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Full Length Research Paper

Characterization of potential ethylene-producing rhizosphere bacteria of *Striga* -infested maize and sorghum

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Three rhizosphere bacteria, *Pseudomonas* sp., *Enterobacter sakazakii* and *Klebsiella oxytoca*, were analyzed for genetic variation. DNA fingerprint patterns of the three bacteria were markedly different when amplified with different primers. In total, 68 bands were produced by the three primers, 62 of which where variable. The number of polymorphic RAPD loci per isolate ranged from one to 13. Cluster analysis indicated that *E. sakazakii* and *K. oxytoca* are the most closely related of the three.

Keywords: Bacteria, RAPD-PCR, *Striga hermonthica.*

INTRODUCTION

Microorganisms are increasingly being considered for the biological control of *Striga hermonthica* (Del.) Benth. Limited knowledge of *Striga* biological control agents has retarded their commercial development and widespread use. Research has shown that certain chemicals such as ethylene gas generators (ethephon), strigol and strigol derivatives can induce abortive germination of *Striga* seeds in the absence of a suitable host, and therefore lead to depletion of the seed reserve in the soil (Eplee, 1981) . Soil living bacteria which produces ethylene offers exciting new tools for *Striga* biocontrol.

There is a dearth of information on the molecular biology of *Striga*-associated bacteria. Rapidly evolving technologies from molecular biology and genetics have provided new insights into the underlying mechanisms by which biocontrol agents function in their natural environments to a degree not previously possible. The application of the polymerase chain reaction (PCR), in particular, have greatly facilitated genomic analyses of microorganisms, provide enhanced capability to characterize and classify strains, and facilitate research to assess the genetic diversity of populations (Louws et al., 1999). These techniques offers exciting new tools for *Striga* biocontrol studies. Variations in random amplified polymorphic DNA (RAPD) by PCR is especially suited for genetic studies where little or no molecular genetics research has been conducted previously (Nybom and Bartish, 2000). This would be of great value to determine genomic profiles of certain bacteria in the rhizosphere of *Striga* infected cereals. This study reports the genetic variation of 3 ethylene-producing soil bacteria from the rhizospheres of maize and sorghum.

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RESULTS AND DISCUSSION

Thirty short primers of arbitrary sequence were initially screened, out of which three were selected based on their ability to amplify all three bacteria isolates. Each lane has a minimum of one and a maximum of 13 discrete visible bands ranging in size from 0.1 to 2.6 kb (Figure 1). The banding patterns were markedly different with each primer. Primer OPA-10 showed the highest polymorphism in the three bacteria species. It was

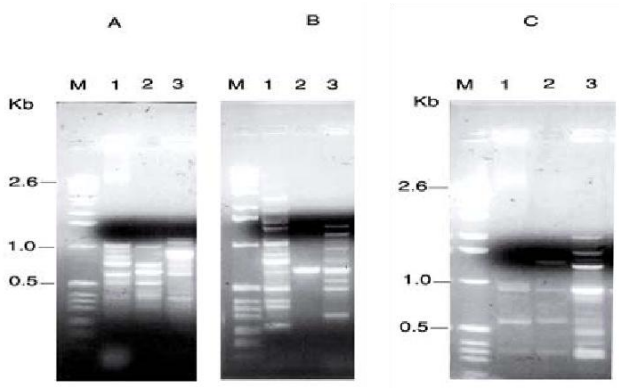

Figure 1: RAPD polymorphism detected in 3 bacteria isolates.

