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Full Length Research Paper

Molecular variation in population structure of *Xanthomonas axonopodis* pv *manihotis* in the south eastern Nigeria

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***Xanthomonas axonopodis* pv *manihotis* (*Xam*) is the causal agent of cassava bacterial blight (CBB) in all cassava growing areas of the world. To develop an efficient disease management strategy, the genetic diversity of the pathogens population ought to be known. Information is scarce on the genetic diversity of *Xam* population in Nigeria. Random amplified polymorphic DNA (RAPD) was used to characterize *Xam* isolates from the southeastern parts of Nigeria. Seventeen *Xam* strains and two reference strains were analyzed with four RAPD primers. RAPD fragment data showed five major clusters at 80% similarity coefficient level. Four strains were not clustered by this analysis (Del5A, Imo38B, Ebon28A and Ebon27A) and were not grouped with the others in the dendrogram. Principal component analysis (PCA) assembly grouped the bacterial strains into three. One of the strains that was separated in the dendrogram was also clustered separately from the others in the major cluster in the PCA. The results showed a high level of genetic diversity in the pathogen.**

Key words: RAPD, DNA, genetic diversity, dendrogram analysis and PCA.

INTRODUCTION

Xanthomonas axonopodis pv *manihotis* (*Xam*) causes cassava bacterial blight, a major disease of cassava, a starchy root crop that feeds about 800 million people throughout the world (Bokanga and Otoo, 1994). It is also used for compounding animal feed and for the manufacture of starch and alcohol (Asiedu, 1989). The useful crop, however, is susceptible to biotic and abiotic stresses that decrease yields. In Africa, for instance, the other destructive disease of cassava are the African cassava mosaic disease and cassava anthracnose (Lozano, 1986; Wydra and Msikita, 1998; Dixon,et al., 2002). Cassava bacterial blight (CBB) contributed immensely to starvation in Zaire during the epiphytotic years of 1970-1975 when losses in central Africa were as high as 80%. This disease is considered as a major destructive disease of cassava in South America and Africa as it could result in complete loss of yield and planting materials under conditions of severity (Lozano, 1986; Nilmanee, 1986).

Bacterial blight of cassava has been reported from several African countries since 1973. The disease is not a recent introduction to Africa and Asia; it may have been endemic but unimportant for a long time. The sudden increase in importance may be due to the importation of highly susceptible varieties from Brazil and Colombia where the disease has been very virulent and destructive (Nilmanee, 1986). The extent of damage caused by CBB on cassava plants varies with locality, variety, and climatic conditions (Terry, 1976). The African regions most severely affected are the savanna of the south and east of Zaire (Maraite and Meyer, 1975) and the mid-west and the southeast of Nigeria (Terry, 1976).

