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Molecular Characterization of *Gluconacetobacter diazotrophicus* Isolated from Maize (*Zea mays* L.) and Its Interaction with Microbial Consortia on Growth and Yield

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Abstract

Gluconacetobacter diazotrophicus is an endophytic, Gram-negative, rod shaped, associative nitrogen fixing bacterium. The bacterium was isolated from roots of maize using LGI-P medium. The bacterium formed orange colour colonies on LGI-P medium. The genomic DNA was isolated and amplified using 16S rRNA primers. The results of the sequence obtained were BLAST searched at NCBI GenBank. The bacterium showed 98 per cent sequence homology with the *G. diazotrophicus* 263-A available in NCBI GenBank. The consortia of *G. diazotrophicus* MZ-01 + *B. megaterium* + *G. fasciculatum* showed significant increase in plant height, number of leaves, number of cob per plant, number of rows per cob, number of grains per cob, test weight, grain yield per plant, cob length, cob diameter, shoot and root biomass contents compared to single inoculation treatments. The un-inoculated maize plants (control) produced least growth and yield indicating the superiority of consortial inoculation.

Keywords: *G. diazotrophicus,* LGI-P medium, *b. megaterium, g. fasciculatum*

Maize (*Zea mays* L.) is one of the important staple food crop of the world and ranks next only to wheat and rice. Maize has been an important cereal because of its great production potential and adaptability to wide range of environment. Maize occupies an important place in Indian economy, like rice, wheat and millets. Besides, being a potential source of food for human being, it is used for feeding cattle, poultry and industries for the production of starch, syrup, alcohol, acetic acid, lactic acid *etc*. In India, maize is grown over an area of 9.18 million ha with a production of 24.17 million tonnes and the productivity is 2.63 t / ha (www.indiastat.com). In Karnataka, it is cultivated in an area of 1.34 million ha with production of 3.98 million tonnes and the productivity is 2.99 t / ha. (www.indiastat.com). Nitrogen gas comprises 78 per cent in the atmosphere. Despite its abundance in the atmosphere, the paradise of nature can't be assimilated by plants unless it is reduced to ammonia by special group of prokaryotic organisms called nitrogen fixers. The group of diazotrophs capable of colonizing the roots of non-legumes gained importance and the association has been termed as associative symbiosis or diazotrophic biocoenosis. *Gluconacetobacter diazotrophicus* (previously known as *Acetobacter diazotrophicus*) is a strict aerobe and a nitrogen fixing endophyte originally isolated from sugarcane roots and stems by Dobereiner (Bertalan *et al.,* 2009).

Gluconacetobacter diazotrophicus is found to live freely in the intercellular spaces of roots, stems and leaves of the sugarcane plant. This endophytic bacterium does not form any specific structures (like the nodules of legume plants) within plant tissues (Oliveira *et al.,* 2009). *Gluconacetobacter diazotrophicus* appears to grow in a wide spectrum of conditions (Bhavanath *et al.*, 2009). The association between *G. diazotrophicus* and plants is not species specific, like Rhizobia. In fact, *G. diazotrophicus* has been found in a number of unrelated plant species (Boddey *et al.,* 2013). The discovery that *G. diazotrophicus* associates as a free-living organism in plants, fixes nitrogen, and supplies nitrogen to plants, has attracted a broad interest in plant science (Oliveira *et al.,* 2009). Since *G. diazotrophicus* lives freely in plant tissues and does not form any special structures (like nodules) in plants and the bacterium appears to be non-species specific in its associations (unlike rhizobia), there is a possibility that the bacterium can be introduced into other plants for nitrogen fixation.

In this study, we isolated *G. diazotrophicus* from maize and studied its interaction effect with P solubilizer and arbuscular mycorrhizal fungus to influence the growth and yield of maize.

