



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 comparison of strains was developed 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
LMG 776, NCPPB2443, HMB72, CFBP2603		1970
ORST1, CIAT1060, CFBP1849		1970
ORST2, CIAT1061, CFBP1850	Venezuela	1971
ATCC 23380, HMB68, NCPPB1159	Brazil	1941
HMB 70, NCPBB1160, LMG5273		1941
HMB 55a, NCPBB1834*, LMF784		1965
ORST7, CFBP1854		1973
HMB23, LMG770		1973
ORST3, CIAT 1120, CFBP1852		1974
ORST5, CFBP1855		1974
ORST6, CFBP1856		1976
HMB79, LMG778		1978
LMG777, HMB78		1978
LMG779, HMB80		1978
HMB25, NCPBB3060, LMG 771	Nigeria	1976
ORST42		1978
ORST43		1978
CFBP1857, ORSTOM A202.1		1978
CFBP1858, ORSTOM A203.1		1978
CFBP1859, ORSTOM A205.1		1978
CFBP1860, ORSTOM A207		1978
ORST34	Benin	1982
ORST35		1982
ORST36		1982
ORST37		1982
ORST38		1982
CFBP1944	Ivory Coast	1979
LMG5249, HMB203		1981
ORST55		1984
ORST56		1984
ORST (198 strains)	Congo	1977-1991
ORST (29 strains)	Togo	1987-1991

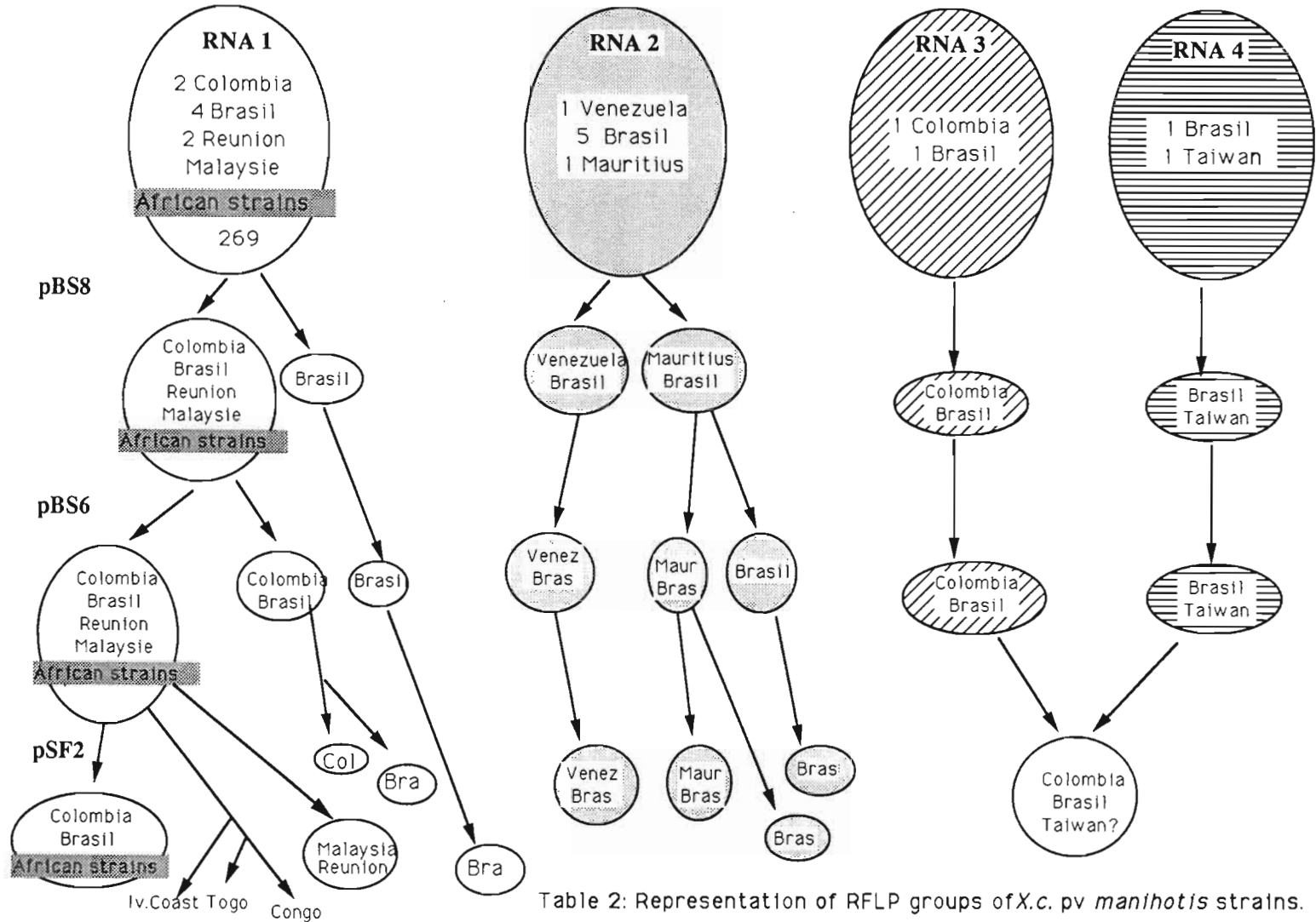
Strain no * and in other collection	Place and year of isolation	Isolated y
HMB6, LMG 767	Zaire	1973
HMB9, LMG 768, NCPBB3058		»
LMG 769, NCPBB3059, HMB10		»
LMG 766, HMB3		»
ORST44		1979
ORST45		»
ORST46		»
ORST47		»
ORST48		»
ORST49		»
ORST50		»
ORST51		»
ORST52		»
ORST53		»
ORST54		»
ORST186		1987
ORST187		»
ORST39	RCA	1977
ORST40		»
ORST41		»
LMG 5287, NCPPB 3161	Cameroon	1976
HMB27, LMG629		1977
LMG780, HMB81	Uganda	1979
LMG782, HMB93		»
LMG783, HMB148	Kenya	1979
LMG5288, NCPPB 3194	Niger	1978
LMG765	Malaysia	1980
LMG774, HMB60	Taiwan	1978
HMB71, NCPBB1161, LMG775	Mauritius	1946
CFBP2624	Reunion	1986
CFBP2635		1987

ATCC : American Type Culture, Rockville, Maryland, USA. CFBP : Collection Française de Bactéries Phytopathogènes, Angers, France.

NCPBB : 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 Internacional 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-*EcoRI*) and **BS8** (8kb-*EcoRI*), two restricted fragments from the chromosomal DNA (*X.c. pv manihotis* strain CNBP1851-CIAT1111) and **pBsF2** derived from the 13kb-*HindIII* fragment of plasmid DNA cloned in the bluescript vector plasmid. DNA probes were labeled *in vitro* by using a random priming kit with ³²P 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 Brazilian 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 *X.c. 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.