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Isolation and genetic characterization of *Acidovorax avenae* from red stripe infected sugarcane in Northwestern Argentina

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Abstract Red stripe is a bacterial disease of sugarcane causing important economic losses in Argentina that affects 30 % of the milling stems and consequently the juice quality. In this study, sugarcane leaves exhibiting red stripe symptoms were sampled in the 2008–09 growing season from 13 different sugarcane producing areas of Tucumán and Salta (northwest of Argentina). To achieve the identification and characterization of the causal agent of red stripe, bacterial isolation was performed. Species-specific PCR using Oaf1/Oar1 primers allowed the amplification of a fragment of 550 bp from approximately 50 % of the isolates; 16S rDNA sequences analysis displayed a similarity greater than 99 % with *Acidovorax avenae* subsp. *avenae.* By means of RAPD-PCR the presence of at least four different biotypes among the analyzed isolates was detected. Results of pathogenicity test allowed us to confirm *A. avenae* subsp. *avenae* as the pathogenic agent for red stripe. This study constitutes the first report on the identification and molecular characterization of this plant pathogen from the Argentina sugarcane production areas. The genetic diversity observed among *A. avenae* is an important factor to be considered to improve an accurate diagnosis and/or the selection of sugarcane tolerant clones.

Keywords Sugarcane · Red stripe · *Acidovorax* · RAPD-PCR · Genetic diversity

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Introduction

Sugarcane is the most important sacchariferous crop worldwide, accounting for 70 % of the total sugar production. In Argentina, sugarcane production is geographically distributed in three regions: Tucumán, Northern (Salta and Jujuy) and Littoral (Santa Fe and Misiones), occupying an approximate area of 300.000 ha and representing one of the main economic resources in Northwestern region (http://www.centroazucarero.com.ar/zafra2009). Sugarcane agroindustry accounts for 1. 5 % of the national gross domestic product (GDP), the strong impact on the provinces GDP (Jujuy 58.5 %, Tucumán 50 % and Salta 25.6 %) has always been considered of substantial importance for social and economic development (Mariotti 2008). In particular, Tucumán has traditionally been the most important region with 60–65 % of the total of Argentina production.

Many factors - climatic, phytosanitary, economic and political - may affect sugarcane productivity. Sugarcane

diseases caused by different pathogens such as viruses, fungi and bacteria (Rott and Davis 2000), may be considered as a major limiting issue affecting crop productivity. At least 10 of these diseases are considered of great importance, both by direct damage or as forthcoming threats that may affect crops of sugarcane (Maccheroni and Matsuoka 2006). Three of the most important pathologies are caused by bacteria such as *Leifsonia xyli* subsp. *xyli*, causing ratoon stunting, *Xanthomonas albilineans,* causing sugarcane leaf scald and *Acidovorax avenae* subsp. *avenae* causing red stripe disease. In particular, red stripe has been found to affect sugarcane crop practically worldwide.

During the last decade, new agricultural techniques were implemented in Argentina such as green-cane harvesting and crop rotation with soybean, which resulted in significant increases in the occurrence of red stripe. The causative organism, *A. avenae* subsp. *avenae*, previously known to be a phytopathogenic species of *Pseudomonas* for sugarcane and rice (Willems et al. 1992), has been recently reclassified to species level as *A. avenae* for sugarcane and *Acidovorax oryzae* for the strains isolated from rice (Schaad et al. 2008). The first symptoms appear on the leaves as water-soaked stripes that gradually turn reddish. Then, symptoms extend to the plant apical meristem which becomes wet resulting in bud rot. Under favourable conditions, bud rot extends along the stem producing cracks from which a liquid with strong odour leaks out and in highly susceptible sugarcane varieties, rot may extend to basal nodes. Infected sugarcane fields exhibiting red stripe and bud rot have a characteristic odour that may be sensed at a distance (Maccheroni and Matsuoka 2006).

Mainly due to the loss of the stems for grinding, red stripe has become a major concern in the sugarcane production areas from the northern of Argentina, being detected in recent years with a marked increase in the incidence of this disease and in the symptom severity in commercial varieties in Tucumán (Pérez Gómez et al. 2010).