Material and Methods

Isolation of *G. diazotrophicus* from Maize roots

Roots were collected from Maize plant and cut into 2-3 cm bits. These root bits were surface sterilized by sodium hypochlorite solution (5%) for 5 minutes and was washed repeatedly using sterile water. Thus sterilized root bits were transferred in to test tubes containing LGI-P medium and incubated at 30º C for 7 days. After incubation, change of the medium to orange colour as well as formation of pellicles on the surface was observed for *G. diazotrophicus*. The bacterium was further purified by repeated streaking on fresh LGI-P agar plates. The pure culture so obtained was preserved on LGI-P agar slants in refrigerator for further use.

Molecular identification of the Bacterium using 16S rRNA gene sequence

Extraction of genomic DNA and PCR amplification : Total genomic DNA of the bacterium was extracted by alkaline lysis method (Nandhini *et al.,* 2014). The bacterium was grown in LGI-P broth for 48 hours at 30°C and 3 ml of bacterial culture was pelleted by centrifugation at 12,000 rpm. The bacterial pellet was re-suspended in 650 µl of extraction buffer (10mM Tris HCl pH 8.0, 20 mM EDTA and 250 mM NaCl) and incubated at 65°C for 30 minutes for lysis. To the extract, 100 µl of 5M Potassium acetate solution was added and placed on ice for 15 minutes for precipitation of protein and carbohydrates and clear supernatant was collected by centrifugation. DNA was precipitated by adding equal volume of ice cold Isopropanol and the DNA pellet was collected by centrifugation at 12,000 rpm. The pellet was twice washed with 70 per cent ethanol, air dried, and dissolved in 10mM TE (10:1) buffer stored in aliquots at -20°C. The quality and quantity of the isolated DNA was checked with 0.8 per cent agarose gel electrophoresis and spectrophotometrically.

Primer designing and PCR amplification using 16S rRNA : The primers already reported 16S rRNA sequences from the NCBI database (http://www.ncbi.nlm.nih.gov) were custom synthesized by Sigma-Aldrich (Sigma, USA) and diluted accordingly for the PCR reactions (A 26 bp of forward primer 5' GTTAGATCTTGGCTCAGGACGAACGC3' and 24 bp of reverse primer 5' GATCCAGCCGCACCTTCCGATACG 3'). PCR was performed in a 40µl reaction volume containing 1X buffer with $MgCl_2$ (1.5mM), dNTPs (200µM), forward and reverse primers (0.5µM each), *Taq* DNA polymerase (3U Genei Bangalaore) and template DNA (50ng). Amplification was carried out with an initial denaturation at 96°C for 3 minutes followed by 35 amplification cycles consisting of 94°C for 1 minute, 50°C for 30 seconds and 72°C for 1 minute and a final extension step at 72°C for 12 minutes. PCR products were separated on 1.0 per cent agarose gel and documented using gel documentation system Hero Lab, Germany (Nandhini *et al.,* 2014).

Pot experiment : Pot experiments were conducted in glass house of Department of Agricultural Microbiology, University of Agricultural Sciences, GKVK campus, Bengaluru. Soil and FYM were filled to 10 kg pot with 2:1 ratio, respectively, treatment details are as follows. T_1 = Soil (Control), T_2 = FYM (Control), T_3 = *G. diazotrophicus* MZ-01, T_4 = *Azotobacter chroococcum* (N_2 fixer ref. strain), T_5=*Bacillus megaterium* (PO_4 solubilizer ref. strain) T_6 = *Glomus fasiculatum* (Arbuscular mycorrhiza), T_7 = *G. diazotrophicus* MZ-01 + *B. megaterium* + *G. fasiculatum* and T_8 = *A. chroococcum* + *B. megaterium* + *G. fasiculatum*. In pot experiment growth and yield observations were recorded *viz*., plant height, number of leaves per plant, seed yield per plant, shoot dry weight and root dry weight.

