MEDWELL PUBLISHING
Research Journal of Biological Sciences 4 (3): 312-319, 2009
ISSN: 1815-8846
© Medwell Journals, 2009

Genetic and Pathogenic Diversity of *Xanthomonas albilineans* (Ashby) Dowson, in Mexico

[1]Manuel Huerta-Lara, [2]Reyna Isabel Rojas-Martinez, [2]Juliana Bautista-Calles,
[3]Delfino Reyes-Lopez, [3]Marcelino Becerril-Herrera, [3]Omar Romero-Arenas,
[4]Omar Franco-Mora, [1]Daniel Jimenez-Garcia,
[1]Agustin Aragon-Garcia, [3]Alfredo Simon-Baez and [5]Dagoberto Guillen-Sanchez
[1]Department of Agoecology and Ambient, Universidad Autonoma de Puebla,
Science Institute of the Benemerita, Calle 14 Sur No. 1601, Colony San Manuel,
C.P. 72570, Puebla, Puebla, Mexico
[2]Colegio de Postgraduados, Montecillo, Edo. Mexico
[3]Benemerita Universidad Autonoma de Puebla, Mexico
[4]Universidad Autonoma del Estado de Mexico
[5]Universidad Autonoma del Estado de Morelos

Abstract: The researching of genetic and pathogenic diversity of *Xanthomonas albilineans* (Xa) support directly the legal control and the genetic improvement of sugarcane (*Saccharum officinarum* L.). This researching was carried out with the objective to analyze genetic and pathogenic variability of the bacteria Xa from Mexico, applying isolated strain of syntomathic sugarcane from Veracruz (XaVer1 y XaVer2) and Chiapas (XaChis1 y XaChis2). The identification of Xa was carried out through PCR. The genetic variability was done using RFLP with the enzymes *Alu*I, *Kpn*I, *Tsp*5091, *Hpa*II y *Hha*I. The pathogenic variability was determined in the variety Mex 64-1487 in the greenhouse using a complete randomized design with 5 replications. The plants were inoculated with 1 mL, of 1.2×10^{8} CFU mL^{-1}, 6 days old bacterial suspensions or distilled water. The sequence of PCR amplified segment DNA of XaVer2 had a homology of 98% with an isolation of Xa coming from Brazil (Access: AF209751). The genetic markers got with *Alu*I, *Tsp*5091 and *Hpa*II allowed to different the Veracruz and Chiapas states, both in origin and in variability. The more pathogenic strains were XaVer2 and XaChis1 ($p\leq0.05$), both of them coming from susceptible varieties.

Key words: Genetic markers, diversity, leaf scald, sugarcane, *Xanthomonas albilineans*, resistance

INTRODUCTION

The bacteria *Xanthomonas albilineans* is the causal agent of the leaf scald diseases of sugarcane, illness that affects the vascular bundle causing severe symptoms; which are accompanied by white lines in the leaves, wilting, necrosis and proliferation of lateral bud (Birch and Patil, 1983; Huerta-Lara *et al*., 2003b; Champoiseau *et al*., 2006). Nevertheless, often it presents latent infection, doing the impossible visual diagnosis (Rott *et al*., 1997; Pan *et al*., 1999; Wang *et al*., 1999; Huerta-Lara *et al*., 2003a). Consistently, the pathogen is disseminated for the infected sugarcane stems used as seed for the propagation of the crops at the local level, as well as with the exchange of sugarcane seed to international level. The studies of genetic and pathogenic diversity can support directly the measures of legal control, as well as to the programs of so much genetic improvement in the national as international environment. Louws *et al*. (1999), they stated that the evaluation of the diversity of populations is necessary to establish a stable taxonomy and to develop durable strategies for the management of bacterial illnesses, on the base that the diversity is the degree of genetic variation among bacterial populations, this fact helps to order the systematic bacterial one in multiple levels taxonomic or filogenetic. The maps of genetic diversity, more probably in the form of dendrogram or filogenetic trees, it can provide orderly images of the natural structure of the bacterial variation in many taxonomic levels. Fundamental aspect for the improved plants varieties creation. The bacteria can be orderly an infrasubspecific level according to its differences in pathogeneticy, characteristic that permits to clarify its genetic variability. The pathogenic

Corresponding Author: Manuel Huerta-Lara, Department of Agoecology and Ambient, Universidad Autonoma de Puebla, Science Institute of the Benemerita, Calle 14 Sur No. 1601, Colony San Manuel, C.P. 72570, Puebla, Puebla, Mexico

races can differ in virulence, causing a symptoms severity rank when they are inoculated individually in host plant species (Young *et al*., 1992). These pathogenic variations can be detected by means of DNA. The DNA marked are genomic sequences located in a same one locus, that differ in their sequence of nitrogenous bases. These variations are consequence of various events of mutation that are declared in the genomes that are compared. The detection of some these mutations can be realize with the technique called RFLP (Restriction Fragment Lenght Polymorphism), which indicate the differentiate in specific places of the DNA that are recognized for enzymes of private restriction or endonucleasa; technical that permits to visualize the population genetic variability with a pattern of products amplified (Valadez and Kalh, 2000).