Despite the documentation of significant production losses, red stripe disease has been poorly studied and no reports on the isolation, identification and characterization of *A. avenae* in Argentina are available. In addition, in the last year a new disease called "false red stripe" exhibiting similar symptomatology to red stripe caused by a *Xanthomonas* sp. (Giglioti and Matsuoka 2000) was detected in Brazil sugarcane production area. In this sense, an accurate diagnosis of these sugarcane diseases is of critical importance to design efficient crop management procedures and requires integrating knowledge of both the etiologic agent as well as the role of factors determining its development. In this study, classical and molecular microbiological methodology was applied for the isolation, identification and genetic characterization of the agent responsible for sugarcane red stripe in the Northwest of Argentina.

Materials and methods

Plant material

Fifteen leaf samples from sugarcane exhibiting red stripe symptoms were collected in the 2008–09 growing season representing six sugarcane genotypes from 13 different sugarcane producing areas of Tucumán and Salta, Argentina (see Table 1). Young plants, less than 4 months after harvesting, were selected since initial symptoms are more easily identified. Samples were placed on filter paper into ziplock plastic bags; half of the leaf fragments were stored at 4–7 °C for processing and the remaining ones were kept at −20 °C for long-term preservation.

Table 1 Samples analyzed in this study

Cultivation region sampled	Sugarcane genotypes	Province
1-Louisiana-north		Tucumán
2-Los Ralos-north		
3-Ranchillos-north		
5-La Trinidad-south	TucCP 77-42	
7-Agua Dulce-central		
8-Las Piedritas-central		
9-Macomitas-north		
15-La Ramada-central		
4-La Trinidad-south	FAM 89-686	Tucumán
10-Famaillá-central		
6-Cruz Alta-central	FAM 91-209	Tucumán
14-Obera Pozo-central	RA 87-3	Tucumán
12-Tabacal	NA 02-2320	Salta
11-Colonia Santa Rosa	NA 85-1602	Salta
13-Colonia Santa Rosa		

Microbiological analysis

Classical microbiological procedures for the isolation of pathogenic microorganisms according to the Laboratory Guide for Identification of Plant Pathogenic Bacteria were followed (Schaad et al. 2001). Starting material, consisting of leaves stored at 4–7 °C for a maximum of 15 days, was cut into small pieces, disinfected twice with 70 % ethanol (1 min) and rinsed with sterile water (1 min). Leaf material (approximately 0.5 g) was manually macerated with pellet pestle-polypropylene (Sigma, Argentina) in sterile 2 ml tubes using saline-peptone solution (0.9 g/l NaCl and 10 g/l bacteriological peptone) as diluting agent, the supernatant was used as a first dilution and successive 1/10 dilutions were prepared. The following culture media were used: (i) Nutritive agar (NA) for mesophilic aerobic bacterial counts incubated at 37 °C for 48 h, (ii) semi selective Sorbitol Neutral Red (SNR) (Schaad et al. 2001) medium, and (iii) Beef-Yeast extract (BYE) and Yeast extract-Dextrose-Calcium Carbonate (YDC) (Schaad et al. 2001) for *A. avenae* incubated at 30 °C for 48 h. Representative colonies from each culture media were randomly selected, transferred to Luria-Bertani broth (LB) (Schaad et al. 2001) at 30 °C in a shaking incubator for 2 days. Further plating on NA was carried out to obtain pure colonies for storage at −20 °C in LB media containing 20 % glycerol. Fresh cell cultures from all isolates were subjected to microscopic observation to record morphology and mobility and Gram staining and oxidase activity using reactive paper discs (Britania, Argentina). Motility was also investigated by growing the cells after inoculation in the bottom of tubes containing semisolid NA (0.7 %) and incubated at 37 °C during 48 h. Unless otherwise specified all media and ingredients were obtained from Britania, Argentina.

DNA extraction

Three ml of a bacterial logarithmic phase culture growth at 30 °C in a shaking incubator over night was used for total DNA extraction according to Ausubel et al. (1992). The obtained DNA was quantified with Qubit® (Invitrogen, Argentina), visualized by electrophoresis through 0.7 % (w/v) agarose gel and stained with Gel Red (Genbiotech, Argentina).

