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SHORT NOTE

Isolation and Characterization of the Endophytic N_2 – Fixing Bacterium, *Acetobacter* Diazotrophicus aAssociated with Sugarcane Plants

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Acetobacter diazotrophicus is an obligate endophytic N_2- fixing bacterium mainly associated with sugarcane and other sugar-rich plants that are propagated vegetatively (Kirchhof *et al*., 1998). This bacterium does not survive in soil or in weeds found in cane fields (Dobereiner, (1995). The presence of this endophyte within the tissues of sugarcane plants no doubt supplements the N-nutrition of the crop. This plants‹ preliminary work, thus attempts, for the first time, to isolate and characterize endophytes within the tissues of sugarcane plants grown in Kenana Sugar Company.

Isolation of the bacterium was carried out using the method employed by Dobereiner (1992) in which a semi- solid LGIP medium was used. Thirteen sugarcane varieties were randomly chosen to test for the presence of the bacterium, and from each variety, sugarcane leaves, stems(top, middle and bottom) and roots were taken for investigation. These parts were cut into small pieces and washed with distilled water and 70% ethanol.

Rhizosphere soil was collected from three plants from each variety at a depth of 5-10cm. Samples were separately collected in sterile plastic bags. A volume of 0.25 ml soil suspension and 0.25 ml leaf, stem and root macerates were used as inocula in I ml of the semi-solid LGIP in small test tubes, replicated three times and incubated at 30^0C for 5 days.

Seven different levels of pH and 7 levels of NaCl were chosen to

examine the effect of pH and salt, respectively, on the growth of the bacterium. Three C sources at three levels were also tested. A multi disk for antimicrobial susceptibility testing was used in the concentrations given below. Viable cell count (Beck et al., 1993) was used for counting on solid agar plates.

After 5 days of incubation at 30^0C, surface pellicles were formed in all test tubes containing inocula from leaf macerate, stem juice and root macerate from all the sugarcane varieties investigated. Identification by light microscopy revealed the presence of small motile rod-shaped organisms, the morphology of which and staining properties conform with that of *Acetobacler diazo-trophicus* identified by Dobereiner (1992). Soil inocula, on the other hand, did not reveal any bacterial growth similar to this bacterium.

Bacterial growth, identified as *Acetobacter diazotrophicus*, was observed in test tubes with PH 2.0, 3.0, 4.0 and 5.5 but not in test tubes with pH 7.0, 8.0 or 10.0, which indicates that it an acid tolerant bacterium. *Acetobacter diazotrophicus* growth was also observed in all samples with different levels of NaCl which indicates that it can tolerate high levels of this salt up to 1.1 g NaCl I^{-l})(Table l). Earlier workers indicated the sensitivity of this bacterium to high pH and its tolerance to high salinity(James *et al.*, 1994).

Table 1. effect of NaCl concentrations, pH of medium, carbon source and concentrations. and antibiotics on the viability of *Acetobacter diazotrophics*.

NaC1 g1	Bacterial Growth	pH of medium	Bacterial growth	Carbon Source		Bacterial growth	Antibacterial Agent ug. 0-1	Bacterial Growth
0.0	+	2.0	+	Glucose	5%	+	Ampicilin 20	-
0.1	+	3.0	+		10%	+	Cefoperazone 75	+
0.3	+	4.0	+		15%	+	Cefotaxime 30	+
0.5	+	5.5	+	Mannitol	5%	+	Piperacilin 100	-
0.7	+	7.0	+		10%	+	30 Ceflazidime	-
0.9	+	8.0	+		15%	+	Ciprofloxacin 5	-
1.1	+	10.0	+	Sourose	5%	+	Celizoxime 30	-
					10%	+	Augmentin 30	-
					15%	+	Ofloxacin 5	+
						+	Gentamicin 10	+
						+	Amikacin 30	-
						+	Peflixacin 10	+

+ Bacterial growth identified as Acetobacter diazotrophicus - No bacterial growth

The bacterium also grew in all petri dishes containing LGIP supplemented with sucrose, mannitol and glucose as C source at 5%‹ 10% at 15% each (Table l). Similar results were obtained by Boddey *et al* (1991).

The antibiotic susceptibility test indicated that the bacterium was resistant to Cefoperazone, Cefotaxime, Ofloxacin, Gentamicin and Pefloxacin at the concentrations used but not to the other seven antibiotics tested (Table l

Table 2. gives the counts of *Acetobacter diazotrophicus* in different parts in different varieties grown in Kenana Estate. There were significant differences among varieties and between different plant parts. Dobereiner (1992) and Baldani (1997) identified significant differences in the colonization of different plant parts and among varieties of sugarcane by *Actobacter diazotrophicus.*

Table 2. Average numbers of bacterial cells per gram fresh weight in different plant parts of different sugarcane varieties.

Sugarcane variety	Root	Stem	Leaf
co 6806	920	820	900
co 997	1280	930	840
co 527	880	640	820
co 775	1080	1100	900
Kn 93046	1300	925	780
BT 74209	493	820	760
TUC 75-3	1020	1270	1620
Kn 93067	740	1110	940
70531	610	1160	680
8532	980	1090	720
Kn 8532	780	800	860
Kn 96167	1460	1180	1380
Kn 94024	1640	780	1060

This research indicates the presence of *Acetobac/er diazolrophicus* in all commercial sugarcane varieties investigated and in all plant parts tested. It was present in appreciable numbers and as such probably contributes. significantly to the N-nutrition of the crop.

Further investigations are needed, however, using methods such as ^{15}N methodology, to determine precisely how much N is added to the crop through associative N_2 fixation with this endphytic diazotroph.

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