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SCREENING FOR THE DINITROGEN FIXING EFFICIENCY OF *GLUCONACETOBACTER DIAZOTROPHICUS* ISOLATED FROM THE PROBLEM SOILS OF NAGAPATTINUM DISTRICT OF TAMILNADU, INDIA

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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 entophytic bacterium NGDZ – 14 fixed about 17.30 ± 0.43 Amount of "N" fixed (mg/g of malate) in our study. All the twenty isolates of *Gluconacetobacter* were graded into three categories 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). The range of 15(mg/g of malate) of isolate in dinitrogen fixing efficiency of 20% (mg/g of malate), 50% of isolate in dinitrogen fixing efficiency of 10- 14.99 (mg/g of malate) and 30% of isolate below 10 (mg/g of malate).

Keywords: *G.diazotrophicus*, Dinitrogen Fixing Efficiency, Sugarcane, Nagapattinam District

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INTRODUCTION

Nitrogen is an essential plant nutrient and, in agriculture, fertilization with nitrogen products is widely and increasingly practiced to increase the production yield of food (Reinhold Hurek, 2003). However, the use of elevated doses of fertilizers, as well as pesticides, 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 plant growth-promoting bacteria (PGPB). These bacteria may provide a natural and harmless means to improve the growth and yield of crops, thereby minimizing the use of agrochemicals. PGPB are defined as free-living soil, rhizosphere, rhizoplane, endophytic, and phyllosphere bacteria that, under certain conditions, are beneficial for plants (Basha 2005). They are capable of promoting plant growth through different mechanisms, such as biological nitrogen fixation (BNF), phytohormone production, phosphate solubilization and siderophore production. *Gluconacetobacter diazotrophicus* is a nitrogen fixing bacterium originally found in monocotyledon sugarcane plants in which the bacterium actively fixes atmosphere nitrogen and provides significant amounts of nitrogen to plants. This bacterium mainly colonizes intercellular spaces within the roots and stems of plants and does not require the formation of the complex root organ like nodule. The bacterium is less plant/crop specific and indeed *G. diazotrophicus* has been found in a number of unrelated plant species. Importantly, as the bacterium was of monocot plant origin, there exists a possibility that the nitrogen fixation feature of the bacterium may be used in many other monocot crops. This paper reviews and updates the research progress of *G. diazotrophicus* for the past 25 years but focuses on the recent research development.

MATERIAL AND METHODS

Determination of the Dinitrogen Fixing Efficiency of *Gluconacetobacter* Isolates Under *Invitro* Condition

Microkjeldahal Assay (Bremner, 1960) Dinitrogen Fixing Efficiency of *Gluconacetobacter* isolates 100 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 CFU/ml) 48h old cultures of *Gluconacetobacter*, *viz*., NGDZ-1 to NGDZ-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 heated 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, stirred 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 categories on the basis of their dinitrogen fixing efficiency determined by Microkjeldhal assay. Ist Category - Above 15 mg 'N' fixed per gram of carbon source II Category - 10-14.99 mg 'N' fixed per gram of carbon source III 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 n moles $C_2H_4h^{-1}$ (mg of cell protein)-1 and it was later confirmed by Gills *et al.* (1989). Raúl O. Pedraza (2008) described the first N_2-fixing acetic acid bacterium (AAB) was described in Brazil.

Table 1. Dinitrogen fixing efficiency of *G. diazotrophicus* isolates (microkjeldhal assay)

S.No	Isolate designation	Location	Amount of 'N' fixed mg/g of Malate
1	NGDZ-1	Kutthalam	11.55±0.43
2	NGDZ-2	Mayiladuthurai	10.87±0.99
3	NGDZ-3	Kilavelur	12.83±1.17
4	NGDZ-4	Vedaranyam	11.15±1.22
5	NGDZ-5	Nagapattinum	12.73±0.87
6	NGDZ-6	Sirkazhi	12.17±0.84
7	NGDZ-7	Tharangambadi	9.66±0.34
8	NGDZ-8	Thirukkuvalai	16.24±0.21
9	NGDZ-9	Tholuthalangudi	11.93±0.60
10	NGDZ-10	Senniyanalore	14.63±0.28
11	NGDZ-11	Thiruvalangadu	8.93±0.39
12	NGDZ-12	Mekkirimangalam	9.84±0.15
13	NGDZ-13	Thiruvaduthurai	16.24±0.54
14	NGDZ-14	Pandaravadai	17.30±0.43
15	NGDZ-15	Nallavore	5.88±0.24
16	NGDZ-16	Kokkur	14.23±0.53
17	NGDZ-17	Maruthur	12.12±0.39
18	NGDZ-18	Palaiyur	0.95±0.59
19	NGDZ-19	Paravore	7.36±0.23
20	NGDZ-20	Palayakoodalore	16.44±0.54

Table 2. Grading the G.diazotrophicus isolates on the basis of their nitrogen fixing efficiency (microkjeldhal assay)

N- fixing mg/g Malate	Positive number isolates	Name of the isolates	% of isolates
15 and above	4	NGDZ-8 NGDZ-13 NGDZ-14 NGDZ-15	20
10-14.99	10	NGDZ-1 NGDZ-2 NGDZ-3 NGDZ-4 NGDZ-5 NGDZ-6 NGDZ-9 NGDZ-10 NGDZ-16 NGDZ-17	50
Below 10	6	NGDZ-7 NGDZ-11 NGDZ-12 NGDZ-18 NGDZ-19 NGDZ-20	30

It was found inside tissues of the sugarcane plant, and first named as *Acetobacter diazotrophicus*, but then renamed as *Gluconacetobacter diazotrophicus*. 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 endophytic bacterium NGDZ-14 fixed about 17.30 ± 0.43 Amount of "N" fixed (mg/g of malate) in our study. All the twenty isolates of *Gluconacetobacter* were graded into three categories on the basis of their dinitrogen fixing efficiency among the isolates tested for its dinitrogen fixing efficiency 20% of them were fixed more than 15 ml/g of malate and 50% of the isolates fixed in the range of 10 to 14.99 mg/g of malate and and the remaining nearly 30% of the isolates fixed below 10 mg/g of malate.

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