Results and Discussion

Isolation and molecular identification of Gluconacetobacter diazotrophicus

The 16S rRNA partial gene sequence having 1089bp showed 98 per cent homology with *Gluconacetobacter diazotrophicus* strain 263-A available in the NCBI data base. Thus, the bacterium was identified as *Gluconacetobacter*

diazotrophicus. Phylogenetic tree constructed with sequence of 10 *Gluconacetobacter diazotrophicus* species present in NCBI showed that the new isolate *Gluconacetobacter diazotrophicus* MZ-01 as similar to *Gluconacetobacter diazotrophicus* strains (strain 232, Ac-C2.5, 176-B2-1, 165, 263-A, 202 and Ac-CF2.2) Fig. 1(a) and 1(b) available at NCBI GenBank.

GTCGCGGGCGCATGCTTACACATGCAGTCGCACGAACCTTTCGGGGTTAGTGGCGGACGGGTGAG
TAACGCGTAGGGATCTGTCCATGGGTGGGGGATAACTCCGGGAAACTGGAGCTAATACCGCATGA
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TTGCAGCATGATGGGTGGACATCTAAGGAAACTGCCGGATGAACAGC

Select: All None Selected:0

Alignments Download GenBank Graphics Distance tree of results

Description	Max score	Total score	Query cover	E value	Ident	Accession
Gluconacetobacter diazotrophicus strain 263-A 16S ribosomal RNA gene, partial sequence	1909	3050	97%	0.0	90%	KP969074.1
Gluconacetobacter diazotrophicus strain 232 16S ribosomal RNA gene, partial sequence	1989	3850	97%	0.0	98%	KP969072.1
Gluconacetobacter diazotrophicus strain 176-B2-1 16S ribosomal RNA gene, partial sequence	1989	3850	97%	0.0	98%	KP969070.1
Gluconacetobacter diazotrophicus strain 165 16S ribosomal RNA gene, partial sequence	1989	3850	97%	0.0	98%	KP969069.1
Gluconacetobacter diazotrophicus strain PAL 5 16S ribosomal RNA gene, complete sequence	1989	3850	97%	0.0	98%	NR_074284.1
Gluconacetobacter diazotrophicus PAL 5 complete genome	1989	154002	98%	0.0	98%	AM889285.1
Gluconacetobacter diazotrophicus strain PAL 5 16S ribosomal RNA gene, complete sequence	1984	3839	97%	0.0	98%	NR_074292.1

Fig. 1(a): *G. diazotrophicus* MZ-01 showing 98 per cent sequence homaology with 16S rRNA sequence present in NCBI database