To better select resistant cassava germplasm

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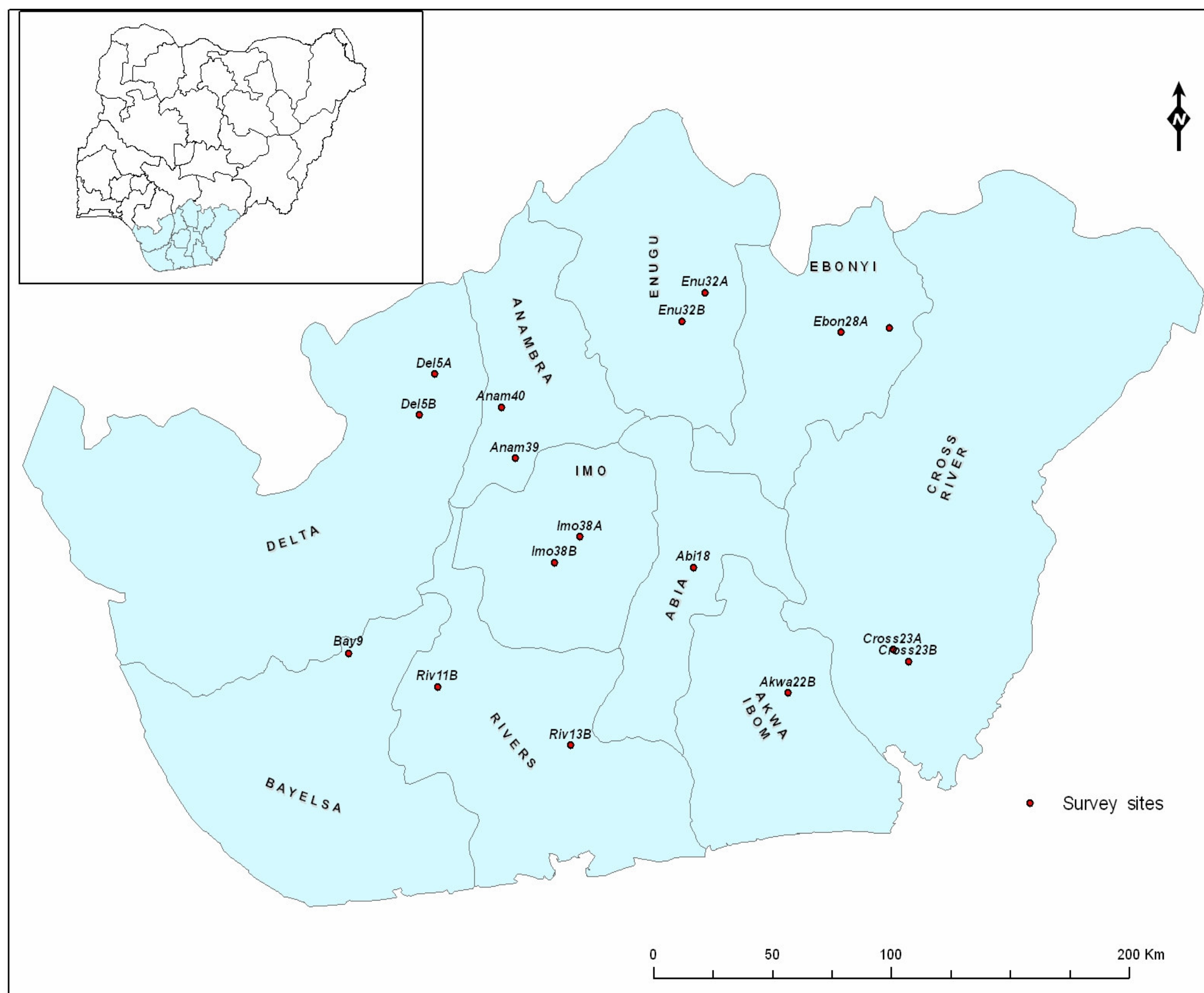

Figure 1. The origin of *Xanthomonas axonopodis* pv *manihotis* strains in the southeast Nigeria used in this study.

adaptable for this region, we examined the population structure of *Xam* in southeastern Nigeria. Until now, no extensive study of the molecular diversity of the bacterial population has been reported for these areas of this country that is the world's largest producer of cassava and a major center of diversity for the crop in Africa.

MATERIALS AND METHODS

Bacterial strains and DNA isolation

We used field isolates collected in the southeast of Nigeria in 2000 (Ogunjobi et al., 2001) and two reference strains from the German Collection of Microorganisms and Cell Cultures (DSMZ). The states and locations of collection for each bacterial strain are shown in Figure 1. The bacteria were cultured on yeast extract dextrose peptone agar (YDPA) containing 5 g yeast extract, 10 g dextrose, 5 g peptone, and 15 g agar per litre of distilled water (pH 7.2).

Extraction of bacteria genomic DNA

The cells of the bacteria were harvested from 1.5 ml suspension of cells by low speed centrifugation at 5000 x g for 4 min. The pellet was washed in 1 ml of 5 M NaCl and once in 1 ml TE buffer (10 mM Tris-HCl, 1 mM EDTA (pH 8)). Genomic DNA was extracted by the method of Sambrook et al. (1989). The DNA quality was checked on agarose and quantified with DNA fluorometer (model TD-700).

Polymerase chain reaction (PCR) amplification

All PCR reactions were performed in a total volume of 25 µl using 20-50 ng DNA. In the random amplified polymorphic DNA (RAPD) amplification, the primers OPAC 5, OPAC 19, OPAC 20, and OPAG 20 (Operon Technologies Inc, Alameda, CA. USA) were used. The modified method of Goncalves and Rosato (2000) was employed in the RAPD analysis. The reaction mixture per PCR tube contained the following: thermo buffer, 2.5 µl; $MgCl_2$, 2.5 µl; 5% Tween 20, 2.5 µl; deoxynucleotide triphosphate (dNTP) made up of 2.5 mM/µl

