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RESEARCH ARTICLE

STUDIES ON THE DINITROGEN FIXING EFFICIENCY OF *GLUCONACETOBACTER DIAZOTROPHICUS* ISOLATES COLLECTED FROM SALT AFFECTED AREAS OF CUDDALORE DISTRICT OF TAMILNADU

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ABSTRACT

In this present study the ability of *Gluconacetobacter diazotrophicus* in dinitrogen fixation associated with sugarcane suggests that the endophyte is of considerable in cane yields as well as sugar recovery. The endophtic bacterium CDZ-8 fixed about 17.26 ± 0.42 Amount of "N" fixed (mg/g of malate) in our study. All the twenty isolates of Gluconacetobacter were graded into three gategories on the basis of their dinitrogen fixing efficiency of 15 (mg/g of malate), above 10- 14 (mg/g of malate) and below 10 (mg/g of malate). A range of 15 percent of isolate in dinitrogen fixing efficiency of 15 (mg/g of malate), 55% of isolate in dinitrogen fixing efficiency of 10- 14 (mg/g of malate) and 30% of isolate below 10 (mg/g of malate).

INTRODUCTION

G. diazotrophicus (previously known as *Acetobacter diazotrophicus*) a nitrogen fixing bacteria, associated with sugarcane as an endophyte exists in high numbers (as high as 10^6 counts g^{-1} plant tissue) in root, shoot and leaves (Cavalante and Dobereiner, 1988). It is primarily responsible for biological nitrogen fixation and seems to contribute substantially to nitrogen nutrition of the plant (James *et al.,* 1994; Dobereiner *et al.,* 1995). *G. diazotrophicus* inoculation experiments involving micro-propagated plants suggest the positive colonization and its contribution to plant growth and development in terms of improved plant height, nitrogenase activity, leaf nitrogen, biomass and yield (James *et al.,* 1994; Sevilla *et al.,* 2001; Oliveira *et al.,* 2002; Muthukumarasamy *et al.,* 2002). Suman *et al.* (2001) reported that the native occurrence of *G. diazotrophicus* in sugarcane varieties of subtropical India is very low but through the inoculation of efficient indigenous isolates, their number, plant N uptake and nutrient use of efficiency could be increased (Suman *et al.,* 2005). However, high N fertilization causes a negative effect on the population of such endophytic diazotrophic bacteria in sugarcane (Suman *et al.,* 2008). Apart from nitrogen fixation, other properties associated with *G. diazotrophicus* are P solubilization, plant growth hormone indole acetic acid (IAA) production and suppression of red rot disease (Muthukumarasamy *et al.,* 1999; Suman *et al.,* 2001).

MATERIAL AND METHODS

Detrmination of The Dinitrogen Fixing Efficiency of Gluconacetobacter Isolates Under Invitro Condition

Microkjeldahal Assay (Bremner, 1960)

Dinitrogen Fixing Efficiency of Gluconacetobacter Isolates

Hundred ml volume of the LGI broth was taken into 250 ml Erlenmeyer flask and sterilized by autoclaving. The flasks were separately inoculated with 1 ml of (1 x 10^7 CFU/mL) 48h old cultures of Gluconacetobacter , *viz*., CDZ-1 to CDZ-20 for one week under stationary condition.

Microkjeldhal Assay of Dinitrogen Fixation

After the incubation period, 1 ml of each above said broth was transferred to 50 ml Pyrex Microkjeldhal flask, separately. A quarter teaspoonful of the digestion mixture (10 g of reagent grade Potassium sulphate , 1 g of Cupric sulphate and 0.1 g of Selenium metal powder) and 4 ml of Salicylic-Sulphuric acid mixture (0.1 g of Salicylic acid, 1.0 g of Sodium thiosulphate and 30 ml of concantrate sulphuric acid) were introduced into it. The contents were slowly heted till frothing ceased and then heated strongly. Completion of the digestion was indicated by the solution turning into bluish green. After cooling, about 15 ml of distilled water was added to the flask, swirled and cooled. The contents were transferred into the distillation unit and 25 ml of 40 per cent Sodium hydroxide was added. The ammonia was steam distilled for 15 min into an excess of 0.1N Sulphuric acid (10 ml) containing two drops of methyl red. The contents were back titrated against 0.1 N Potassium hydroxide till the appearance of golden yellow color. The nitrogen content of the sample was calculated using the following factor.

1 ml of 0.1 N of H_2SO_4 = 0.0014 g of N

Grading the Gluconacetobacter Isolates on the Basis of their Dinitrogen Fixing Efficiency

All the twenty isolates of Gluconacetobacter were graded into three gategories on the basis of their dinitrogen fixing efficiency determined by Microkjedahl assay.