The analysis of the sequence in the transcribed intergenic region between the 16 and 23 S gene of ribosomal DNA has come be a standard technique for the identification of unknown bacteria, since is a highly conserved region with smaller variations among these organisms. The advantage to use genotypic information to identify bacteria is due that these characters are not influenced for nutritious, environmental and temperature conditions neither age of the microorganism; by which, the data obtained with these methodologies are less variable and can be interpreted with greater precision that the data obtained with the physiological or biochemical tests (Alvarez *et al*., 1996; Davis *et al*., 1997; Louws *et al*., 1999; Pan *et al*., 1999; Wang *et al*., 1999; Valadez and Kahl, 2000; Schaad *et al*., 2001, 2003). The bacteria, or other organisms, are identified comparing the and sequence previously amplified with the nucleotic sequences stored in the databases of the Gene-Bank available in internet (Schaad *et al*., 2001).

The genetic variation has been observed so much in the populations of plants as in those of the pathogen. In the case of sugarcane-*X. albilineans* pathosystem, different levels of sensitivity or resistance have been observed to international level among identical sugarcane varieties. In the case of the bacteria has been observed like changes in aggressiveness or virulence, so much at the local level as international (Alvarez *et al*., 1996), observation that have stimulated the investigators to study the variability of *X. albilineans* and the sugarcanes in detail. Rott *et al*. (1994), they carried out studies of genetic diversity of *X. albilineans* on a worldwide basis with serological methods and they determined three groups to which they called them serovars. The serovar I included bacterial stock of Australia, United States, Island Guadalupe, India, Mauritania and south Africa. The serovar II included bacterial stock originating from Africa and the serovar III that grouped stock originating from Caribbean Islands, Fiji and Sri Lanka in Asia. Pan *et al*. (1997), they studied the genetic variability in this same bacteria, utilizing molecular methods as the Polymerase Chain Reaction or PCR (by their acronyms in English), including strains type of each serovar determined by Rott *et al*. (1994). These investigators found correspondence with the results of Rott *et al*. (1994), upon determining also three greater groups, but with the difference that they determined eight subgroups more; it permitted them to conclude that the molecular methods, based on DNA, have greater sensibility for the studies of genetic diversity. Observation that coincide with Alvarez *et al*. (1996), who indicated that the molecular methods have the advantage on the serological and nutrimental methods, because it permit us to differentiate more quickly among strain of *X. albilineans* that were considered equals.

Actually, the leaf scald, caused by the bacteria *X. albilineans*, remains as a primary problem due to the lack of study of genetic and pathogenic variability in this bacteria, that help to carry out the safe evaluation of sugarcane varieties resistance for this illness. Preceding, that combined with the importance of the sugarcane agroecosystem and the existence of international and local quarentenary measures, were reason to carry out the present research with the objective to analyze the genetic and pathogenic variability in the *X. albilineans* populations including strain from Veracruz and Chiapas state only, because of this bacteria was not found in other place of Mexico.

MATERIALS AND METHODS

***Xanthomonas albilineans* isolation:** The sugarcane plants with symptoms of leaf scald were collected in the geographical regions of Mexico, that to the date, presented outbreak of the illness. The variety Mex 64-1487 was collected in the Municipality of Ursulo Galvan, Veracruz and the variety Q 117 in the Municipality of Manlio Flavio Altamirano, Veracruz, Mexico, both were collected in August of the 2001. The varieties CP 31-294 and Co 380 they were collected in July of the 2002, in different lots of the National Station of Sugar Cane Hybridization in Tapachula, Chiapas, Mexico. The bacteria *X. albilineans* (Xa) was isolated in the "Instituto de Fitosanidad de el Colegio de Postgraduados", utilizing the selective basal media (Davis *et al*., 1994). The bacterial strains isolated were called: XaVer1 (*X. albilineans* obtained from the variety Mex 64-1487), XaVer2 (*X. albilineans* obtained from the variety Q 117), XaChis1 (*X. albilineans* obtained from the variety CP 31-294) and XaChis2 (*X. albilineans* obtained from the variety Co 380).

Genotypic characterization of *X. albilineans*: The genotypic characterization was carried out with DNA extracted of strain of Xa originating in Veracruz and Chiapas according to the technique described by Huerta-Lara *et al.* (2003b). After of quantified the DNA, it carry out the PCR, employing the specific primers to *X. albilineans*: PGBL1 (5'CTT TGG GTC TGT AGC TCA GG3') and PGBL2 (5' GCC TCA AGG TCA TAT TCA GC3'), designed by Pan *et al.* (1999) for amplify a segment of 288 bases pairs in the transcribed intergenic region between the 16S and 23 S gene of ribosomal DNA. As negative control was utilized DNA purified (20 ng μL^{-1}) and bacterial cells in suspension of a yellow saprophytic bacteria Gram negative (obtained from sugarcane together with *X. albilineans*). The reaction of PCR was prepared according to the diagnosis of Xa carried out by Huerta-Lara *et al.* (2003b).