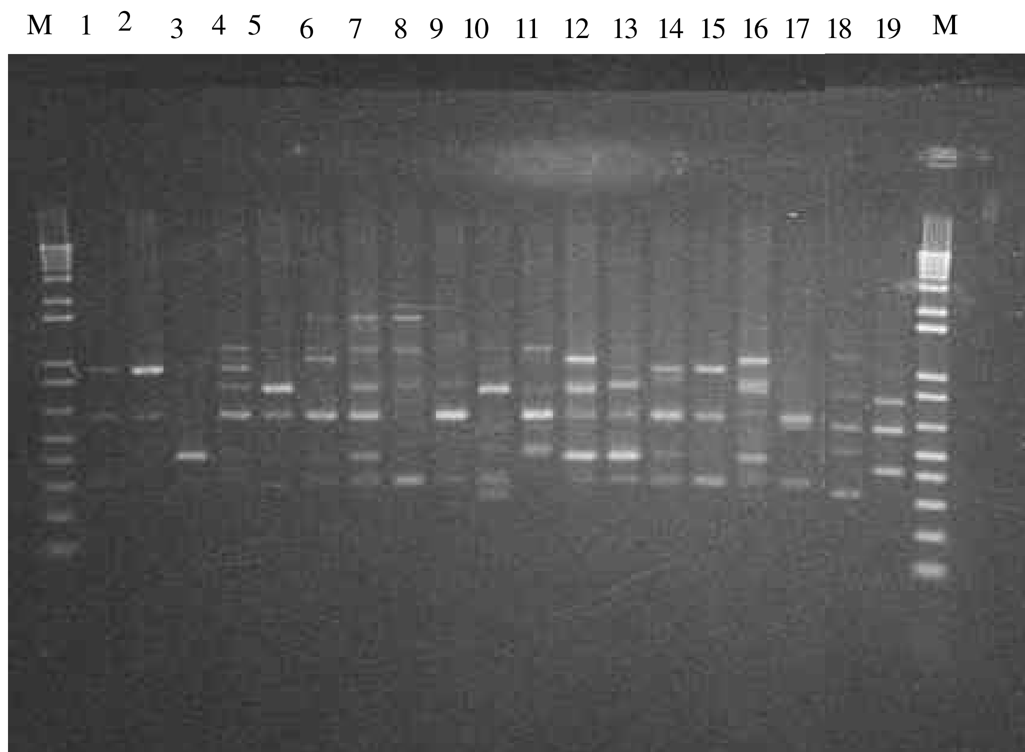

Figure 2. The fragments generated from Primer OPAC 20 (Operon Technologies USA) after PCR amplification and electrophoresis on agarose gel. Lanes 1- 17 were *Xanthomonas axonopodis* pv *manihotis* strains; lane M was Lambda 1 kb DNA marker; lanes 18 and 19 were the reference isolates.

each of dATP, dCTP, dGTP, and dTTP, 2.0µl; 2 µM RAPD Primer, 1.0 µl; Taq polymerase, 0.2 µl (2 units); DNA, 5.0 µl; distilled H_2O, 12.3 µl. The reaction mixture was placed in the PCR tube and run in PCR machine (Model: PTC-200 Peltier Thermal Cycler). The PCR program used was 94°C for 30 s; 44 cycles of 94°C for 20 s, 37°C for 40 s, 72°C for 60 s; and final 72°C for 7 min. Soaking temperature was 4°C. Thereafter, the products were run on 2% agarose gel and stained in ethidium bromide. The pictures were captured with Polaroid film for documentation and scoring. Gels were scored, based on presence or absence of bands and later analyzed using the Numerical Taxonomy and Multivariate analysis system (NTSYS-PC version 2.0) software. This involved clustering, using unweighted pair group method of arithmetic average clustering (UPGMA) and a phylogenetic tree or dendrogram was obtained from the data. The data obtained were also subjected to statistical analysis using “proc prin com” of SAS version 8.0 of 1998 to find the principal component analysis (PCA) which revealed the relationship and variations that existed among the bacterial population in a scatter plot.

RESULTS

DNA fragments obtained per primer differed in number and polymorphism structures. The primer OPAC 19 yielded fragments ranging from 200 bp to 2 kb and nine fragments were considered for analysis. A band approximate to 850 bp was common to all strains and variation was detected with the smaller fragments. Six different patterns were obtained; two major patterns were shown by more than 75 % of the strains. Primer OPAC 20 amplified up to seven fragments per strain (0.5–2.0 kb) and six bands were included in the cluster analyses (Figure 2). Primer OPAG 20 produced few polymorphisms and visualization of the fragments was difficult. Bands between 400 and 2000 bp yielded most of the differences among the strains, and only five different bands were considered. OPAC 5 primer showed the highest level of polymorphisms, amplifying fragments of 0.25–2.0 kb.