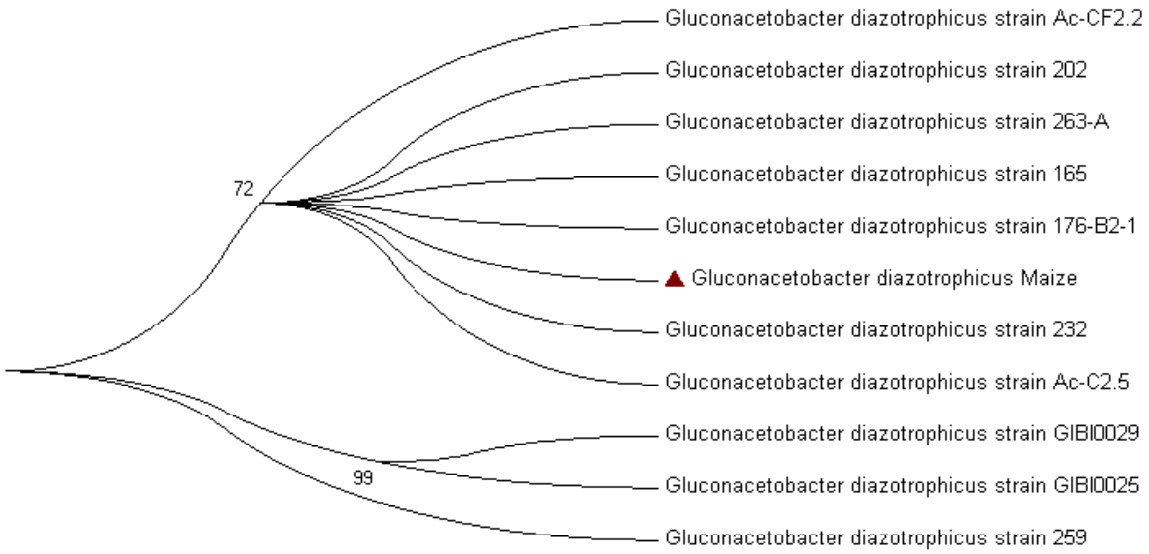

Fig. 1 (b): Molecular Phylogenetics of *G. diazotrophicus* with ten different species available at NCBI GenBank.

Microorganisms can be identified using both classical methods and molecular tools. The genes encoding for 16S rRNA in prokaryotes are most widely used in molecular phylogenetic. The small sub unit of 30S ribosomal RNA (SSU rRNA) genes have been used extensively for sequence based evolutionary analysis because they are 1) Universally distributed, 2) Functionally constant, 3) Sufficiently conserved and 4) Have adequate length to provide a view of evolution encompassing all living microorganisms (Madigan *et al*., 2009). Tian *et al.* (2009) studied colonization of *Gluconacetobacter diazotrophicus* in corn (*Zea mays*), the presence of the bacterium in plant tissues was evaluated via polymerase chain reaction using specific primers. The bacterium was detected in 11 grain corn lines, 9 sweet corn varieties and the overall colonization in corn was 74.1 per cent. This indicates that corn can be a potential new host plant for *G. diazotrophicus*.

Effects inoculation of *G. diazotrophicus, B. megaterium* and *G. fasiculatum* on plant growth and yield

The plant height of maize at different growth stages were significantly higher in the treatment number T_7 (*G. diazotrophicus* MZ-01 + *Bacillus megaterium* + *Glomus fasciculatum*) as compared to the T_8 (*A. chroococcum* + *B. megaterium* + *G. fasciculatum*). Among the signal inoculation treatments the highest plant height was recorded in the treatment T_4 (*G. diazotrophicus* MZ-01). The treatment T_1 (Control) is the least recorded of plant height in all the growth stages. Similarly, observation recorded on number of leaves per plant was significantly higher in treatment that received *G. diazotrophicus* MZ-01 + *B. megaterium* + *G. fasciculatum* (8.33, 12.67, 13.67 and 12.67 at 30 DAS, 60 DAS, 90 DAS and 110 DAS, respectively) and the lowest number of leaves per plant was recorded in the treatment soil control (5.00, 8.00, 9.33 and 6.67 at 30 DAS, 60 DAS, 90 DAS and 110 DAS respectively) in all the stages of growth (Table I).

The production of growth hormones by diazotrophs plays a vital role in enhancing the growth of grasses. It has also been shown that *G. diazotrophicus* is beneficial to sugarcane through production of growth promoting factors. Khan and Pariar (2012) reported that combined inoculation of *Azospirillum brasilense*, VAM (*Glomus fasciculatum*) and P-solubilizing *Pseudomonas striata* and *Serratia* spp. increased the plant height, top root length, stem girth and weight of coffee seedlings at all stages of growth as compared to un-inoculated control. The increased cell elongation and multiplication due to enhanced nutrient uptake by plants following inoculation of Nitrogen fixer and P solubilising bacteria may have caused the increased plant height **(**Laheurte and Berthelin., 2013**).** Iman (2008) reported that the inoculation of the efficient PSB strains significantly increased the plant height up to 81 per cent. The obtained results in our studies is agreed with those of Mohod *et al.* (2012) who mentioned that phosphate dissolving bacteria posses the ability to bring insoluble phosphate in soluble forms by secreting organic acids which lower the pH and bring about the dissolution of bonds of phosphate.