Molecular identification

Species-specific PCR was performed using the primers Oafl and Oarl (Table 2) for *A. avenae* as previously described by Song et al. (2003). Amplification of a 550 bp fragment corresponding to the 16S-23S intergenic spacer was performed in a GeneAmp PCR System 9700 thermocycler (Applied Biosystems, Italy). Reactions were carried out in a final volume of 25 μl containing 1× Master Mix PCR (Promega, Italy), 0.1 μM of each primers, 10 –20 ng sample DNA. Amplifications consisted of 1 cycle of denaturation for 5 min at 94 °C, 25 cycles at 94 °C for 1 min, annealing at 55 °C for 30 s, extension at 72 °C for 1 min, and a final step at 72 °C for 7 min. PCR fragments were visualized by electrophoresis using 1.5 % agarose and stained with Gel Red (Genbiotech, Argentina).

RAPD analysis

Genetic diversity of *A. avenae* was achieved by RAPD using the primers M13 and RAPD2 (Table 2), selected on the bases of their discriminative ability among strains of a single species as well as on their reproducibility. RAPD reactions were performed according to Fontana et al. (2005) in a reaction volume (50 μl) containing 3 mM $MgCl_2$, buffer reaction (1×), deoxynucleoside triphosphate (200 μM each), 1 μM of each primer, DNA (20 ng), and Taq polymerase (0.5 U; Promega, Italy). RAPD products were electrophoresed at 100 V on 2.5 % agarose gel and stained with Gel Red (Genbiotech, Argentina). RAPD profiles were normalized and submitted to Cluster Analysis with Bio Numerics software (www.applied-maths.com/bionumerics/bionumerics.htm). Dice similarity coefficient was used for strains similarity matrix calculation and dendrograms were obtained by the unweighted pair group method with arithmetic averages.

16S rRNA gene sequence analysis

16S rRNA gene fragments of 500 bp and 1500 bp were amplified by PCR using Plb - Mlb and P0f - P6r pairs of primers, respectively (Table 2). Amplification programs were: initial denaturation of DNA for 5 min at 95 °C, 30 cycles of 30 s at 95 °C, 30 s at 52 °C, and 45 s at 72 °C, for the primer sets Plb/Mlb and 5 min at 95 °C, 25 cycles of 30 s at 95 C, 30 s at 55 °C, and 1 min at 72 °C, when P0/P6 primers sets were used. PCR

Table 2 Primers used in this study

Primer names	Sequence 5′–3′	Use	Reference
P0f	GAGAGTTTGATCCTGGCTCAG	16S (universal primer)	Klijn et al. (1991)
P6r	CTACGGCAACCTTGTTACGA	16S (universal primer)	
Plb	AGAGTTTGATCCTGGCTCAG	16S (universal primer)	Hébert et al. (2000)
Mlb	GGCTGCTGGCACGTAGTTAG	16S (universal primer)	
Oaf1	GTCGGTGCTAACGACATGG	ITS	Song et al. (2003)
Oar1	AGACATCTCCGCTTTCTTTCAA	ITS	
M13	GAG GGT GGC GGT TCT	RAPD	Huey and Hall (1989)
RAPD2	AGC AGC GTG G	RAPD	Cocconcelli et al. (1995)

f primer forward; *r* primer reverse

products were purified using ExoSAP-IT (Amersham USB Corporation, Italy) and directly sequenced (BMR-Genomics, Italy).

All PCR amplifications were performed with the PCR Master Mix (Promega, Italy) in GeneAmp PCR System 9700 (Applied Biosystems, USA).

Sequence analysis

Nucleotide sequences were analyzed using BLAST (Altschul et al. 1997) to determine the closest known relatives of the partial 16S rRNA gene sequences and of species-specific PCR products. Taxonomical identification and similarity rank (S_ab) calculation were also performed, using Ribosomal Database Project (http://rdp.cme.msu.edu/).

Nucleotide sequence accession numbers

The 16S rDNA sequences from *Acidovorax avenae* T10_59, T8_23, S12_5 and S13_11 strains isolated in this study were deposited in the GenBank database under the following accession numbers: JQ390307, JQ390308, JQ390309 and JQ390310 respectively.