These bacterial strains were clustered, based on the similarity in the polymorphisms of the DNA fragments generated from the RAPD analysis. The dendrogram generated from the RAPD data analyzed grouped the bacterial strains into 5 clusters (Figure 3). For ease of differentiation, 80% coefficient level of similarity was used and the *Xam* strains were separated clearly on the dendrogram. The first group was a cluster of 5 strains comprised 26.3% of the bacteria and the second was a cluster of 2 bacterial strains with the 2 reference strains also assembled along in this cluster while others were just 2 strains per cluster of the *Xam* population in this region. Four of the bacterial strains (Del5A, Imo38B, Ebon28A and Ebon27A) were not grouped along with the others. The diversity observed in the bacterial population

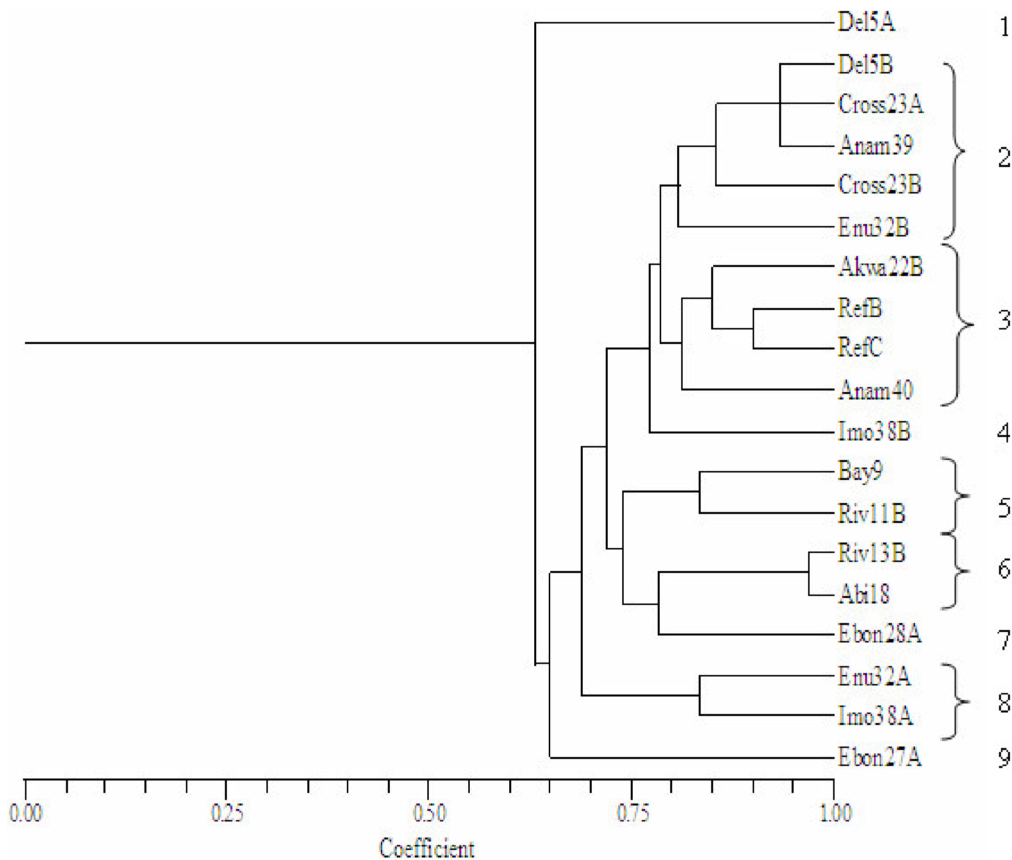

Figure 3. Dendrogram obtained from RAPD marker analysis of *Xanthomonas axonopodis* pv *manihotis* strains from southeastern Nigeria.

at 80 percent similarity coefficient level suggested that the *Xam* strains are not homogenous within this agroecological zone. The PCA, however, assembled the *Xam* strains into 3 clusters (Figure 4). Here the reference strains were grouped together in cluster 1 and the majority of the bacterial strains were crowded together in cluster 2. Cluster 3 separated strains Del5A, Del5B, Bay9, and Riv11B from the other *Xam* strains. The 4 bacteria that were not clustered in the dendrogram were grouped along with the other strains in the PCA, except for Del5A that was separated in both analyses.

