by the recommended chemical N fertilizers (Muthukumarasamy *et al.*, 1999). Hence, the study was conducted to isolate endophytic nitrogen-fixing bacteria, determine the nitrogen-fixing ability, test the indole acetic acid production and its performances on sugarcane growth and yield in combination with inorganic fertilizer as well as maintenance of soil fertility.

MATERIALS AND METHODS

The study was conducted both in the laboratory and field at Bangladesh Sugarcrop Research Institute during 2013-2014 cropping year. In the laboratory the following studies were performed.

Isolation of bacterial endophytes

Sugarcane plant samples were collected from experimental field of Bangladesh Sugarcrop Research Institute. Separated root, stem and rhizospheric soil samples were used for bacterial isolation. For bacterial isolation outer layer of stem was removed, washed with sterilized distilled water and cut into pieces, sterilized with 70% alcohol for 5 min and washed thrice with sterilized water, then with 2% $HgCl_2$ for 2 min and washed 5-6 times with sterilized distilled water. The plant material was macerated in water containing 10% sugar to isolate endophytes in semisolid LGI vials according to Dobereiner *et al.* (1987). Root pieces were also washed with sterilized distilled water followed by sterilization with 70% alcohol for 5 min. After washing three times with sterilized water, root samples were placed in 0.2% $HgCl_2$ for 5 min followed by several washings with sterilized distilled water. Hand homogenized root material was placed as described for shoots. Soil samples were serially diluted and used for bacterial isolation similarly as described for roots and stem samples.

Morphology of the strains

Bacterial growth from semi-solid LGI vials, with a white, pink, yellowish pellicle below the surface, were streaked on LGI agar plates and incubated for 72 h. The colony morphology and estimate colony forming unit were counted by colony counter. The gram stain reactions were observed on an optical microscope.

Acetylene reduction assay

The nitrogenase activity of the isolates was carried out by acetylene reduction assay. The 10 fold serial dilutions of the suspension were used to inoculate N-free semisolid LGI media in triplicate (Estrada *et al.*, 2002). 1% acetylene gas was injected. After 96 h of incubation, vials were assayed for acetylene reduction activity (Mascarua-Esparza *et al.*, 1988).

Colorimetric estimation of indoleacetic acid production

Indoleacetic acid production was estimated by growing the isolates in NFb medium supplemented with NH_4Cl and 100 µg/mL DL-tryptophan at 30°C with shaking for 72 h in the dark. Five milliliter of each culture was centrifuged (20 min, 6,000×g), and indoleacetic acid production was measured as indolic compounds in 2 ml of supernatant by mixing with 2 mL of Salkowski reagent and following the absorbance at 535 nm after 30 min in the dark (Glickman and Dessaux, 1995). A standard curve was used for calibration.

DNA extraction

The isolates were grown in nutrient agar medium at 28^0C for 24 h and centrifuged at 12,300×g. The pellets were washed with TE buffer and resuspended in 10 mL of TE (1×) containing 3 mL of 5% SDS in TE (1×) and 3 ml of proteinase K (2.5 $mgml^{-1}$). The suspensions were incubated 37^0C for 1 h. After 1 h, the cleaned lysates were extracted with phenol: chloroform: isoamyl alcohol (25:24:1). DNA were precipitated by adding 0.1 volume of 3M sodium acetate (pH 5.2) and 2.5 volumes of ethanol to the supernatant. The dried pellets were dissolved in TE (1×) buffer (Govindarajan *et al.*, 2007).

PCR amplification and sequencing of 16S rRNA genes

The 16S rRNA genes were amplified by PCR using universal primers F27 (5)-AGAGTTTGATCATGGCTCAG-3) and R1492 (5-TACGGTTACC TTGTTACGACTT-3). The PCR amplifications were carried out in 50 µL reaction volumes with ddH_2O, 10×buffer, 2.5 $mmoll^{-1}$ dNTP, 10µ$moll^{-1}$ primer, DNA template and Pyrobest TM DNA polymerase. The reaction was set up. Products were visualized on a 1.5% agarose gel in 1×TBE buffer (Figure 1). The amplicons were purified with a PCR purification kit. The purified PCR products were sequenced bi-directionally with F27 and R1492 from Center for Advanced Research in Sciences (CARS), University of Dhaka. The BLAST was used for alignment analysis of the nucleotide sequences.

