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Molecular epidemiology of *Xanthomonas campestris* pv. *manihotis* causal agent of cassava bacterial blight

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Abstract

In order to detect and assess genetic and evolutionary relationships among strains of *Xc* pv. *manihotis* a comparison of strains of distinct geographical origin, representing 18 countries, was performed using a range of assays including restriction fragment length polymorphism (**RFLP**) analysis. The probes used were: 16+23S rRNA genes from *E.coli* and three restriction fragments from the chromosomal or plasmid DNA of *Xc* pv. *manihotis*.

Hybridization with the probe corresponding to the rRNA genes allowed the distinction of four RFLP groups. Subgroups were identified based on hybridization profiles with the three others probes.

Genetic variability of *Xc.* pv. *manihotis* was extensive in strains from the area of origin of the host plant and limited elsewhere. These results are in agreement with the hypothesis of the recent introduction of the pathogen to these latter areas and suggests that the African strains have not yet diversified genetically at the chromosomal level.

Our results indicate that RNA and DNA probes are useful tools for epidemiological studies and in following the genetic evolution of strains.

Keywords: *Xanthomonas campestris* pv. *manihotis*, cassava, RFLP, rRNA probe, DNA probe.

I. INTRODUCTION

Cassava (*Manihot esculenta*), family *Euphorbiaceae* is a root stock crop native from South and Central America. Portuguese traders introduced it to West Africa in the sixteenth century and to East Africa in the eighteenth century (SILVESTRE & ARRAUDEAU, 1983). It became one of the most important tropical food in countries of Tropical Africa.

Cassava bacterial blight (C.B.B) caused by *Xanthomonas campestris* pv. *manihotis* is one of the most important diseases of cassava. The disease was first reported in Brazil in 1912 (BONDAR, 1912) but has also been observed in Colombia and Venezuela (LOZANO & SEQUEIRA, 1974), as well as in most of African (MARAITE & MEYER, 1975) and Asian countries (BOOTH & LOZANO, 1978).

Cassava originated from South America and its related bacterial pathogens could have been propagated to others countries through the cuttings and seeds. To be able to detect and assess evolutionary relationship among pv. *manihotis* a comparaison of strains was developped using a wide range of assays.

II. MATERIAL AND METHODS.

X.c. pv. *manihotis* collection.

The bacterial strains used in this study, their geographical origin and their sampling collecting places are listed in Table 1.

Physiological characteristics.

Different phenotypic features were examined: the *in vitro* susceptibility to 20 antibiotics was determined, the utilization of carbon sources (19 tested), and the amylase activity according to described methods (GROUSSON et *al*, 1990).

Phytopathogenicity test.

Pathogenicity of all strains was tested on cassava plants, Congo's cultivar PMB, multiplied from cuttings. The stem inoculation was done according to previously described methods (MARAITE et *al*, 1981).

Table 1 : *Xanthomonas campestris* pv. *manihotis* strains used and information on their origin and isolation.

Strain no * and in other collection	Place and year of isolation		Isolated y
CFBP1851,CIAT1111	Colombia	1974	Lozano.J
LMG 776, NCPPB2443,HMB72,CFBP2603		1970	Lozano.J
ORST1, CIAT1060, CFBP1849		1970	Lozano.J
ORST2, CIAT1061, CFBP1850	Venezuela	1971	Lozano
ATCC 23380, HMB68,NCPPB1159	Brazil	1941	Burkholder
HMB 70, NCPBB1160, LMG5273		1941	Drumond Hipolito
HMB 55a,NCPBB1834*, LMF784		1965	Robbs C.
ORST7, CFBP1854		1973	Neto J.R.
HMB23, LMG770		1973	Pereira A.
ORST3, CIAT 1120, CFBP1852		1974	Lozano.J
ORST5, CFBP1855		1974	Neto J.R.
ORST6, CFBP1856		1976	Neto J.R.
HMB79, LMG778		1978	Takatsu.A.
LMG777, HMB78		1978	Takatsu.A.
LMG779, HMB80		1978	Takatsu.A.
HMB25, NCPBB3060, LMG 771	Nigeria	1976	Maraite H.
ORST42		1978	Daniel J.F
ORST43		1978	«
CFBP1857, ORSTOM A202.1		1978	«
CFBP1858, ORSTOM A203.1		1978	«
CFBP1859, ORSTOM A205.1		1978	«
CFBP1860, ORSTOM A207		1978	«
ORST34	Benin	1982	Daniel J.F
ORST35		1982	«
ORST36		1982	«
ORST37		1982	«
ORST38		1982	«
CFBP1944	Ivory Coast	1979	Ridé M.
LMG5249, HMB203		1981	Maraite H.
ORST55		1984	Daniel J.F
ORST56		1984	«
ORST (198 strains)	Congo	1977-1991	Daniel J.F Boher B. Verdier V.
ORST (29 strains)	Togo	1987-1991	Boher B.