Shoot and root dry weight of maize were significantly higher in treatment T_7 (shoot 118.41 g and root 18.73 g) as compared to treatment T_8 (shoot 110.33 g and root 16.67 g). Among the single inoculation treatment T_3 (shoot 104.27 g and root 16.54 g) were recorded maximum (Table II). Similarly, Meenakshisundaram and Santhaguru (2011) reported that the combining ability of *G. diazotrophicus* with AM fungi on *Sorghum bicolour*, where in fresh weight, dry weight, soluble sugars and photosynthetic pigments in leaves was significantly higher in dual inoculated plants. The highest values were recorded with *Glomus fasciculatum* + *G. diazotrophicus* combination. AM fungal infection was significantly higher in dual inoculated plants. Nitrogen concentration was significantly increased by *G. diazotrophicus* even more in association with the efficient fungal strains.

Treatment T_7 (*G. diazotrophicus* MZ-01 + *B. megaterium* + *G. fasiculatum*) were recorded significantly higher seed yield per plant (152.50 g per plant) as compare to treatment T_8 (*A. chroococcum* + *B. megaterium* + *G. fasiculatum*) (134.05 g per plant), Among the single inoculation treatments T_3 (*G. diazotrophicus* MZ-01) recorded maximum to yield (117.75 g per plant) and treatment T_1 (Control) recorded the lowest yield (89.19 g per plant) (Table II). Kaeppler *et al.* (2013) studied and identified maize

TABLE I

Effect of inoculation of G. diazotrophicus, B. megaterium *and* G. fasciculatum *on plant height and number of leaves*

Treatments	Average plant height (cm) at different growth intervals				Average number of leaves per plant at different growth intervals			
	30 DAS	60 DAS	90 DAS	110 DAS	30 DAS	60 DAS	90 DAS	110 DAS
Control (Soil)	33.00 d	90.67 g	125.00 g	126.00 h	5.00 c	8.00 e	9.33 e	6.67 d
Control (Soil FYM)	33.67 d	94.00 f	134.17 f	135.17 g	5.00 c	8.00 e	10.67 d	8.33 c
G. diazotrophicus MZ-01	37.50 c	116.67 c	150.17 c	154.67 c	6.67 b	10.33 c	12.33 bc	9.67 b
Azotobacter chroococcum	34.67 d	103.33 d	141.33 d	143.33 d	5.33 c	8.67 de	11.33 cd	8.67 c
Bacillus megaterium	34.25 d	101.67 de	139.58 e	141.08 e	5.67 c	9.00 d	11.00 cd	9.00 c
Glomus fasciculatum	34.00 d	100.00 d	138.33 e	139.33 f	5.33 c	8.33 de	11.00 cd	8.67 c
G. diazotrophicus MZ-01 + Bm + Gf	44.17 a	146.00 a	172.33 a	178.33 a	8.33 a	12.67 a	13.67 a	12.67 a
A. chroococcum + Bm + Gf	40.67 b	120.33 b	160.33 b	162.33 b	7.00 b	11.67 b	12.67 b	10.00 b
SEm±	0.62	0.63	0.46	0.46	0.26	0.26	0.29	0.29
LSD	1.863	1.90	1.37	1.37	0.79	0.79	0.87	0.87

Note : Means with same superscript, in a column do not differ significantly at P=<0.05 as per Duncan Multiple Range Test. DAS: Days After Sowing. Bm: *Bacillus megaterium,* Gf: *Glomus fasciculatum*

TABLE II

Effect of inoculation of G. diazotrophicus, B. megaterium *and* G. fasciculatum *on yield*