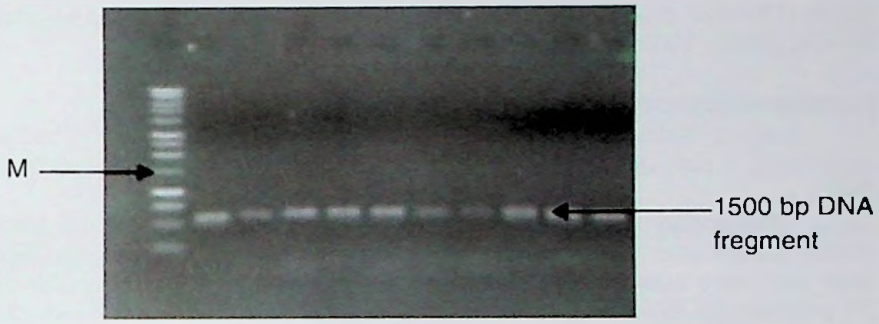

Figure 1. PCR product of the 16S rDNA bacterial strains M: Trans 15 K DNA marker.

Field trial

A field trial was conducted at experimental field of Bangladesh Sugarcrop Research Institute during 2013-2014 cropping season. The isolated two diazotrophic bacterial strains *Klebsiella pneumoniae* and *Pantoea agglomerans* were used in the experiment. Two eyed sugarcane setts were used in this study. The treatments of the experiment comprised of factor A: Fertilizer and bacterial strains: T_1 = No fertilizers and bacterial strains, T_2 = Recommended Dose of Fertilizers (RFD), T_3 = 50% N of RFD + *Klebsiella pneumoniae*, T_4 = 50% N of RFD + *Pantoea agglomerans*, T_5 = 75% N of RFD + *Klebsiella pneumoniae*, T_6 = 75% N of RFD + *Pantoea agglomerans* and factor B: Sugarcane variety: V_1: Isd 37 and V_2: Isd 39 used for test crop. The experiment was laid out in a Randomized Complete Block (RCB) in factorial design with three replications. There were twelve treatment combinations. The plot size of the experiment was 8m × 6 m. The recommended fertilizer rates $N_{165}P_{55}K_{120}S_{30}Mg_{10}Zn_{2.5}$ and $B_{1.5}$ were used. The crop was planted on 21 December 2013 and harvested on 28 December 2014.

Preparation of inoculants

The bacterial strains were grown in respective broth culture for two days. Then the broth was centrifuged at 6000 rpm for 5 minutes. After that supernatant was discarded and cell pellets were washed in to two times with sterile water and were collected in a 30 ml bottle. This solution was centrifuged by vortex mixture to homogenize the strains in sterile water. Bacterial concentration was adjusted using a spectrophotometer at 540 nm and 0.1 ml of suspension containing 10^8 cells was inoculated.

Setts soaking with inoculants

Two eyed sugarcane setts were soaked in 40 L suspension containing 500 ml bacterial culture for 30 minutes for the bacteria to adhere in the setts.

Fertilizer application

One third of nitrogen and one half of potassium and total phosphorus, sulphur and zinc were applied as basal dose and thoroughly mixed with the soil before planting. Remaining potassium and one third nitrogen were applied as top dressing at tillering

stage (120-150 days). The rest amount nitrogen was top dressed after completion of tillering (about 180 days).

Inoculants application rate

40 L bacterial suspension was prepared with 500 ml bacterial culture for inoculating 1 acre of land. Inoculants were applied at the base of seedling at 120 and 180 DAP.

Necessary intercultural operations were done throughout the cropping season for proper growth and development of the crop. Data were compiled and tabulated in proper form and were subjected to statistical analysis by using the computer package Statistix 10 program for Windows Version. Computation was done by the use of Microsoft Excel 2003 program.