Pathogenicity tests

For the pathogenicity tests, four strains representative of each RAPD group, were used. Young plants (less than 2 months) from three sugarcane cultivars TucCP 77-42, RA 87-3 and LCP 85-384, susceptible, intermediate and resistant, respectively were inoculated. Bacterial suspensions adjusted to approx. 10^8 CFU/ml were applied on leaves adaxial and abaxial surfaces by rubbing the leaf surfaces with sterile cotton wetted with the suspension. Control plants were inoculated in an identical manner with sterile water and with a bacterial suspension of a non *A. avenae* isolate. Approximately 40 young seedlings of each variety for each isolate were placed in pots of 300 ml with soil, no pasteurized and compost (Famaillá, Tucumán) in a ratio 70/30 and were maintained under high relative humidity (>90 %) in plastic tunnels and at constant temperature (30 °C). The plants were examined for symptoms every day up to 10 days after inoculation to determine the evolution of symptoms. The occurrence of red stripe on leaves were referred as "typical symptoms" (localized striations often extended up to the third leaf) and "severe symptoms" when the apical bud rot and death of the apical shoot occurs. Leaves showing red striations were subjected to microbiological and molecular analysis as described above, to confirm that red stripe symptoms were caused by the same *A. avenae* strains inoculated (data not shown). All experiments were performed in triplicate.

Direct detection of *A. avenae* from infected sugarcane leaves

Total DNA was obtained from infected leafs (approximately 100 mg) resulting from pathogenicity tests by using NucleoSpin Plant Kit (Macherey-Nagel- www.mn-net.com). ITS PCR amplification was performed using species-specific primers for *A. avenae* as previously described above.

Results

A. avenae isolation and phenotypic characterization

All agar culture media used were effective for the isolation of *Acidovorax*, however none were selective. Two different colonies morphology were observed on NA, SNR and YDC agar media, one circular, translucent, white-cream coloured with entire margins and yellow colonies, circular with entire margins too (Fig. 1). Preliminary characterization of the 150 isolated (100 white-cream and 50 yellow colonies) by microscopy and Gram reaction, indicated that both creamy and yellow colonies presented a singly or two- or three-rods chains morphology and were Gram-negative. In addition, white creamy colonies were oxidase positive rods and showed to have motility on semisolid NA (0.7 %) and microscopic observation, while yellow colonies were all oxidase negative.

A. avenae identification by species-specific PCR

The species-specific PCR amplification from all white creamy colonies showed that approximately 50 % (50/100) of the isolates exhibited a positive signal amplifying a 550 bp fragment (Fig. 2). This result indicated the presence of other bacterial groups with similar *A. avenae* colony characteristics as morphology and colour that may be associated with the infection. When the PCR product obtained using species-specific primers was sequenced and analyzed by comparison with available sequences in the GenBank database, an identity >99 % with *A. avenae* subsp *avenae* was obtained (data not shown). This result was confirmed by 16S rDNA sequences analysis for the following strains: T10_59, T8_23, S12_5 and S13_11 (GenBank accession number: JQ390307, JQ390308, JQ390309 and JQ390310, respectively). These strains were used as positive control for further molecular analysis performed in this study.

PCR negative strains (white creamy and yellowish colonies) were identified by means of the 16S rDNA sequences analysis; identity more than 99 % with *Erwinia* and *Pantoea* genera was found.

Genetic diversity of isolates by RAPD-PCR

Intraspecies diversity of *A. avenae* isolates were evaluated by RAPD-PCR using the primers RAPD2 and M13. The reproducibility of RAPD-PCR assay and running conditions estimated from duplicated PCR reactions using DNA prepared from separate pure cultures of the same strain was greater than 92 % (data not shown). The RAPD profiles showed that primer M13 was more discriminative than RAPD2, enabling the differentiation of four biotypes among 39 isolates of *A. avenae* analyzed. A high polymorphism (differences in the number of bands, fragment size and intensity) was observed among isolated strain from the different sugarcane production region. Figure 3 shows the dendrogram obtained from RAPD profiles using