The products of PCR obtained of *X. albilineans* strain originating in Veracruz, were cleaned and purified with the Kit Wizard® PCR preps. The nucleic acid sequencing was carried out in the "Instituto de Biologia Celular de la Universidad Nacional Autonoma de Mexico. The sequences were compared way Internet using the databases in the gene-bank, employing the BLAST program (www.ncbi.BLAST.nr).

Restriction Fragment Lenght Polymorphism (RFLP): The genetic variability in the different strains of *X. albilineans* from Veracruz and Chiapas state: XaVer1, XaVer2, XaChis1 and XaChis2, were analyzed submitting the product of PCR to the digestion with the restriction endonucleasas: *Alu*I and *Kpn*I (Biolabs®), *Tsp*5091, *Hpa*II and *Hha*I (Gibco®) and *Mse*I (Mbi Ferment®). The reaction mixture was prepared in a final volume of 20 μL by enzyme of restriction. To each tube were added 10 μL of PCR product, 2 μL of buffer, 0.2 μL of each enzyme, except for *Mse*I that 0.4 μL were added. All tubes were gauged to 20 μL with deionized and sterile water. The tube with the *Alu*I, *Kpn*I, *Hpa*II, *Hha*I and *Mse*I enzymes were incubated 12 h to 37°C and the pipe with *Tsp*5091 was incubated 3 h to 65°C. The DNA fragments obtained with the enzymatic digestion were separated in a 5% polyacrilamyde gel. Then were charged 20 μL of the reaction more 4 μL of loading buffer, the gel ran in electrophoresis camera for 4 h at 85 volts (Sambrook *et al.*, 1989; Valadez and Kahl, 2000). The molecular markers utilized were 1 kb (Gibco BRL®) and Ø (MCA®) and were charged 2 μL each one. The gel was stained with ethidium bromide(bromide 3,8-diamino-5-etil-6-fenilfenantridinium) for 15 min and was visualized with ultraviolet light in a computerized photodocumented (Bio Rad®).

Dissimilarity analysis: To determine the genetic similarity in the isolations of *X. albilineans* originating from Veracruz and Chiapas, was elaborated a master record of data with the patterns of restriction. From the patterns of bands obtained a binary matrices was built; values were assigned that described the presence (1) or absence (0) of these bands. The program NTSyS® (pc. 2,0) was used to elaborate the similarity matrices with the Dice coefficient. Finally, the conglomerates analysis was carried out used the Unweighted Pairs Group Method with Arithmetic mean (UPGMA), with the programs S+4.0 for Windows®. The data obtained were submitted for an analysis of sturdiness of the tree diagram or dendrograms, for which 2000 repetitions were carried out. The dissimilarity analysis was done used the coefficient of matching method bootsstraps (random samples with replacement of bands). By the graphic observation in the genetic relations among the strains and to evaluate the goodness of the conglomerates analysis, a master record was built with cophenetic values (matrices of ultranumerics values) from the master record of similarity.

Pathogenic variability in *Xanthomonas albilineans*: The experiment was carried out in the greenhouses of the Colegio de Postgraduados using the sugarcane variety Mex 64-1487 (susceptible variety to *X. albilineans*), in a complete randomized design with 5 repetitions. The plants were cultivated in January 2003 in black plastic bags utilizing stems of a same stump of cane. The plants were maintained 3 months in the Campus Veracruz of the Colegio de Postgraduados and subsequently they were transferred to Colegio de Postgraduados in Texcoco, Mexico state. In June 2003, the different strain of *X. albilineans* bacteria (XaVer1, XaVer2, XaChis1 and XaChis2), were inoculated by injection in the half of the stem of five sugarcane plants, using 1 mL of a 6 days old bacterial suspensions, with an approximate concentration of 1.2×10^{8} CFU mL^{-1} (Colony Forming United for mL). The witness plants were treated similarly, but with sterile and distilled water.

RESULTS

***Xanthomonas albilineans* identification:** The DNA sequencing in the fragment of 288 bases pairs amplified with PCR (Fig. 1), permitted to corroborate the identification of *X. albilineans* by comparison with the sequences GENEBANK, because the XaVer2 strain (*X. albilineans* from Veracruz state), presented a 98% of homology with a strain from *X. albilineans* native of Brazil (Access: AF209751, reported by Goncalves and

Table 1: Genetic variability in *Xanthomonas albilineans* strains from Veracruz and Chiapas states, Mexico, detected by Restriction Fragment Lenght Polymorphism (RFLP) in the transcribed intergenic region between the 16 and 23 S of ribosomal DNA gene amplified with PCR. 2003. Mexico