RESULTS AND DISCUSSION

Isolation and morphological characteristics of N_2-fixing bacteria

After incubation of the N_2-fixing bacteria, it was found that the colonies on LGI agar plates were large, yellow, round and translucent with entire margin (Figure 2). These observations were supported by Govindarajan *et al.* (2007). In microscopic examination, the strains were gram negative and rod shaped. Many genera of endophytic nitrogen fixing bacteria were actually gram-negative (Reis *et al.*, 2004). The numbers of bacteria in the stem sample were of $0.1\text{-}2.4\times10^3$ CFU/g of dry weight. The nitrogen fixing bacteria, including *Klebsiella pneumoniae, Klebsiella oxytoca*, *Azospirillum* spp., *Herbaspirillum* spp., *Burkholderia* spp., *Enterobacter cloacae*, and *Pantoea* sp. were reported to have been isolated from the roots, stems and leaves of sugarcane (Mendes *et al.*, 2007).

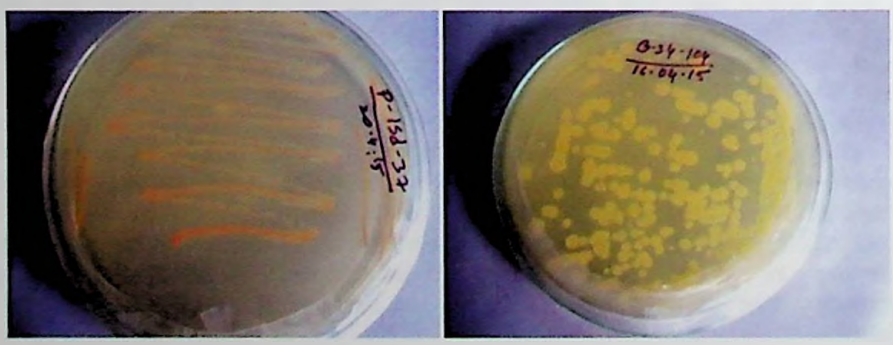

Figure 2. Colony of *Pantoea agglomerans* and *Klebsiella pneumoniae*

Acetylene reduction assay

After 96 hours of incubation, vials were assayed for acetylene reduction. The nitrogenase activity of *Klebsiella pneumoniae* was 7.60 nmol C_2H_4 ml^{-1} h^{-1} and the *Pantoea agglomarence* was 6.98 nmol C_2H_4 ml^{-1} h^{-1} (Table 1). Liu *et al.* (2011) stated that the acetylene reduction assay of *K. pneumoniae* NG14 was 47.56 nmol C_2H_4 ml^{-1} h^{-1}, which was isolated on the root surface of rice.

Table 1. **Estimation of acetylene reduction assay and indole acetic acid from nitrogen fixing bacteria**

Bacterial strain	nmol C_2H_4 ml^{-1} h^{-1}	IAA (µg ml^{-1})
Pantoea agglomarence	6.98	11.0
Klebsiella pneumoniae	7.60	62.0

Indole acetic acid (IAA) production

The strains grown in the minimal medium supplemented with tryptophan exhibited considerable production of IAA with amount of 11.0 µgml^{-1} from *Pantoea agglomarence and* 62.0 µgm^{-1} from *Klebsiella pneumoniae* (Table 1). According to the IAA production, it was found that the capacity to synthesize IAA is wide-spread among soil plant-growth-promoting bacteria such as *Azospirillum* spp. *Klebsiella* sp. and *Enterobacter cloace* (Spaepen *et al.*, 2007).