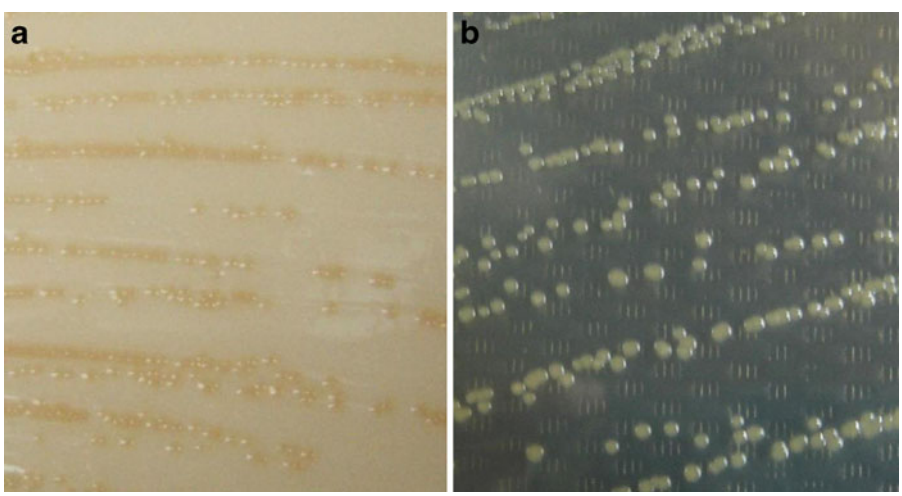


Fig. 1 Colony appearance of *Acidovorax avenae* on different culture media. **a** circular, beige coloured colonies with entire margins grown on Yeast extract-Dextrose-Calcium Carbonate (YDC), and **b** circular, translucent, white-cream coloured colonies with entire margins grown on Nutritive agar (NA)

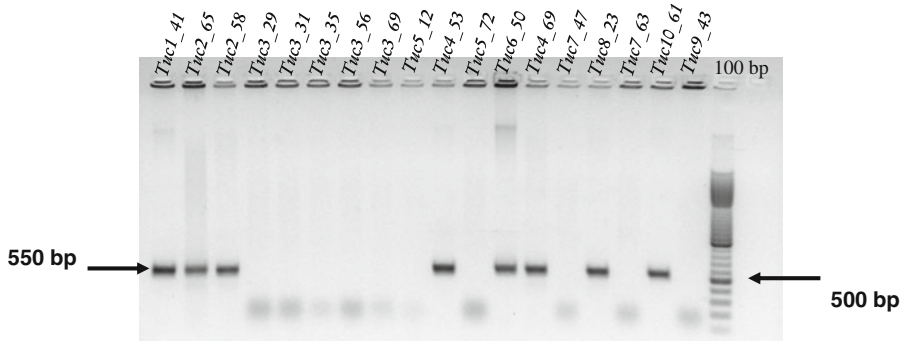


Fig. 2 Electrophoresis on 1.5 % (w/v) agarose gel of PCR amplification products (550 bp) obtained using species-specific primers Oaf1/Oar1 for *A. avenae*. Molecular weight marker of 100 bp was used

the BioNumerics software in which *A. avenae* strains were assembled into two major clusters at a similarity level of >85 %. Cluster 1 grouped 12 *A. avenae* strains which were isolated from TucCP 77-42 (10 strains), RA 87-3 (one strain), Fam 91-209 (one strain) represented the biotype "a" and 5 strains isolated from Fam 89-686 Tucumán sugarcane commercial genotypes (biotype "b"). The cluster 2 grouped biotypes "c" and "d" isolated from NA 85-1602 and NA 02-2320 genotypes, respectively, from Salta region.

Pathogenicity tests

Four *A. avenae* strains coming from the different biotypes, "a" (T8_23) and "b" (T10_59) isolated from Tucumán sugarcane leaves, and "c" (S13_11) and "d" (S12_5) from Salta samples were used as inoculum. All four *A. avenae* biotypes were observed to successfully reproduce the red stripe symptoms on sugarcane leaves; the inoculated seedlings did not show any differences in pathogenicity or aggressiveness for each of the *A. avenae* biotypes (Fig. 4). The intensity of the lesions developed on leaves was called "typical symptoms" with localized striations that extended up to the third leaf. Seedling death and/or apical bud rot (top rot) due to infection were not observed; no trace of disease was observed in plants inoculated with non *Acidovorax* strains.