Restriction enzyme	Fragments number obtained in the *X. albilineans* strains			
	*XaVer1	*XaVer2	**XaChis1	**XaChis2
AluI	1	2	3	3
KpnI	3	2	4	4
TSp5091	4	4	3	3
HpaII	3	3	4	4
HhaI	4	4	3	2
MseI	--	3	4	2

* = *X. albilineans* isolated from Mex 64-1487 variety collected in Veracruz state. + = *X. albilineans* isolated from Q 117 variety collected in Veracruz state. ** = *X. albilineans* isolated from CP 31-294 variety collected in Chiapas state. + + = *X. albilineans* isolated from Co 380 variety collected in Chiapas state

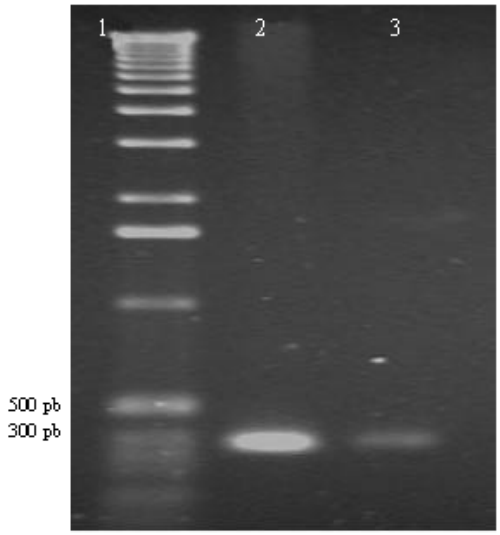

Fig. 1: Amplification of the intergenic region between the 16 and 23 S of ribosomal DNA gene on *Xanthomonas albilineans* (Xa), lanes: 1 = Molecular marker 1KB, 2 = Strain XaVer2 from Veracruz, Mexico and 3 = Strain XaChis1 from Chiapas, Mexico 2003

Rosato, 2002). The strain of Xa from Chiapas state was not possible to sequencing, due to the little concentration of DNA purified (Fig. 1: Rail 3).

Genetic variability in *Xanthomonas albilineans*: The four strains of *X. albilineans*: XaVer1, XaVer2, XaChis1 and XaChis2 showed genetic variability by comparing the patterns bands obtained with the restriction enzymes *Alu*I, *Kpn*I, *Tsp*5091, *Hpa*II, *Hha*I and *Mse*I (Table 1). The genetic variation was observed between isolates from the same region, XaVer1 and XaVer2, but it was more evident among isolates from different geographical regions of Mexico: XaChis1 and XaVer2.

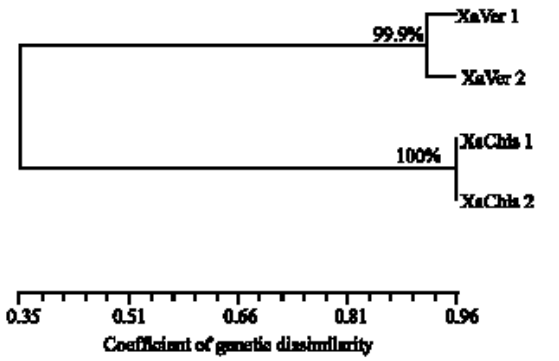

Fig. 2: *Xanthomonas albilineans* dendrogram isolated from different geographical regions of Mexico. State of Veracruz: XaVer1 and XaVer2. State of Chiapas: XaChis1 and XaChis2

Dissimilarity analysis: The dendrogram developed with the restriction patterns obtained with different enzymes, made clear the existence of genetic variability among isolates of *X. albilineans* (Fig. 2). The populations studied were divided into two groups genetically highly counterparts according to place of origin: One formed by the isolated Veracruz: XaVer1 and XaVer2, with a probability of 99.9% of the group and a second group formed by the isolated Chiapas: XaChis1 and XaChis2, with a likelihood of a grouping of 100%.

The pathogenic variability in *Xanthomonas albilineans*: The four strains of *X. albilineans* (XaVer1, XaVer2, XaChis1 and XaChis2) inoculated in Mex 64-1487 sugarcane plants in greenhouse, presented white lines parallel to the central venation of the leaf (Fig. 3). The 4 strains showed pathogenic variability, both in virulence as aggressiveness (Table 2). The most aggressive isolates were XaVer2 and XaChis1 ($p \leq 0.05$), which were taken from more susceptible sugarcane varieties (Q 117 and CP 31-294, respectively). The highest bacterial population found at the time in the obtained the different strain of *X. albilinean*, in July 2002, was the XaChis1 isolated from the variety CP 31-294, which reached 34'450,000 cfu $g^{-1}$of fresh tissue, followed by the strain XaVer2 obtained from the variety Q 117, with 5'518,333 cfu g-1 fresh tissue. The strain XaVer1 was significantly similar in aggressive than the strain XaChis2 ($p \leq 0.05$), as it had a similar bacterial population (Table 2), which was 1'165,500 cfu g^{-1} fresh tissue for XaVer1 and 926,600 cfu g^{-1} fresh tissue for XaChis2. The single most virulent, in terms of average number of white lines per sheet, was XaVer2 ($p \leq 0.05$), followed by XaChis1 and XaVer1, which were statistically