Bacterial position

The 16S rRNA gene sequenced results were submitted to National Center for Biotechnology Information of BLAST. The submitted sequenced results were highest 99% similarity to the bacteria of *Klebsiella pneumoniae* and *Pantoea agglomarence.*

Varietal effect

Due to sugarcane variety all growth parameters influenced significantly over control (Table 2). The highest germination was found in variety Isd 37 (70.99%) and the lowest was in Isd 39 (50.72%). The highest number of tiller was obtained in Isd 39 (140.58×10^3 ha^{-1}) and the lowest was in variety Isd 37 (121.88×10^3 ha^{-1}). The highest Brix (%) was found in variety Isd 37 (17.42 %) and the lowest was in Isd 39 (13.83%). The highest pol (%) cane was obtained in Isd 39 (11.93 %) and lowest was in Isd 37 (8.95 %). The highest yield of cane was obtained in Isd 37 (89.37 tha^{-1}) and lowest yield was in Isd 39 (81.90 tha^{-1}). Morais *et al.* (2011) reported that inoculation of nitrogen fixing bacteria in sugarcane varieties had significant differences between genotypes (G) and genotype xtreatment interaction (G×T) for all the traits.

Table 2. Effect of diazotrophic bacteria on growth and yield of sugarcane

	Germination (%)	Tiller ($\times 10^3 ha^{-1}$)	Millable Cane ($\times 10^3 ha^{-1}$)	Brix (%)	Pol (%)	Yield ($t ha^{-1}$)
Varietal Effect						
V_1	70.99 a	121.88 b	89.35	17.42 a	8.95 b	89.67 a
V_2	50.72 b	140.58 a	92.20	13.83 b	11.93 a	81.90 b
LSD (0.05)	1.62	3.06	NS	0.41	0.32	2.82
Treatment Effect						
T_1	55.36 b	101.01 b	67.13 b	16.60 a	11.14 a	61.16 b
T_2	61.44 a	139.81 a	92.68 a	15.18 ab	10.09 ab	87.73 a
T_3	61.76 a	139.15 a	96.29 a	15.48 ab	10.30 ab	86.24 a
T_4	64.00 a	135.41 a	97.86 a	14.38 b	9.51 b	93.18 a
T_5	62.72 a	135.37 a	96.41 a	16.13 a	10.84 a	95.31 a
T_6	59.86 ab	136.62 a	94.26 a	15.97 a	10.78 a	91.10 a
LSD (0.05)	2.80	5.30	3.13	0.70	0.56	4.88
Interaction Effect (Variety × Treatment)						
V_1T_1	66.24 b	100.81 d	67.83 c	15.43 bc	10.32 bc	61.91 c
V_1T_2	71.78 ab	129.81 bc	88.39 b	12.93 de	8.32 de	95.33 a
V_1T_3	71.57 ab	126.21 c	94.64 ab	13.73 cde	8.63 de	91.68 ab
V_1T_4	74.67 a	126.76 c	95.20 ab	12.00 e	7.53 e	95.43 a
V_1T_5	70.19 ab	121.48 c	96.04 ab	14.30 cd	9.22 cd	99.26 a
V_1T_6	71.51 ab	126.20 c	93.99 ab	14.57 cd	9.70 cd	94.42 ab
V_2T_1	44.48 d	101.20 d	66.43 c	17.77 a	11.96 a	60.41 c
V_2T_2	51.09 cd	149.81 a	96.97 ab	17.43 ab	11.86 ab	80.13 b
V_2T_3	51.95 cd	152.09 a	97.93 a	17.23 ab	11.97 a	80.79 b
V_2T_4	53.33 c	144.07 ab	100.51 a	16.77 ab	11.48 ab	90.93 ab
V_2T_5	55.25 c	149.26 a	96.79 ab	17.97 a	12.47 a	91.36 ab
V_2T_6	48.21 cd	147.04 a	94.53 ab	17.37 ab	11.85 ab	87.79 ab
LSD (0.05)	3.96	7.49	4.42	0.99	0.79	6.90
CV (%)	7.98	6.99	5.97	7.80	9.27	9.85

Fertilizer and bacterial strains: T_1 = No fertilizers and bacterial strains, T_2 = Recommended Dose of Fertilizers (RFD), T_3 = 50% N of RFD + *Klebsiella pneumoniae*, T_4 = 50% N of RFD + *Pantoea agglomerans*, T_5 = 75% N of RFD + *Klebsiella pneumoniae*, T_6 = 75% N of RFD + *Pantoea agglomerans* and sugarcane variety: V_1: Isd 37 and V_2: Isd 39.