Treatments	Seed yield per plant (g)	Shoot dry weight (g/plant)	Root dry weight (g/plant)
Control (Soil)	89.19 e	95.87 g	14.34 e
Control (Soil FYM)	92.93 e	97.50 f	14.47 e
G. diazotrophicus MZ-01	117.75 c	104.27 c	16.54 b
Azotobacter Chroococcum	105.50 d	99.33 e	15.31 d
Bacillus megaterium	103.33 d	99.67 de	15.57 cd
Glomus fasciculatum	102.21 d	101.00 d	16.14 bc
G. diazotrophicus MZ-01 + Bm + Gf	152.50 a	118.41 a	18.73 a
A. chroococcum + Bm + Gf	134.05 b	110.33 b	16.67 b
SEm±	1.46	0.50	0.16
LSD	4.38	1.50	0.59

Note : Means with same superscript, in a column do not differ significantly at P=<0.05 as per Duncan Multiple Range Test. Bm: *Bacillus megaterium,* Gf: *Glomus fasciculatum*

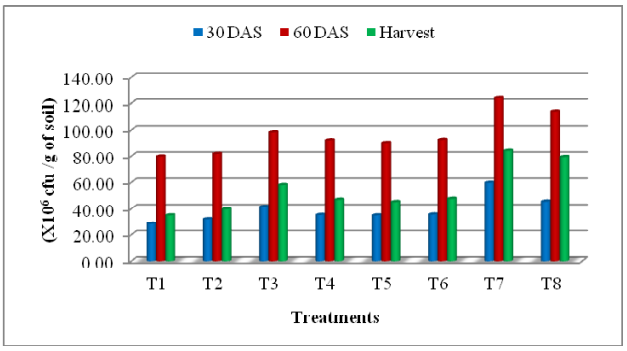

Fig. 02: Bacterial population in the rhizosphere soil of maize at different growth intervals

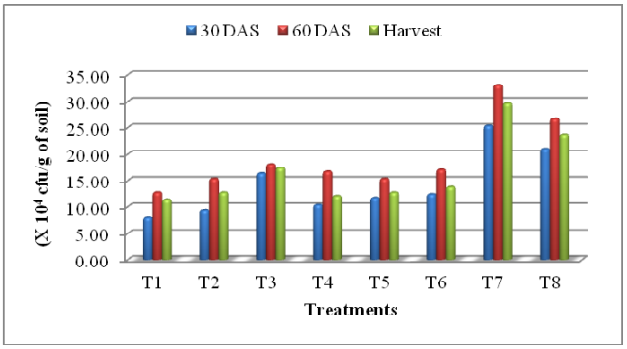

Fig. 03: Fungal population in the rhizosphere soil of maize at different growth intervals

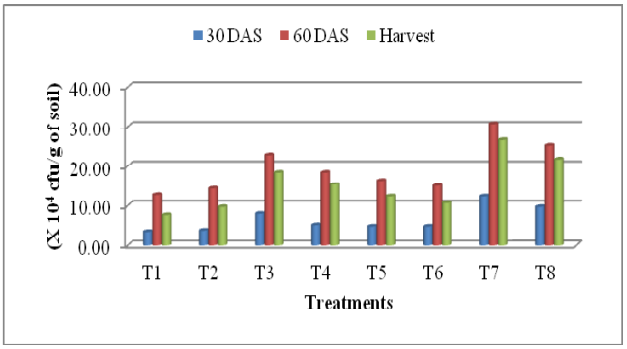

Fig. 04: Actinomycetes population in the rhizosphere soil of maize at different growth intervals

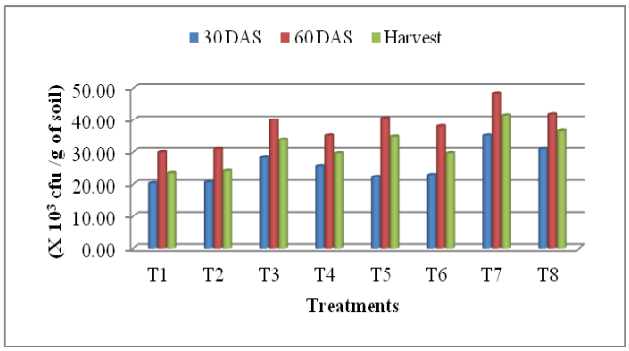

Fig. 05: *Azotobacter* population in the rhizosphere soil of maize at different growth intervals