Table 2: Pathogenic variability of *Xanthomonas albilineans* from different geographical regions of Mexico, inoculated by injection into the susceptible variety Mex 64-1487 under greenhouse in Montecillo, Mexico, 2003

Xanthomonas albilineans Strain[y]	Inoculation (CFU mL^{-1})	Average white lines per plant				CFU g^{-1} fresh tissue after 48 ddi[x]	
		10 ddi	22 ddi	33 ddi	48 ddi	Stem	Apical section
XaVer1	1.2×10^8	0.20a[z]	3.02a	3.40[a]	4.20b	8'274,000c	1'236,250c
XaVer2	1.2×10^8	0.20a	2.64a	2.69[a]	5.27a	45'131,333a	2'100,000a
XaChis1	1.2×10^8	0.0a	2.30a	2.85a	4.01b	19'300,667b	1'316,666bc
XaChis2	1.2×10^8	0.0a	1.70a	1.21b	2.93c	7'002,333c	1'455,417b
Testigo	0.0	0.0a	0.0b	0.0c	0.0d	0.0d	0.0d

CFU = Colony Forming Units. [X]= Days after inoculation. [y] = From Veracruz state: XaVer1 and XaVer2; from Chiapas state: XaChis1 and Xachis2. [z]= Equal letters in the same column indicate that the values are not statistically different (Tukey, p≤0.05)

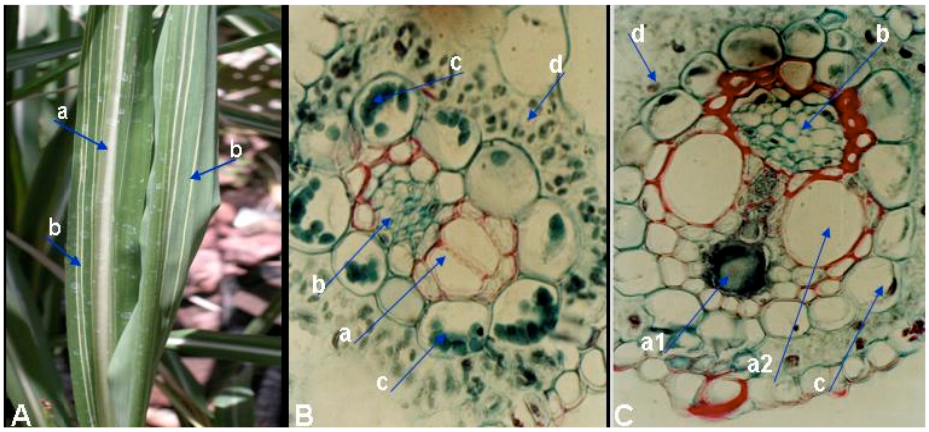

Fig. 3: Chronic symptom of the leaf scald in sugarcane Mex 64-1487, after 48 day of inoculated with the bacterium *Xanthomonas albilineans* (Xa) under greenhouse conditions in Montecillo, Mexico. 2003. A): leaves showing symptoms of scald: a = Central venation, b = White lines parallel to the central venation of the leaf, B): Cross-leaf healthy: a = Xylem, b = phloem, c = Parenchyma cells with completely developed chloroplasts and d = Parenchyma cells with chloroplasts, C): Cross-leaf infected that presented white lines of scald: a = Xylem (a1 = Protoxylem lacuna occluded for Xa bacteria and a2 = Metaxylem vessels), b = Phloem, c = Sheath cells without chloroplasts or with reduction in number and size of chloroplast and d = Parenchyma without chloroplasts or with reduction in number and size of chloroplast

equal (p≤0.05). The less virulent strain XaChis2 was obtained originally from Co 380 resistant variety leaf scalf.

DISCUSSION

***Xanthomonas albilineans* identification:** The analysis in the intergenic region between the 16S and 23S of ribosomal DNA gene is a standard technical for identification of unknown bacteria, because of it is a highly conserved region, therefore, gives greater accuracy than the physiological or biochemical tests (Pan *et al*., 1997; Sasaki *et al*., 1997; Schaad *et al*., 2001, 2003). The identification of Xa with DNA, using primers designed by Pan *et al.* (1999), was positive for all strain of Xa to amplify a segment of 288 base pairs. Fact that it agreed with the colonies morphology, which were circular, honey-colored, convex, no mucous, with a diameter less than 0.5 mm, smooth and shiny, as described by Davis *et al.* (1994). These phenotypic characteristics of colonies strengthen the homology of 98% found with an isolated *X. albilineans* originating in Brazil (GeneBank Access: AF209751, reported by Goncalves and Rosato, 2002). The strain of Xa from Chiapas state was not possible to sequencing, due to the little concentration of DNA purified (Fig. 1: Rail 3). However, the fragment amplified by PCR was equal to the reported by Pan *et al.* (1999).