Treatment effect

Due to different treatments all the growth parameter differed significantly over control (Table 2). The highest germination (%) was found in treatment T_4 (64.00 %) where 50 % N of RFD + *Pantoea agglomerans* was inoculated. All other treatment except control showed statistically similar germination (%). The highest number of tiller was found in T_2 (139.81 $\times 10^3$ ha^{-1}) which was statistically similar with all other treatments except control. Highest brix (%) was obtained in T_1 (16.60%) which was statistically at par

with all other treatments except T_4 (14.38 %). T_4 produced statistically similar brix (%) followed by T_3 (15.48%) and T_2 (15.18%). The highest pol percent cane was also found in T_5 (10.84%) which was statistically similar with all other treatment except T_4 (9.51%). The highest yield of cane was obtained in T_5 (95.31 tha^{-1}), which was statistically similar with all other treatments except control (61.16 tha^{-1}). Hari (1995) reported that inoculation of N fixing microbes to sugarcane have increased the cane yield by 5-15%, saved 25 kg fertilizer N ha^{-1} and also improved the juice quality parameter *viz*, sucrose and purity.

Interaction effect of variety × treatment

Due to interaction of sugarcane variety and different treatments all growth parameters influenced significantly (Table 2, Figure 3). Highest germination (%) was found in interaction V_1T_4 (74.67 %), which was statistically similar with interaction V_1T_2 (71.78 %), V_1T_3 (71.57 %), V_1T_5 (70.19 %) and V_1T_6 (71.51 %). The highest number of tiller was obtained in interaction V_2T_3 (152.09 $\times 10^3$ ha^{-1}) which was statistically similar with that of V_2T_2 (149.81 $\times$ 10^3 ha^{-1}), V_2T_5 (149.26 $\times$ 10^3 ha^{-1}), V_2T_6 (147.04 $\times 10^3$ ha^{-1}) and V_2T_4 (144.07 $\times$ 10^3 ha^{-1}). The highest number of millable canes was obtained in interaction of V_2T_4 (100.51 $\times$ 10^3 ha^{-1}) which was statistically at par with that of V_2T_3 (97.93 $\times 10^3$ ha^{-1}), V_2T_2 (96.97 $\times$ 10^3 ha^{-1}), V_2T_5 (96.79 $\times 10^3$ ha^{-1}), V_1T_5 (96.04 $\times$ 10^3 ha^{-1}), V_1T_4 (95.20 $\times 10^3$ ha^{-1}), V_1T_3 (94.641 $\times$ 10^3 ha^{-1}), V_2T_6 (94.53 $\times$ 10^3 ha^{-1}) and V_1T_6 (93.99 $\times$ 10^3 ha^{-1}). The highest pol percent cane was obtained in interaction of V_2T_5 (12.47 %) which was statistically at par with that of interactions V_2T_3 (11.97 %), V_2T_1 (11.96 %), V_2T_2 (11.86 %), V_2T_6 (11.85 %) and V_2T_4 (11.48 %). The highest yield of cane was obtained in interaction of V_1T_5 (99.26 tha^{-1}) which was statistically at par with that of interactions V_1T_4 (95.43 tha^{-1}), V_1T_2 (95.33 tha^{-1}), V_1T_6 (94.42 tha^{-1}), V_1T_3 (91.68 tha^{-1}), V_2T_5 (91.36 tha^{-1}), V_2T_4 (90.93 tha^{-1}) and V_2T_6 (87.79 tha^{-1}). Chauhan *et al.* (2010) supported our result where they reported that inoculation of diazotroph (*Gluconacetobacter*) to sugarcane significantly increased the plant height, cane length, number of millable canes resulting the cane yield increase by 42% over control.

Figure 3. Performance of diazotrophic bacteria in sugarcane

Soil fertility

Table 3 showed that the post harvest nitrogen content of some treatments was found much higher (0.06±0.006%) than initial soil nitrogen (0.05±0.005%). Inoculation of nitrogen fixing bacteria in sugarcane indicated that the post harvest nitrogen nutrient were also higher over control plot (0.05±0.005%). Chauhan *et al.* (2010) reported that application of nitrogen fixing bacteria to sugarcane significantly increased total nitrogen and chlorophyll content over control.