Bacteria were isolated (Babalola, 2002) and a small amount of the bacteria, just enough to be clearly visible by the naked eye, was picked from a colony (Nowrouzian et al., 2001) and suspended directly in the PCR mixture. Tubes were placed in a styrofoam and heated at 95^{o}C for 15 min in the PCR mixture to disrupt the bacteria before taq polymerase was added. In the present study, the amount of bacteria added to the PCR mixture was found to vary between 1.6 x 10^{6} and 4.7 X 10^{6} CFU, in which range the PCR patterns showed satisfying reproducibility. Amplification were carried out in a programmable thermal cycler (PTC- 100^{TM} MJ-Research Inc.) using the following parameters: an initial denaturing step at 94^{o}C for 1 min, 40 cycles of denaturation at 94^{o}C for 1 min, annealing at 40^{o}C for 1 min, and 72^{o}C for 2 min. A final extension step of 10 min at 72^{o}C was included. The amplification products were analysed by electrophoresis on 1.5% agarose gels and detected on ethidium bromide visualized under UV light. 1kb size marker (Boehringer Mannheim, Germany) was loaded in marker lane M. Ten-microlitre aliquots of the PCR products were loaded, *Pseudomonas* sp. (lane 1), *E. sakazakii* (lane 2) and *K. oxytoca* (lane 3). The three arbitrary decamer primers used in this study were OPA-10 (Fig. 1a), OPA-09 (Fig. 1b) and OPA- 16 (Fig. 1c), purchased from Operon Technologies, Inc. (Alameda, CA).

assumed that bands of the same molecular weight in different individuals were identical in nucleic acid sequence. The results of the scoring are presented in Table 1. A common band is shared by the isolates in each of the three primers within the range of 0.6 to 0.7 kb. In total, 68 bands were produced by the 3 primers, 62 of which where variable. These DNA bands were significantly different (P<0.0002).

The aim of this study was to provide some general information about the genetic variation of 3 soil bacteria with potential for use as *S. hermonthica* biocontrol agent. The potential use of bacteria inoculums for the biological control of *S. hermonthica* is only recently being considered. The RAPD-PCR reaction yields a series of products of varying size, which were separated by gel electrophoresis. The band pattern of RAPD-PCR reactions represents a "genetic fingerprint" that can be used to characterize a particular bacterial strain (Welsh and McClelland, 1990). The low similarity index among the bacteria isolates was not unexpected since they are of different species with different habitats. *Pseudomonas* sp. was isolated from the exorhizosphere while *E. sakazakii* and *K. oxytoca* were from the endorhizosphere. Based on summary data presented (Figure 2 and Table 2) we agree with the report of Waugh and Powell (1992) that polymorphism as revealed by molecular markers could help to select priority areas for conservation and provide vital information for development of genetic sampling improvement. The study is currently being re-evaluated with specific primers targeted toward specific sequences of the microbial enzyme, 1-aminocyclopropane-1–carboxylic acid (ACC) deaminase gene (Penrose and Glick, 1997). This enzyme catalyses the hydrolytic cleavage of ACC, the immediate precursor of ethylene. This will allow for better resolution of closely related, morphologically similar species. These findings could be

Table 1: Number of bands and percentage of DNA polymorphic bands in three bacteria isolates amplified with three oligonucleotide primers.

Primer	Primer sequence	Bacteria isolates	Number of fragments scored	Percentage of polymorphic loci
OPA-09	5'GGGTAACGCC3'	*Pseudomonas* sp.	11	64.71
		E. sakazakii	1	5.88
		K. oxytoca	9	52.94
OPA-10	5'GTGATCGCAG3'	*Pseudomonas* sp.	9	52.94
		E. sakazakii	8	47.06
		K. oxytoca	9	52.94
OPA-16	5'AGCCAGCGAA3'	*Pseudomonas* sp.	5	29.41
		E. sakazakii	3	17.65
		K. oxytoca	13	76.47

Table 2: Nei and Li coefficient values (below diagonal) and Jaccard similarity index (above diagonal) computed from RAPD profiles obtained with three primers, OPA-09, OPA-10, OPA-16.