Strain no * and in other collection	Place and year of isolation		Isolated y
HMB6, LMG 767	Zaïre	1973	Maraite H.
HMB9, LMG 768, NCPBB3O58		»	»
LMG 769, NCPPB3O59, HMB10		»	»
LMG 766, HMB3		»	»
ORST44		1979	Daniel J.F
ORST45		»	»
ORST46		»	»
ORST47		»	»
ORST48		»	»
ORST49		»	»
ORST50		»	»
ORST51		»	»
ORST52		»	»
ORST53		»	»
ORST54		»	»
ORST186		1987	Boher B
ORST187		»	»
ORST39	RCA	1977	Daniel J.F
ORST40		»	»
ORST41		»	»
LMG 5287, NCPPB 3161	Cameroon	1976	Persley G.
HMB27, LMG629		1977	Maraite H.
LMG780, HMB81	Uganda	1979	Maraite H.
LMG782, HMB93		»	»
LMG783, HMB148	Kenya	1979	Maraite H.
LMG5288, NCPPB 3194	Niger	1978	Bradbury.J
LMG765	Malaysia	1980	Lan G.
LMG774, HMB60	Taïwan	1978	Maraite H.
HMB71, NCPBB1161, LMG775	Mauritius	1946	Orian G.
CFBP2624	Reunion	1986	Pruvost O.
CFBP2635		1987	Girard J.C

ATCC : Americain Type Culture, Rockville, Maryland, USA. **CFBP** : Collection Française de Bactéries Phytopathogènes, Angers, France.
NCPPB : National Collection of Plant Pathogenic Bacteria, Harpenden, U.K. **HMB** : H. Maraite's Bacterial Collection, LOUVAIN La Neuve, Belgium.
LMG : Laboratorium voor Microbiologie Gent culture Collection, Gent, Belgium. **ORST** : Collection du Laboratoire de Phytopathologie, ORSTOM, Brazzaville, Congo.
CIAT : Centro International de Agricultura Tropical, Cali, Colombia. * : Pathovar reference strain.