Genetic variability of *Xanthomoans albilineans*: The genetic variability of *X. albilineans* has been demonstrated at the global level, which stronger the results of genetic variation obtained here. Rott *et al.* (1994), identified 3 groups worldwide using serological methods, on the other hand, Pan *et al.* (1997), identified three major groups as well, but with eight subgroups, using strains of each serovar type determined by Rott *et al.* (1994). Davis *et al.* (1997) found genetic

variation among 218 strains of *X. albilineans* from 31 geographic regions in different parts of the world, using genomic DNA with restriction enzyme *Spe*I. They indicated that this genetic variation may be associated with the factors that govern the spreading of the pathogen from one plant to another in nature, as well as to environmental conditions and the interchange of genetic material in plasmid (Schaad *et al*., 2001), a phenomenon that could have happened between the strains from Chiapas, which were collected in one area, but in different sugarcane varieties.

The strains from Veracruz showed bigger genetic dissimilarity that the strains from Chiapas, because they were clustered at a distance of 0.92, unlike the strains of Chiapas, which were consolidated at a distance of 0.96. Indeed showed that the genetic diversity of populations of *X. albilineans* in Mexico, emphasizing at the same time, that the strains from Chiapas are more closely related and have closer proximity genetic that Veracruz strains; aspect that may be because both strains from Chiapas were collected in the same region, within the experimental plots in the National Hybridization Station of Tapachula. Therefore, the small genetic variation detected with the restriction enzymes *Hha*I and *Mse*I, may be because it comes from different sugarcane varieties: XaChis1 from variety CP 31-294, susceptible to *X. albilineans* and XaChis2 from variety Co 380, which is similar to Co 997 classified as resistant to *X. albilineans* (Huerta-Lara *et al*., 2003a, b). This aspect is consistent with Davis *et al.* (1997), who concluded that the variation in the races of *X. albilineans* may be associated with the factors that govern the transfer or spread of the pathogen from one plant to another. This also explains, the difference in the strains from Veracruz, which have a greater genetic dissimilarity because both are from different regions and different sugarcane varieties: XaVer1 strain comes from the variety susceptible Mex 64-1487 (Huerta-Lara *et al*., 2003a, b), in the municipality of Ursula Galvan and XaVer2 comes from the variety Q 117, also designated as susceptible, the Municipality of M.F. Altamirano. *Tsp*5091 and *Hpa*II enzymes allowed to clearly distinguishing between strains of *X. albilineans* from Veracruz and Chiapas states (Table 1). The Veracruz strains of Xa presented four bands with the enzyme *Tsp*5091 and the Chiapas strains only three bands. But the result was totally reversed when using the enzyme *Hpa*II. The enzyme *Alu*I allowed to clearly distinguishing between strains of Veracruz and the enzyme *Mse*I between strains collected in Chiapas (Table 1). The use of enzymes *Alu*I and *Mse*I or *Hha*I appears to be enough to detect the origin and genetic variability of different strains of *X. albilineans* studied here, as indicated Alvarez *et al.* (1996), who concluded that the molecular methods to distinguish quickly between strains of *X. albilineans* that seemingly are equal. The use of restriction enzymes in different Mexican strain of *X. albilineans*, from different geographical regions, identified mutations in the intergenic region between the 16 and 23 S of ribosomal DNA gene amplified with PCR. These mutations detected with RFLP indicated genetic differences at the level of DNA bases in a single locus, that although, mutations of a single base in a gene can encode for the synthesis of a new protein, which could result in a virulence factor and lead to the emergence of a new race more pathogenic and aggressive (Valadez and Kahl, 2000). Several authors who studied the genetic variability of *X. albilineans* in the world concluded that knowledge of the genetic variability of populations of *X. albilineans* allows substantiate the establishment of national and international quarantine measures (Rott *et al*., 1994; Alvarez *et al*., 1996; Davis *et al*., 1997). Those activities may prevent disease outbreaks that are destructive and costly to the sugarcane industry (Hoy and Grisham, 1994; Rott *et al*., 1994; Alvarez *et al*., 1996; Davis *et al*., 1997; Pan *et al*., 1999; Wang *et al*., 1999 and Huerta-Lara *et al*., 2003b). As noted in the case reported by Davis *et al.* (1997), who attributed the leaf scald outbreak in Florida, Texas, Louisiana, Mexico, Guatemala, Isla Guadalupe and Mauritania to the introduction of a new race of *X. albilineans*. This is due to the disease had been endemic for many years before the outbreak in Florida and Mauritania.

Pathogenic variability of *Xanthomonas albilineans*: The four isolates of *X. albilinean* inoculated in the sugarcane plants Mex 64-1487 under greenhouse, presented the typical white lines parallel to the central nervure of the leaf, as noted Rott *et al*. (1994, 1997), Davis *et al*. (1997) and Huerta-Lara *et al*. (2003a, b) for the leaf scald disease in sugarcane. This symptom is due to the lack of completely developed chloroplasts in sheath cells surrounding the vascular bundles (Fig. 3), which are inhibited in their development by the albicidin toxin that affects the DNA replication in prokaryotic organisms (Birch and Patil, 1983; Champoiseau *et al*., 2006).