Direct detection of *A. avenae* from infected sugarcane leaves

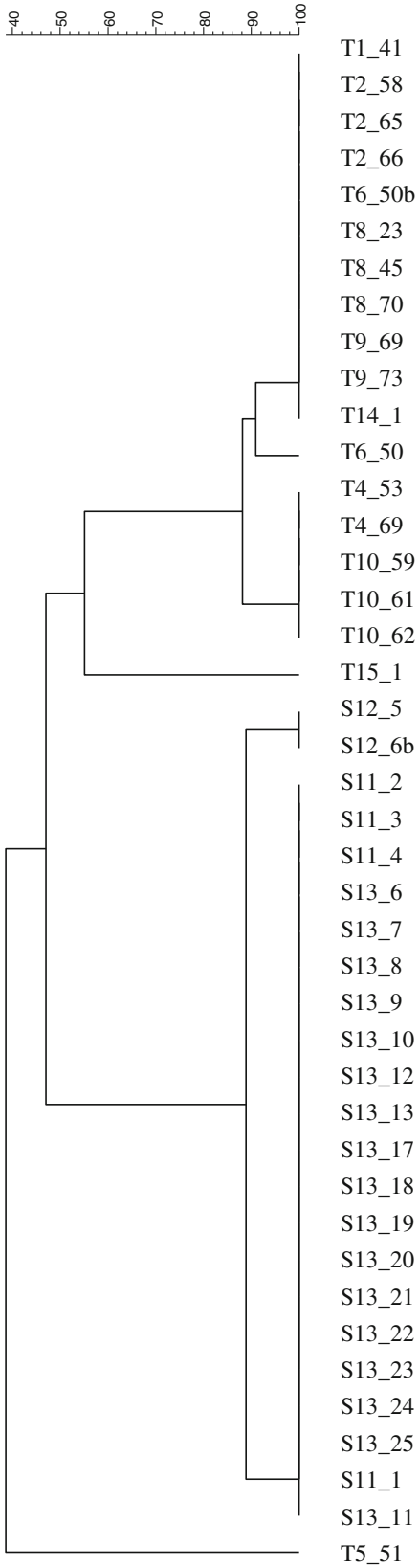
PCR species-specific amplification was performed from total DNA extracted from infected sugarcane leaves as result of the pathogenicity tests. The obtained 550 bp-specific product confirmed the presence of *A. avenae* in the infected leaves; in addition, these results confirm Koch's postulates (Fig. 5). This technique may constitute a rapid and reliable molecular tool for the direct identification of this phytopathogen without colony isolation.

Discussion

Acidovorax avenae is considered the etiological agent of red stripe in the main sugarcane regions of the world; however, in the sugarcane production areas of Argentina, no data confirming the association between this organism and the red stripe disease are available. The lack of a precise identification of this organism within the pathosystem sugarcane-red stripe represents a serious concern in the last years due to the presence in Brazil of a new disease with similar symptoms, the "false red stripe" of sugarcane caused by other bacterial species, *Xanthomonas* sp. (Giglioti and Matsuoka 2000).

In this study, *Acidovorax avenae* was isolated and identified from sugarcane leaves with typical symptoms of red stripe, from a representative survey carried out in

Fig. 3 Dendrogram obtained from RAPD-PCR patterns using M13 primer from *A. avenae* strains isolated from sugarcane samples analyzed by BioNumerics software. The similarity matrix was calculated using Dice coefficient; the dendrogram was constructed with UPGMA (Unweighted Pair Group Method with Arithmetic Mean). The letter on the strain code represents the sugarcane producing areas S— Salta, T— Tucumán, the first number represents the sample and the second the progressive number of isolated strains. For example T10_61 represents the strain number 61 isolated from the sample numbers 10 from Tucuman, while T15_1 and T5_51 represents strains profiles from non *Acidovorax* genera



different areas of sugarcane of Tucumán and Salta. The identification and characterization of the pathogen was carried out by combining classical microbiological techniques, such as microscopic observations, biochemical tests and Gram reaction, with rapid and effective molecular methods. Previous studies, reported the difficulty in identifying plant pathogenic bacteria at the species or subspecies levels using only classical phenotypic tests (Schaad et al. 2001; Jones et al. 2001); moreover, species of the genera *Acidovorax* could be identified only with a combination of phenotypic and pathogenicity tests. In this study, two dominant colony types were observed on NA, SNR and YDC agar media. Circular, translucent, white-cream coloured and entire margin colonies exhibited the typical morphology described previously for *Acidovorax* (Martin and Wismer 1989; Jones et al. 2001) while those yellow, circular with smooth margin colonies presented *Xanthomonas*-like morphology (Schaad et al. 2001). Because *A. avenae* is frequently overgrown by other seed-borne saprophytic bacteria, its recovery and identification is often difficult. Isolation results in semi selective SNR medium containing sorbitol as carbon source are in agreement with those previously reported by Summer and Schaad (1977) who recovered *A. avenae* subsp. *avenae* as causative agent of bacterial leaf blight of corn. Unfortunately, since this medium was not able to inhibit a large number of saprophytes, it was not useful for *A. avenae* isolation from rice seeds (Shakya and Chung 1983; Kadota et al. 1991). Genera such as *Erwinia* and *Pantoea* together with some *Pseudomonas* species such as *Pseudomonas fluorescens* and *Pseudomonas putida* are described as epiphytic bacteria coexisting as competing microorganisms with *A. avenae* in/on rice seeds (Song et al. 2004; Cottyn et al. 1996).