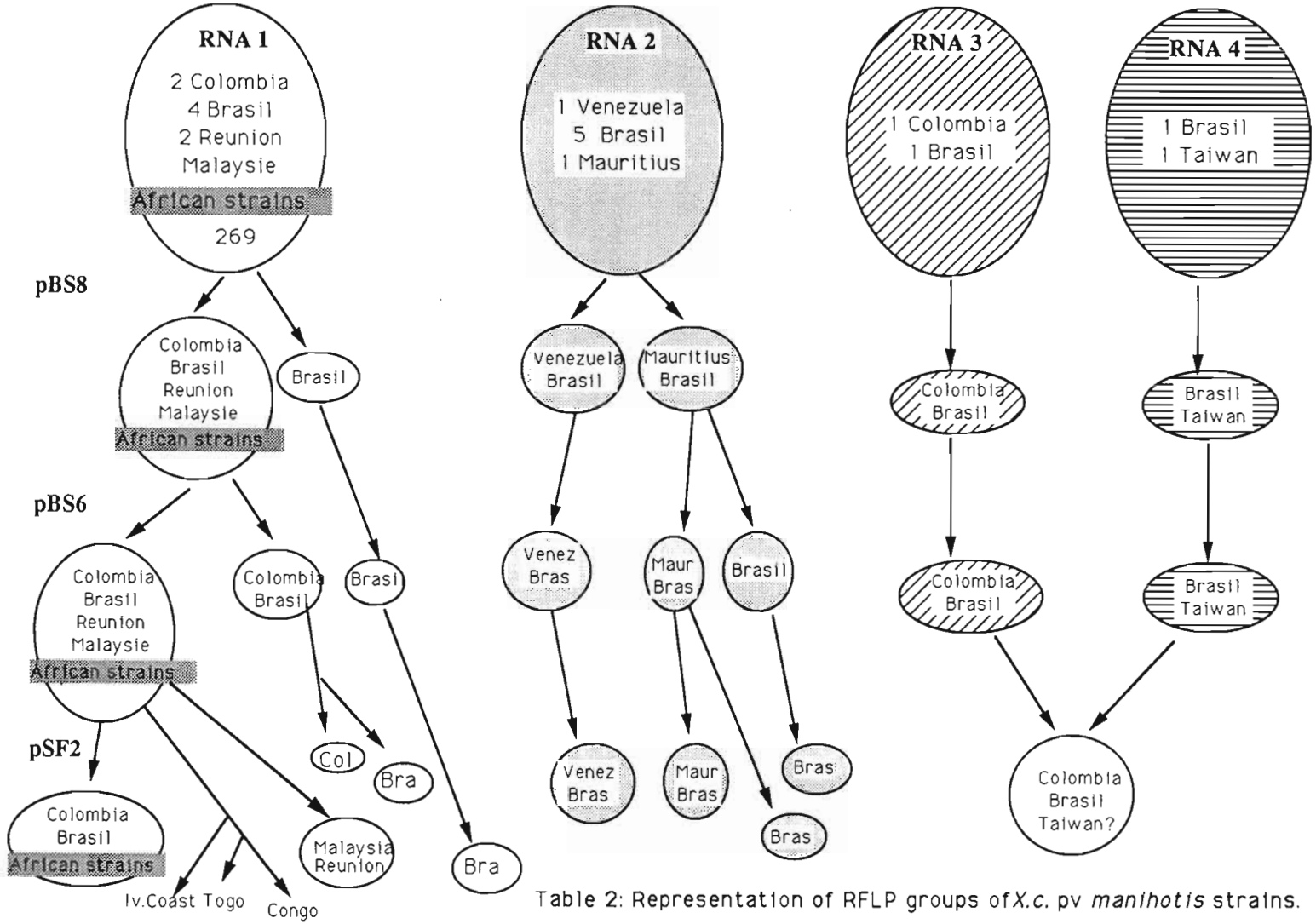

Table 2: Representation of RFLP groups of *X.c.* pv *manihotis* strains.

RFLP analysis.
Total genomic DNA isolation, endonuclease digestion, electrophoresis and Southern blot were done according to previously described methods (BERTHIER et *al*, 1992). Hybridization was made with different probes. Acetyl Amino Fluorene labeled ribosomal 16+23S RNA genes from *E.coli* (Eurogentec, Liege, Belgium) hybridized with the genomic DNA of bacteria. The rRNA - rDNA duplexes were detected using the anti-AAF monoclonal antibody (GRIMONT et *al*, 1989).
The DNA probes used in this study were: **BS6** (7kb-*Eco*RI) and **BS8** (8kb-*Eco*RI), two restricted fragments from the chromosomal DNA (*Xc.* pv *manihotis* strain CNBP1851-ClAT1111) and **pBsF2** derivated from the 13kb-*Hin*dIII fragment of plasmid DNA cloned in the bluescript vector plasmid. DNA probes were labeled *in vitro* by using a random priming kit with ^{32} P deoxycytidine triphosphate (Multiprime Amersham).

III. RESULTS

RFLP patterns.
Using the rRNA probe, the distinction of 4 RFLP groups among the 290 strains tested could be possible. Strains from South America were heterogenous and gave different patterns, on the contrary no polymorphism was noticed in African strains (Table 2).
Hybridization profiles with DNA probes could differentiate 6 groups with BS8 probe and 8 groups with BS6 probe, each group representing strains with identical RFLP pattern (Table 2). Polymorphism could be noticed in South American strains which are represented in groups mentioned above. In contrast, no polymorphism was observed in African strain with BS8 and BS6 probes suggesting that these regions are well conserved into the genome.
Variability among RFLP patterns of African strains was only noticed with the plasmid DNA probe pSF2.
Pathogenic characteristics.
Variability among pathogenic characteristics exists but was not related with the geographical origin of strains.
Phenotypic features.
Same results were obtained for two of the three phenotypic features tested (sensitivity to antibiotics and utilization of carbon sources). Starch hydrolysis was observed for all strains but two groups were differentiated. All African Reunion and Malaysian strains showed a low amylase activity similar to that found in 3 Brasilian and Colombian strains.