The variation of the races of *X. albilineans,* in virulence or aggressiveness, may be associated, in part, with the factors that govern the spreading of the pathogen in nature (Alvarez *et al*., 1996). In this case, the strains of Xa more aggressive and virulent were the bacterial strains obtained from the initially more susceptible sugarcane varieties: Q 117 for the strain XaVer2 and CP 31,294 for the strain XaChis1 (Table 2). The analysis of aggressive colonization of the stem,

based on number of cfu g^{-1} of fresh tissue, showed two groups of the population *X. albilineans*: One formed by XaVer2 and XaChis1, which are more aggressive and another formed by XaVer1 and XaChis2, which are less aggressive (Table 2). The virulence test (average number of white lines per leaf), allowed to differentiate between isolated XaVer1 and XaChis2 ($p \geq 0.05$) to 48 days after inoculation (ddi), but no difference between strains XaVer1 and XaChis1, which showed virtually the same number of white lines ($p \leq 0.05$). However, the RFLP technique based on DNA allowed to differentiate each of the strains of *X. albilineans* as contusive (Table 1), while that providing an image of the naturally grouping of *X. albilineans* in the field (Fig. 2), as indicated Alvarez *et al.* (1997), Davis *et al.* (1997), Pan *et al.* (1999), Louws *et al.* (1999), Wang *et al.* (1999) and Schaad *et al.* (2001). The molecular technique of RFLP showed the genetic diversity of *X. albilineans* between geographic areas studied, clustering the strain in two populations groups: One formed by the isolated from Veracruz state and another formed by the isolated from Chiapas state.

CONCLUSION

Based on the results fined in this study were concluded, that populations of the *Xanthomonas albilineans* in Mexico presented bigger pathogenic variability when it was develop in susceptible sugarcane varieties. The most aggressive and virulent strains were XaVer2 and XaChis1, both isolated from susceptible varieties: Q 117 and CP 31-294, respectively. The less aggressive and virulent strain was XaChis2 isolated from the Co 380 variety. The use of restriction enzymes *Alu*I and *Tsp*5091o *Hpa*II may be sufficient to detect as the origin as the genetic variability of different strains of *X. albilineans* in Mexico. The determination about 2 population groups of *X. albilineans*, coupled with the variability in the different pathogenic strains fined, are results that strengthen the quarantine measures that restrict the mobilization of sugarcane plants diseases to free zones of scald, both at national and international levels.

REFERENCES

Alvarez, A.M., S. Schenck and A.A. Benedict, 1996. Differentiation of *Xanthomonas albilineans* strains with monoclonal antibody reaction patterns and DNA fingerprints. Plant Pathol., 45: 358-366. DOI: 10.1046/j.1365-3059.1996.d01-118.x. http://cat.inist.fr/?aModele=afficheN&cpsidt=3043287.

Birch, R.G. and S.S. Patil, 1983. The relation of blocked chloroplast differentiation to sugarcane leaf scald disease. Phytopathology, 73: 1368-1374. DOI: 10.1094/Phyto-73-1368.

Champoiseau, P., J.H. Daugrois, J.C. Girard, M. Royer and P.C. Rott, 2006. Variation in albicidin biosynthesis genes and in pathogenicity of *Xanthomonas albilineans*, the sugarcane leaf scald pathogen. Phytopathology, 96: 33-45. INIST-CNRS, Cote INIST: 674, 35400011519312.0010.

Davis, M.J., P. Rott, P. Baudin and J.L. Dean, 1994. Evaluation of selective media and immunoassays for detection of *Xanthomonas albilineans*, causal agent of sugarcane leaf scald disease. Plant Dis., 78: 78-82. DOI: 10.1094/PD-78-0078. http://www.apsnet.org/pd/SEARCH/1994/PD_78_78.asp.

Davis, M.J., P. Rott, C.J. Warmuth, M. Chatenet and P. Baudin, 1997. Intraspecific genomic variation within *Xanthomonas albilineans*, the sugarcane leaf scald pathogen. Phytopathology, 87: 316-324. http://cat.inist.fr/?aModele=afficheN&cpsidt=2597832.

Goncalves, E.R. and Y.B. Rosato, 2002. Phylogenetic analysis of Xanthomonas species based upon 16S-23S rDNA intergenic spacer sequences. Int. J. Syst. Evol. Microbiol., 52: 355-361. DOI: 10.1099/ijs.0.01886-0. http://ijs.sgmjournals.org/cgi/content/abstract/52/2/355.

Hoy, J.W. and M.P. Grisham, 1994. Sugarcane leaf scald distribution, symptomatology and effect on yield in Louisiana. Plant Dis., 78: 1083-1087. Cote INIST: 12673, 35400005740965.0140.