Due to difficulties for the identification of plant pathogenic bacteria at species or subspecies level using only classical phenotypic tests, other methods have been proposed to identify and differentiate *Acidovorax* from *Xanthomonas* and from other plant saprophytes. Although with limited success, culture test (Kihupi et al. 1996), isolation on selective agar media (Zeigler and Alvarez 1989; Kadota 1996), blotter test (Shakya and Chung 1983) as well as serological tests (Shakya 1987; Kadota et al. 1991) were evaluated. Preliminary characterization of the 150 isolated colonies by microscopy and Gram reaction indicated that both creamy and yellow colonies presented a single rod or two- or three-rods chains morphology and were Gram-negative. In addition,

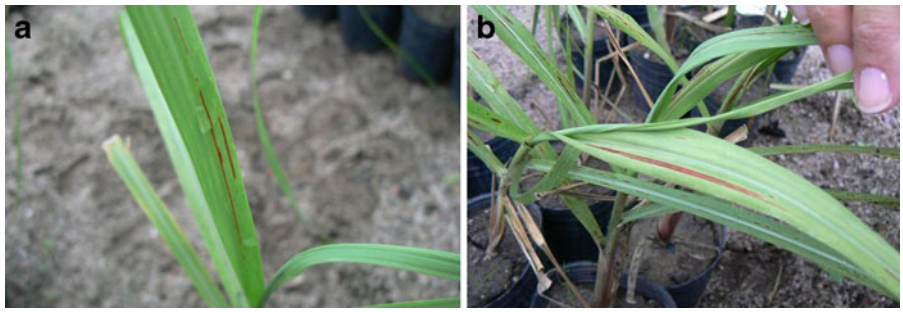


Fig. 4 Sugarcane cultivar TucCP 77-42 used for the pathogenicity test showing the red stripe symptom on sugarcanes leaves as result of the artificial infection: **a**) and **b**) correspond to typical symptoms developed at 72 and 120 h after inoculation, respectively

creamy colonies were mobile and oxidase positive rods as reported for *Acidovorax* species (Jones et al. 2001) while yellow colonies were oxidase negative. The molecular techniques used in this study such as species-specific PCR and RAPD fingerprinting have been widely used for genetic identification of several plant pathosystems including different pathovars of the genus *Xanthomonas* (Pooler et al. 1996; Khoodoo and Jaufeerally-Fakim 2006). Furthermore, the use of RAPD to generate polymorphic markers was previously described in race differentiation (Mesquita et al. 1998), genetic characterization of different genera of plant fungi (Kageyama et al. 1998) as well as food pathogen bacteria (Parent et al. 1996; Hilton and Penn 1998). The strategy used in this study (RAPDs and PCR amplification of ITS regions) for the rapid and consistent discrimination among *A. avenae* strains from sugarcane has been also applied for variability studies of *Ustilago scitaminea* causing smut disease in sugarcane (Singh et al. 2005). The Oaf1/Oar1 primers used in our study were developed and successfully applied for a rapid identification of more than 46 strains of *A. avenae* subsp *avenae* from oat, maize, rice, sugarcane and millet (Song et al. 2003). The 16S rDNA sequencing of PCR negative strains showed more than 99 % identity with genera *Erwinia* and *Pantoea.* No *Xanthomonas* strains, known to be the causative agent of "false red stripe" disease were identified from sugarcane leaf samples in this study.