IV. DISCUSSION

Based on numerical analysis of protein gel electrophoregrams and, 267 phenotypic features, VAN DEN MOOTER et *al*, (1987) and VAUTERIN et *al*, (1991) indicate that the pv *manihotis* strains constitute a phenotypically and genetically homogeneous group. In this study, using the RFLP analysis, small changes in DNA organization were observed. Genetic variability of pv *manihotis* was more extensive in strains from the area of origin of the host plant and more limited in those coming from elsewhere. Among African strains homogeneity was observed with the probe corresponding to the rRNA genes and thus was confirmed with genomic probes used in this study. In our previous data based on plasmid DNA study we have indicated the hypothesis of one common geographic origin within strains of *Xc.* pv. *manihotis* (VERDIER, 1988). The results presented here agree with the hypothesis of the recent introduction of this pathogen from South America to the other countries, and suggest that African strains are not already diversified at chromosomal level. Using the DNA plasmid fragment as a probe, this study revealed that DNA polymorphisms exist in African strains. Plasmids are mobile elements which easily perform genetic exchange in bacterial strains (COPLIN, 1989; EBERHARD, 1990). Presence of essential pathogenicity genes on these plasmid fragment was previously demonstrated (VERDIER et *al*, 1989).
RNA and DNA probes used here were particularly useful in our epidemiological studies, providing information on the genetic population structure of these pathogens and its ability to identify clonally related individuals.

REFERENCES

BERTHIER Y., VERDIER V., GUESDON J.L., CHEVRIER D., DENIS J.B, DECOUX G., LEMATTRE M., 1993. Characterisation of *Xanthomonas campestris* pathovars by rRNA gene restriction patterns. App. Environ. Microbiol., 3, 851-859.

BOOTH R., LOZANO J.C., 1978. Cassava bacterial blight in south east asia. Plant Disease Reporter, 62, 6, 529-530.

BONDAR G., 1912. Una nova molestia bacteriana das hastes da mandioca. Characas e Quintais Sao paulo, 5, 15-18.

COPLIN D., 1989. Plasmids and their role in the evolution of plant pathogenic bacteria. Ann. Rev. Phytopathol., 27, 187-212.

EBERHARD W., 1990. Evolution in bacterial plasmids and levels of selection. The Quarterly Review of Biology, 65 (1), 3-22.

GRIMONT F., CHEVRIER D., GRIMONT P.A.D., LEFEVRE M., GUESDON J.L., 1989. Acetylaminofluorene-labelled ribosomal RNA for use in molecular epidemiology and taxonomy. Ann.Inst. Pasteur/ Microbiol 140:447-454.

GROUSSON F., PAGES J., BOHER B., 1990. Etude de la variabilité d'un agent pathogène, *Xanthomonas campestris* pv. *manihotis*, par l'analyse factorielle multiple. Agronomie, 4, 627-640.

LOZANO J.C., SEQUEIRA L., 1974. Bacterial blight of cassava in Colombia: epidemiology and control. Phytopathology, 64,83-88.

MARAITE H., MEYER J.A, 1975. *Xanthomonas manihotis* (Arthaud, Berthet) Starr causal agent of bacterial wilt and leaf spot of cassava in Zaïre. PANS, 21, 27-37.

MARAITE M., WEYNS J., YINKWAN O., LIPEMBRA P., PERREAUX D., 1981. Physiological and pathogenic variations in *Xanthomonas campestris* pv. *manihotis* p.358-368 in: Proceedings of the Fifth International Conference on Plant Pathogenic Bacteria, 16-30 August, Cali, Colombia, ed CIAT.

SILVESTRE P., ARRAUDEAU M., 1983. Le manioc. 262p. Techniques agricoles et productions tropicales. ACCT. Maisonneuve et Larose, Paris.

VAN DEN MOOTER M., MARAITE H., MEIRESONNE L., SWINGS J., GILLIS M., KERSTERS K., DE LEY J., 1987. Comparaison between *Xanthomonas campestris* pv. *manihotis* (ISPP list 1980) and *Xanthomonas campestris* pv. *cassavae* (ISPP list 1980) by means of phenotypic, protein electrophoretic, DNA hybridization and phytopathological techniques. J.Gen. Microbiol., 133, 57-71.

VAUTERIN L., SWINGS J., KERSTERS K., 1991. Grouping of *Xanthomonas campestris* pathovars by SDS-Page of proteins. J.Gen. Microbiol.,137, 1677-1687.

VERDIER V, 1988. Contribution à l'étude de la variabilité de *Xanthomonas campestris* pv. *manihotis* (Arthaud Berthet et Bondar) Starr agent causal de la bactériose vasculaire du manioc (*Manihot esculenta* Crantz). Thèse, Université de Paris Sud, Orsay, 216p.

VERDIER V., BOUCHER C., BARBERIS P., BOHER B, 1989. Plasmid borne phytopathogenicity genes in *Xanthomonas campestris* pv. *manihotis*. In abstracts of 7th International Conference on Plant Pathogenic Bacteria, Budapest, Hongrie, 11-16 Juin 1989.