Huerta-Lara, M., L.D. Ortega-Arenas, C. Landeros-Sanchez, L. Fucikovsky-Zak and M. Marin-Garcia, 2003a. Response of 10 sugarcane varieties to leaf scald [*Xanthomonas albilineans* (Ashby) Dowson], in the central cost region of Veracruz. Agrocienc., 37: 511-519. http://www.colpos.mx/agrocien/Bimestral/2003/sep-oct/sep-oct-03.html.

Huerta-Lara, M., J.S. Sandoval-Islas, E. Cardenas-Soriano, R.I. Rojas-Martinez, S. Flores-Caceres and M. Marin-Garcia, 2003b. Resistance evaluation of sugarcane varieties Co 997 and Mex 64-1487 using colonization and poblacional dynamic of *Xanthomonas albilineans* in the stem. Mex. Rev. Phytophatol., 21: 316-322. http://www.cababstractsplus.org/google/abstract.asp?AcNo=20053147946.

Louws, F.J., J.L.W. Rademaker and F.J. De Bruijn, 1999. The three Ds of PCR-based genomic analysis of phytobacteria: Diversity, detection and disease diagnosis. Ann. Rev. Phytopathol., 37: 81-125. DOI: 10.1146/annurev.phyto.37.1.81.

Pan, Y.B., M.P. Grisham and D.M. Burner, 1997. A polymerase chain reaction protocol for detection of *Xanthomonas albilineans*, the causal agent of sugarcane leaf scald disease. Plant. Dis., 81: 189-194. DOI: 10.1094/PDIS.1997.81.2.189. http://apsjournals.apsnet.org/doi/abs/10.1094/PDIS.1997.81.2. 189.

Pan, Y.B., M.P. Grisham, D.M. Burner, B.L. Legendre and Q. Wey, 1999. Development of polymerase chain reaction primers highly specific for *Xanthomonas albilineans*, the causal bacterium of sugarcane leaf scald disease. Plant Dis., 83: 218-222. DOI: 10.1094/PDIS.1999.83.3.218. http://apsjournals.apsnet.org/doi/abs/10.1094/PDIS.1999.83.3.218.

Rott, P., M. Abel, D. Soupa, P. Feldmann and P. Letourmy, 1994. Population dynamics of *Xanthomonas albilineans* in sugarcane plants as determined with an antibiotic-resistant mutant. Plant Dis., 78: 241-247. Cote INIST: 12673, 35400002563956.0050.

Rott, P., I.S. Mohamed, P. Klett, D. Soupa, A. De Saint-Albin, P. Feldmann and P. Letourmy, 1997. Resistance to leaf scald disease is associated with limited colonization of sugarcane and wild relatives by *Xanthomonas albilineans*. Phytopathology, 87: 1202-1213. DOI: 10.1094/PHYTO.1997.87.12.1202. http://apsjournals.apsnet.org/doi/abs/10.1094/PHYTO.1997.87.12.1202.

Sambrook, D.J., E.F. Fritsch and T. Maniatis, 1989. Molecular Cloning: A Laboratory Manual. 2nd Edn. Cold Spring Harbor Laboratory Press, N.Y. 7.84 secciones. http://www.ist-world.org/ResultPublicationDetails.aspx?ResultPublicationId=0100e96258da4ba0a07bc909f3e26abc.

Sasaki, T., T. Nishiyama, M. Shintan and T. Kenri, 1997. Evaluation of a new method for identification of bacteria based on sequence homology of the 16S rRNA gene. PDA J. Pharm. Sci. Technol., 51: 242-247. PMID: 9448434.

Schaad, N.W., J.B. Jones and W. Chun, 2001. Laboratory Guide for Identification of Plant Pathogenic Bacteria. 3rd Edn. APS Press. St. Paul, Minnesota, pp: 373. ISBN: 0-89054-263-5.

Schaad, N.W., R.D. Frederick, J. Shaw, W.L. Schneider, R. Hickson, M.D. Petrillo and D.G. Luster, 2003. Advances in molecular-based diagnostics in meeting crop biosecurity and phytosanitary issues. Ann. Rev. Phytopathol., 41: 305-324. Cote INIST: 10829, 35400011305225.0140.

Valadez, E. and G. Kahl, 2000. DNA Marking in Plant Genomes. In: Mundi Prensa, C.V. (Ed.). Mexico, D.F., pp: 147. ISBN: 9687462221.

Wang, Z.K., J.C. Comstock, E. Hatziloukas and N.W. Schaad, 1999. Comparison of PCR, Bio-PCR, ELISA and isolation on semiselective medium for detection of *Xanthomonas albilineans*, the causal agent of leaf scald of sugarcane. Plant Pathol., 48: 245-252. Cote INIST: 7414, 35400008352917.0130.

Young, J.M., Y. Takikawa, L. Gardan and D.E. Stead, 1992. Changing concepts in the taxonomy of plant pathogenic bacteria. Ann. Rev. Phytopathol., 30: 67-105. DOI: 10.1146/annurev.py.30.090192.000435.