Table 3. Initial and post harvest nutrient status at BSRI Farm, Ishurdi

Treatment	OC (%)	pH	N (%)	P (ppm)	K (meq%)	S (ppm)
Initial Soil						
	0.62±0.06	7.20±0.72	0.05±0.005	7.0±0.70	0.12±0.01	42.0±4.2
Post Harvest Soil						
V_1T_1	0.60±0.06	7.30±0.73	0.05±0.005	6.5±0.65	0.12±0.01	30.0±3.0
V_1T_2	0.60±0.06	7.25±0.72	0.06±0.006	7.5±0.75	0.13±0.01	32.0±3.2
V_1T_3	0.59±0.06	7.35±0.73	0.06±0.006	7.0±0.70	0.12±0.01	30.0±3.0
V_1T_4	0.61±0.06	7.30±0.73	0.05±0.005	7.5±0.75	0.13±0.01	31.0±3.1
V_1T_5	0.60±0.06	7.25±0.72	0.06±0.006	7.0±0.70	0.14±0.01	30.0±3.0
V_1T_6	0.61±0.06	7.35±0.73	0.06±0.006	7.5±0.75	0.13±0.01	30.0±3.0
V_2T_1	0.59±0.06	7.30±0.73	0.05±0.005	6.0±0.60	0.11±0.01	29.0±2.9
V_2T_2	0.61±0.06	7.35±0.73	0.06±0.006	7.5±0.75	0.13±0.01	31.0±3.1
V_2T_3	0.60±0.06	7.30±0.73	0.05±0.005	7.8±0.78	0.12±0.01	32.0±3.2
V_2T_4	0.60±0.06	7.25±0.72	0.05±0.005	7.5±0.75	0.12±0.01	31.0±3.1
V_2T_5	0.59±0.06	7.30±0.73	0.06±0.006	7.0±0.70	0.13±0.01	31.0±3.1
V_2T_6	0.61±0.06	7.35±0.73	0.06±0.006	7.5±0.75	0.12±0.01	32.0±3.2

Fertilizer and bacterial strains: T_1 = No fertilizers and bacterial strains, T_2 = Recommended Dose of Fertilizers (RFD), T_3 = 50% N of RFD + *Klebsiella pneumoniae*, T_4 = 50% N of RFD + *Pantoea agglomerans*, T_5 = 75% N of RFD + *Klebsiella pneumoniae*, T_6 = 75% N of RFD + *Pantoea agglomerans* and sugarcane variety: V_1: Isd 37 and V_2: Isd 39.

The nitrogen fixing bacteria *Klebsiella pneumoniae* and *Pantoea agglomerans* were isolated from sugarcane stem. After the confirmation of identification, the bacteria were used for inoculation showing their potentiality in respect of sugarcane yield and quality. The varietal effect showed that highest germination, brix percent and maximum yield of cane was found in variety Isd 37 whereas the highest number of tiller and pol percent cane was found in variety Isd 39. The highest pol percent cane and yield of cane was found in treatment T_5 (10.84 % and 95.31 tha^{-1}, respectively). In case of interaction effect, the highest number of tiller was obtained in V_2T_3 (152.09×10^3 ha^{-1}) and the highest number of millable canes was obtained in V_2T_4 (100.51×10^3 ha^{-1}). But the highest brix percent (17.37%), pol percent (11.97%) was found in V_2T_5 on the other hand the highest yield of cane was obtained in interaction of V_1T_5 (99.26 tha^{-1}). Thus, N_{124} $P_{55}K_{120}S_{30}$ $Zn_{2.5}Mg_{10}B_{1.5}$ + *Klebsiella pneumoniae* was found the best treatments combination of nitrogen-fixing bacteria *Klebsiella pneumoniae* and inorganic fertilizer for higher sugarcane yield. Furthermore, the combined use of diazotrophic bacteria and inorganic

fertilizer was found as an eco-friendly that enhanced sugarcane productivity and maintained soil health in our results.

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