Intraspecies diversity of *A. avenae* isolates were evaluated by RAPD-PCR using the primers RAPD2 and M13. From these results it can be inferred that clustered strains were mostly associated with the two main sugarcane production areas: Tucumán (biotypes "a" and "b") and Salta (biotypes "c" and "d"). The TucCP 77-42 sugarcane genotype is the most susceptible to the red stripe infection in Tucumán; in fact, more than half of the infected leaves sampled in this study correspond to TucCP 77-42 sugarcane genotype, while NA 85-1602 in the most susceptible sugarcane genotype in Salta. Nevertheless, it was observed that when TucCP 77-42 was grown in the Salta region it behaved as a resistant cultivar to this pathogen; the same behaviour was observed by cultivating NA 85-1602 in Tucumán (Rago AM unpublished data). The cluster analysis of the *A. avenae* strains isolated in this study confirmed these data, showing the presence of specific biotypes by cultivation areas.

Regarding to the pathogenicity test, all biotypes were able to successfully reproduce the red stripe symptoms on sugarcane leaves. In no case the death of seedlings or apical bud rot, the typical symptoms in the field, was observed; in the artificial infection conditions used the occurrence both symptoms are hardly observed. Results showed that even LCP 85-384

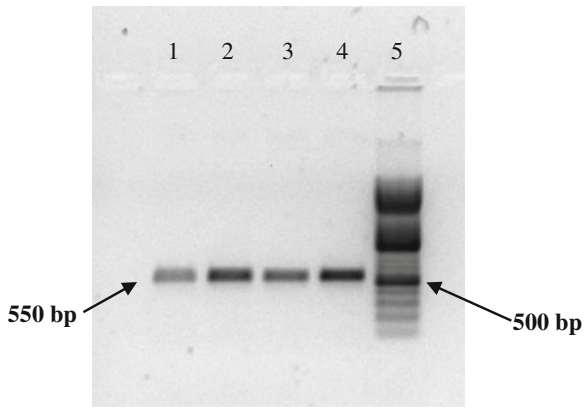


Fig. 5 Direct detection of *A. avenae* from infected sugarcane leaves: lane 1, 2, 3 and 4 species-specific PCR product using total DNA from artificial infected leaves, lane 5: molecular weight marker of 100 bp

sugarcane variety which behaved as resistant to field, under a high inoculum pressure in artificial conditions, was infected. A pathogenic specialisation has been reported for *A. avenae*, since strains isolated from rice showed to only infect rice while strains isolated from finger millet were not able to infect rice (Che et al. 1999). On the other hand, when the relative virulence of *A. avenae* strains to their reported hosts was investigated, *A. avenae* subsp. *avenae* exhibited more virulence in sweetcorn than in maize or sugarcane and was only weakly virulent to oat (Hu et al. 1997).

In addition, when *A. avenae* cross pathogenicity was investigated in the present study, red stripe symptoms were observed to develop earlier in Tucumán sugarcane variety (TucCP77-42) inoculated with strain S13-11 (biotype from Salta sugarcane) than in Salta sugarcane variety (NA 85-1602) inoculated with biotype T10-59 from Tucumán samples (Fontana P.D., personal communication). Strains belonging to the genera *Erwinia* and *Pantoea* were not isolated as pathogenic bacteria from the assayed sugarcane. These results are consistent with the fact that they are not described as pathogenic for sugarcane although being considered endophytic contaminants.

In this study, the use of identification and characterization methods based on molecular techniques in combination with traditional cultivation methods resulted useful for a rapid and efficient detection of the species *A. avenae,* both from pure bacterial cultures and from total DNA leaves with red stripe disease. Direct detection of *A. avenae* from infected sugarcane leaves by PCR species-specific method was performed from total DNA extracted from infected sugarcane leaves. In addition results of the pathogenicity test confirmed *A. avenae* as the causative agent of red stripe in the infected leaves. This technique may constitute a rapid and reliable molecular tool for the direct identification of this phytopathogen without colony isolation.

This is the first report on the identification of *Acidovorax avenae* as the causal agent for sugarcane red stripe in Argentina; the presence of other pathogens such as *Xanthomonas* sp., described as causal agent of sugarcane "false red stripe", was discarded. These findings contribute to the knowledge of the genetic diversity of this phytopathogen as well as its relationship with different sugarcane genotypes. Since in the last 10 years, an increase in the impact of red stripe disease throughout the sugarcane growing area in Argentina has been detected, the data here obtained will be useful for the design of regional crop management strategies.

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