I[st] Category - Above 15 mg 'N' fixed per gram of carbon source
II[nt] Category - 10-14.99 mg 'N' fixed per gram of carbon source
III[rt] Category - below 10 mg 'N' fixed per gram of carbon source

RESULTS AND DISCUSSION

The nitrogen fixation by *G. diazotrophicus* was first reported by Dobereiner (1988), it was proved by Cavalcante and Dobereiner (1988) who reported production of 240 nmoles $C_2H_4h^{-1}$ (mg of cell protein)$^{-1}$ and it was later confirmed by Gills *et al.* (1989). Raúl O. Pedraza (2008) described The first N2-fixing acetic acid bacterium (AAB) was described in Brazil. It was found inside tissues of the sugarcane plant, and first named as *Acetobacter diazotrophicus*, but then renamed as *Gluconacetobacter diazotrophicus*.

Table I: Dinitrogen fixing efficiency of *G. diazotrophicus* isolates (microkjeldhal assay)

Name of isolation	Name of location	Amount of "N" fixed (mg/g of malate)
CDZ-1	Annamalai nagar	11.53 ± 0.41
CDZ-2	Bhuvanagiri	10.87 ± 0.98
CDZ-3	Chidambaram	12.82 ± 1.16
CDZ-4	Cuddalore	11.14 ± 1.20
CDZ-5	Kollidam	12.73 ± 0.87
CDZ-6	Kuringipadi	12.16 ± 0.83
CDZ-7	Marudur	9.65 ± 0.33
CDZ-8	Mutlur	17.26 ± 0.42
CDZ-9	Nellikuppam	14.23 ± 0.20
CDZ-10	Neyveli	11.92 ± 0.48
CDZ-11	Orathur	0.95 ± 0.59
CDZ-12	Palur	14.62 ± 0.27
CDZ-13	Panruti	8.92 ± 0.17
CDZ-14	Parangipettai	13.04 ± 0.39
CDZ-15	Pennadam	9.83 ± 0.14
CDZ-16	Pichavaram	16.23 ± 0.53
CDZ-17	Pinnalur	16.43 ± 0.53
CDZ-18	Pudhuchathram	12.11 ± 0.38
CDZ-19	Sethiathop	7.36 ± 0.25
CDZ-20	Vadalur	5.88 ± 0.24

Later, two new species within the genus Gluconacetobacter, associated to coffee plants, were described in Mexico: *G. johannae* and *G. azotocaptans*. A salt-tolerant bacterium named Swaminathania salitolerans was found associated to wild rice plants. Recently, N2-fixing *Acetobacter peroxydans* and *Acetobacter nitrogenifigens*, associated with rice plants and Kombucha tea, respectively, were described in India. Their natural habitats, physiological and genetic aspects, as well as their association with different plants and contribution through BNF.

Table II: Grading the g.daizotrohicus isolates on the basis of their nitrogen fixing efficiency (microkjeldhal assay)

N-fixed mg/g malate	Positive number isolates	Name of the isolate	% of isolate
15 and above	3	CDZ-8, CDZ-16, CDZ-17	15
10-14	11	CDZ-1, CDZ- 2, CDZ-3, CDZ-4, CDZ-5, CDZ-6, CDZ-9, CDZ-10, CDZ-12, CDZ-14, CDZ-18	55
Below-10	6	CDZ-7, CDZ-11, CDZ-13, CDZ-15, CDZ-9, CDZ-10	30

In this present study The ability of *Gluconacetobacter diazotrophicus* in dinitrogen fixation associated with sugarcane suggests that the endophyte is of considerable in cane yields as well as sugar recovery. The endophtic bacterium CDZ-8 fixed about 17.26 ± 0.42 Amount of "N" fixed (mg/g of malate) in our study. All the twenty isolates of Gluconacetobacter were graded into three gategories on the basis of their dinitrogen fixing efficiency of 15 (mg/g of malate), above 10- 14 (mg/g of malate) and below 10 (mg/g of malate). A range of 15 percent of isolate in dinitrogen fixing efficiency of 15 (mg/g of malate) , 55% of isolate in dinitrogen fixing efficiency of 10- 14 (mg/g of malate) and 30% of isolate below 10 (mg/g of malate).

REFERENCES

Cavalcante VA and Dobereiner J (1988). A new acid-tolerant nitrogen fixing bacterium associated with sugarcane. *Plant soil*. 108 : 23-31.

Muthukumarasamy, R., Revathi, G., Seshadri, S., Laksminarasimhan,C. 2002. *Gluconacetobacter diazotrophicus,* A promising diazotrophic endophyte in trophics. *Current Science* 83 : 137-145.

Subbarao, M. and M.A.E. Shaw, 1985. A review of research on sugarcane soils, of Jamaica. Proc. *Meeting west indies Sugar Technol.*, 2: 343-55.

Suman, A., Gaur, A., Shrivastava, A.K., Yadav, R.L., 2005. Improving sugarcane groeth and nutrient uptake by inoculating *Gluconacetobacter diazotrophicus.* Plant growth Regulation 45: 155-162.

Shrivastava, A.K., K. Singvh, A.K. Ghosha, R. Darash, R.K. Rai, S.P. Shnkla and K. Singh, 1989. Uptake and of sodium and chloride ions in sugarcane. *Sugarcane.* 4: 3-6

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