Primer	Bacteria	*Pseudomonas* sp.	*E. sakazakii*	*K. oxytoca*
OPA-09	*Pseudomonas* sp.	-	0.09	0.54
	E. sakazakii	0.17	-	0.11
	K. oxytoca	0.70	0.20	-
OPA-10	*Pseudomonas* sp.	-	0.55	0.46
	E. sakazakii	0.71	-	0.42
	K. oxytoca	0.67	0.59	-
OPA-16	*Pseudomonas* sp.	-	0.33	0.29
	E. sakazakii	0.50	-	0.30
	K. oxytoca	0.44	0.38	-
Average	*Pseudomonas* sp.	-	0.32	0.43
	E. sakazakii	0.46	-	0.29
	K. oxytoca	0.60	0.39	-

RAPD bands were treated as binary (presence/absence) characters. The Nei and Li coefficient (Nei and Li, 1979) was used to score the DNA polymorphisms; NL = 2a/(b+c). Pair-wise comparisons of genotypes were also used to obtain genetic distances using Jaccard similarity index (Jaccard, 1912); C_j = a/(a+b+c). Where 'a' is the number of similar bands from two isolates, 'b' is the number of bands in bacteria 2, but not bacteria 1, and 'c' is the number of bands in bacteria 1, but not bacteria 2.

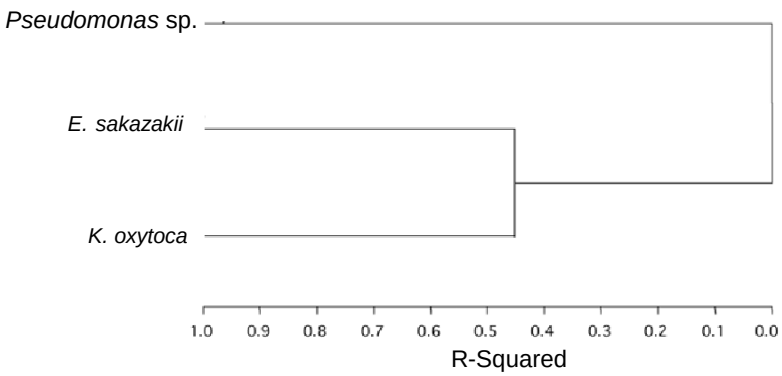

Figure 2: Dendogram showing relationship between three plant-associated bacteria species using SAS (proc cluster) based on Jaccard similarity index from RAPD-PCR data.

exploited as genetic markers to identify particular strains of bacteria isolates of soil rhizosphere for use in biocontrol of *S. hermonthica* through *Striga* suicidal germination.

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REFERENCES

Babalola OO (2002). Interactions between *Striga hermonthica* (Del.) Benth. and fluorescent rhizosphere bacteria Of Zea mays, L. and *Sorghum bicolor* L. Moench for *Striga* suicidal germination In *Vigna unguiculata* . PhD dissertation, University of Ibadan, Ibadan.

Eplee RE (1981) *Striga*'s status as a plant parasite in the United States. Plant Dis. reporter 65:951-954.

Jaccard P (1912). The distribution of the flora in the alpine zone. New phytol. 11:37-50.

Louws FJ, Rademaker JLW, de Bruijn FJ (1999). The three Ds of Pcr-based genomic analysis of phytobacteria: Diversity, Detection, and Disease Diagnosis. Annu. Rev. Phytopathol. 37:81-125.

Nei M, Li WH (1979). Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc. Natl. Acad. Sci 76:5269-5273.

Nowrouzian F, Wold AE, Adlerberth I (2001). Computer-Based Analysis of RAPD (Random Amplified Polymorphic DNA) Fingerprints for Typing of Intestinal Escherichia coli. Mol. biol. Today 2(1):5-10.

Nybom H, Bartish IV (2000). Effects of life history traits and sampling strategies on genetic diversity estimates obtained with RAPD markers in plants. Perspectives in plant ecology, evolution and Systematics. Volume 3/2, pp 93-114.

Penrose DM, Glick BR (1997). Enzymes that regulate ethylene levels-1-aminocyclopropane-1-carboxylic acid (ACC) deaminase, ACC synthase and ACC oxidase Indian J. Exp. Biol. 35(1):1-17.

Waugh R, and Powell W (1992). Using RAPD markers for crop improvement. TIBTECH 10:186-191.

Welsh J, McClelland M (1990). Fingerprinting genomes using PCR with arbitrary primers. Nucl. Acids Res. 18:7213-7218.