DISCUSSION

The genetic analysis presented here contributed to our effort to understand the genetic structure of the *Xam* population in southeastern Nigeria and will help in selecting strains of the pathogen for screening cassava germplasm for resistance to the disease within this area. Our study showed that the regional *X. axonopodis* pv. *manihotis* population had a high degree of genetic diversity. In this study, strain Del5A was not clustered with other strains either in the phylogenetic tree or the PCA analysis. The strain had been identified as a non-pathogenic strain in a previous study (Ogunjobi et al., 2001). This result was contrary to the work of Assigbetsé et al. (1998) which reported that RAPD analysis did not show high level polymorphisms within the *Xam* strains in their study. The RAPD pattern observed here was discriminatory enough within the same species to have revealed minute differences in the bacteria strains. Terry (1976) reported that the Nigerian regions most severely affected by CBB are the midwest and the southeast. The high disease pressure reported in this zone may be related to the existence of different strains of the bacteria, as revealed by the genetic analysis carried out in this study. The quantitative estimate of genetic similarity obtained here did not show any of the bacterial strains to be perfectly identical. They were all slightly different from one another in their genetic structures. This signifies that the *Xam* populations in Nigeria are not perfectly homogeneous, as supposed by previous workers (Restrepo and Verdier, 1997). If the diversity that existed in the analysis of *Xam* population within southeast Nigeria was this high, then the African population could not have been homogeneous. The assertion that the CBB causal agent in Africa had been from a single haplotype from South America can no longer be reliable, since within South America alone, changes in *Xam* population structure have

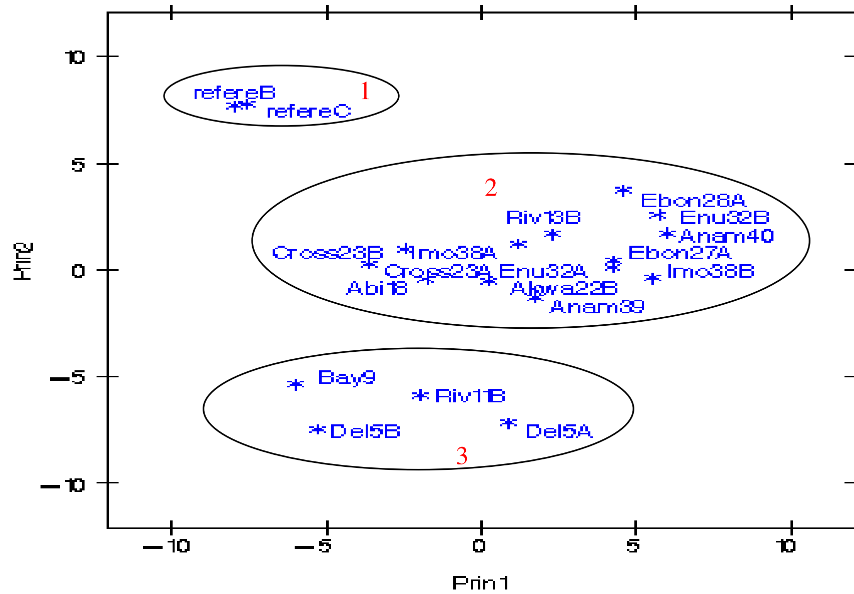

Figure 4. Principal component analysis (PCA) of data generated from RAPD fingerprint of *Xanthomonas axonopodis* pv *manihotis* strains from southeastern Nigeria.

have been reported (Restrepo et al., 2004).

A combination of virulence investigation and molecular genetic characterization is therefore necessary to evaluate existing biodiversity among strains of *Xam* in Africa to determine the true state of this pathogen. Understanding pathogen population structure is essential for formulating long-term disease management strategies. Information on pathogen diversity can be used in characterizing, developing, and deploying resistant germplasm. Therefore, monitoring the bacteria population requires repeated diagnostic surveys of all ecological zones in the country. Molecular typing in surveillance is essential and must be used for a have better understanding of the true pathogenic structure of a virulence or group of virulent bacteria in epidemiological investigation within Nigeria and Africa at large.

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