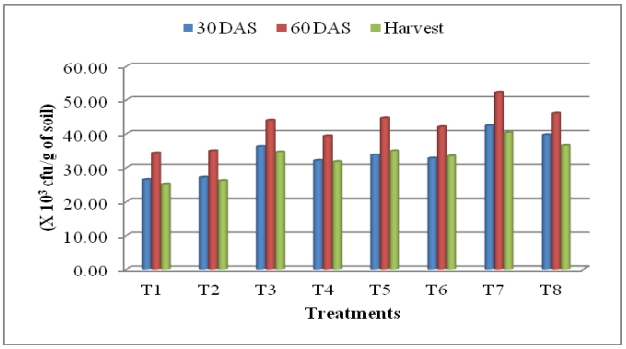

Fig. 06: PSB population in the rhizosphere soil of maize at different growth intervals

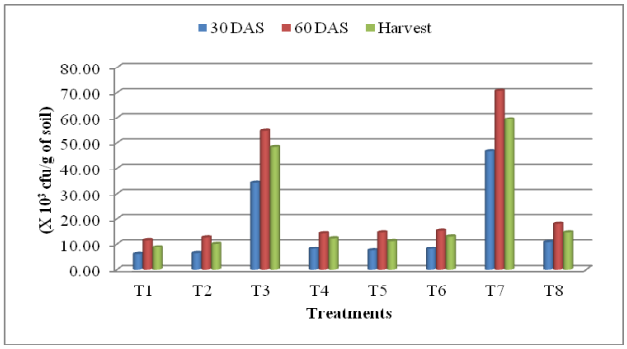

Fig. 07: *G. diazotrohicus* population in the rhizosphere soil of maize at different growth intervals

endophyte associations with increased plant productivity compared with un-inoculated control, where they used a collection of endophytes isolated by several groups. Significant yield enhancements of N-fertilized maize were obtained with bacterial endophytes isolated from N-efficient lines of maize (such as *Klebsiella pneumonia* 342) or Switch grass (*Pantoea agglomerans* P101 and P102). Several other strains from other groups were also tested with best yield enhancements from two Brazilian strains, *Gluconacetobacter diazotrophicus* PAl5 and *Herbaspirillum seropedicae* Z152. No strains were capable of relieving the N-deficiency symptoms of unfertilized maize either in field or in greenhouse.

Microbial population in the rhizosphere soil after inoculation

All treatments were showed maximum microbial populations at flowering stage as compared to vegetative and harvest stage. However, maximum microbial population were at harvest stage as compared to vegetative stage (Fig. 2-7). The higher

bacterial population followed by fungal and actinomycetes populations were found at flowering stage (Jachson and Ilamurugu, 2014).

So far, studies on the occurrence of *G. diazototrophicus* have shown that it has a restricted host range. The study support the hypothesis that in nature, there are many more nitrogen-fixing bacteria to be identified and also strongly suggest that endophytic diazotrophicus bacteria are more prevalent than previously thought. In view of the economic importance of maize to this region and the difficulties of obtaining nitrogen fertilizers, maize-associated nitrogen-fixing *G. diazototrophicus* may be agronomically important because they could supply part of the nitrogen that the crop requires.

REFERENCES

BERTALAN, M., ALBANO, R., PA´DUA, V., ROUWS, L., ROJAS, C., HEMERLY, A., TEIXERA, K. AND SCHWAB, S., 2009, Complete genome sequence of the sugarcane nitrogen-fixing endophyte Gluconacetobacter diazotrophicus Pal5. *Genomics,* **10** : 450.

BHAVANTH JHA, MUKUND C. THAKUR., ITI GONTIA, VALERIE ALBRECHT, MARION STOFFELS, MICHAEL SCHMID AND ANTON HARTMANN, 2009, Isolation, partial identification and application of diazotrophic rhizobacteria from traditional Indian rice cultivars. *Eur. J. Soil Biol*., **45** : 62 - 72.

BODDEY, R. M., URQUIAGA, S., REIS, V. AND DOBEREINER, J. 2013, Biological nitrogen fixation associated with sugarcane. *Dev. Plant Soil Sci*., **48** : 105 - 111.

IMAN, M., 2008, Effect of phosphate solubilising fungi on growth and nutrient uptake of Soybean (*Glycine max* L.) plants. *J. Appl. Sci. Res*., **4** (6) : 592 - 598.

JACHSON, M. K. AND ILLAMURUGU, K., 2014, Metabolic profiling of rice root exudates and its impact on rhizosphere microbial dynamics under aerobic conditions. *Res. J. Agric. Sci*., **5** (4) : 777 - 781.

KAEPPLER, S. M., RIGGS, P. J., CHELIUS, M. K., INIGUEZ, A. L. AND TRIPLETT, E. W., 2013, Enhanced maize productivity by inoculation with diazotrophic bacteria. *Aust. J. Plant Physiol*. 28 : 829 - 836.

KHAN, S. AND PARIAR, P., 2012, Effect of n- fixing biofertilizers on growth, yield and quality of chilli (*Capsicum annum* L.). *Int. J. Life Sci.,* **7** (3) : 481 - 482.

LAHEURTE, F. AND BERTHELIN, J., 2013, Effect of phosphate solubilising bacteria on maize growth and root exudation over four levels of labile phosphorus. *Plant Soil,* **105** : 11 - 17.

MADIGAN, M. T., MARTINCO, J. M., DUNLAP, P. V. AND CLARK, D. P., 2009, Microbial evolution and systematic In brock biology of microorganisms. Pearson *Benjamin cummings*, San Franscisco, p. 358 - 393.

MEENAKSHISUNDARAM, M. AND SANTHAGURU, K., 2011, Studies on association of Arbuscular Mycorrhizal fungi with *Gluconacetobacter diazotrophicus* and its effect on improvement of *Sorghum bicolor* (L.). *Int. J. Cur. Sci. Res*., **1** (2) : 23 - 30.

MOHOD, S. P., GUPTA, D. N. AND CHAVAN, A. S., 2012, Enhancement of phosphate availability and phosphorus uptake in rice by phosphate solubilising culture. *J. Maharashtra. Agric. Univ*., 14 : 178 - 181.

NANDHINI, K., PREETHI, U. AND EARANNA, N., 2014, Molecular identification of phosphate solubilizing bacterium (*Alcaligenes faecalis*) and its interaction effect with *Bradyrhizobium japonicum* on growth and yield of Soybean (*Glycine max* L.). *Afr. J. Biotechnol*., 13 : 3450 - 3454.

OLIVEIRA, A. L. M., STOFFELS, M., SCHMID, M., V. M. REIS., BALDANI, J. I. AND HARTMANN., 2009, Colonization of sugarcane plantlets by mixed inoculations with diazotrophic bacteria. *European J. Soil Biolo*., 45 : 106 - 113.

SHERIF, SAKIT, J. AND CHIRU, S., 2010, Influence of PSB on plant parameters. *Plant Soil,* **24** : 231 - 239.

TIAN, G., PAULS, P., DONG, Z., REID, L. M. AND TIAN, L., 2009, Colonization of the nitrogen fixing bacterium *Gluconacetobacter diazotrophicus* in a large number of Canadian corn plants. *Can. J. Plant Sci*., **89** : 1009 - 1016.

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