Identification of Novel Potential Causal Agents of *Fusarium* Wilt of *Musa* sp. AAB in Southern Mexico

Luis David Maldonado-Bonilla*, Mónica Alicia Calderón-Oropeza, José Luis Villarruel-Ordaz and Ana Claudia Sánchez-Espinosa

Institute of Genetics, Universidad del Mar Campus Puerto Escondido, Carretera via Sola de Vega, San Pedro Mixtepec, Mexico

**ABSTRACT**

A major threat for bananas and plantains production is the Panama Disease or *Fusarium* Wilt caused by *Fusarium oxysporum* f. sp. *cubense*. In order to characterize the causal agents of *Fusarium* wilt in Mexico, a sampling was performed in symptomatic plantations growing in fields of Oaxaca, a coastal southern state of Mexico. A phylogenetic analysis based on the sequences of TEF 1-α and IGS revealed that three isolates belonged to the *Fusarium oxysporum* species complex, while two other isolates were identified as members of the *Fusarium fujikuroi* species complex. Furthermore, isolates from the same complex shared the same ITS2 sequence. Inoculation using spores of each isolate on the roots of *Musa* sp. AAB cv. Manzano produced wilting symptoms of varying severity, suggesting that the *Fusarium* wilt might not be caused only by *Fusarium oxysporum* f. sp. *cubense*. PCR-based detection of Secreted in Xylem (SIX) genes showed that each *Fusarium* isolate harbored a unique combination of genes typically found in banana pathogens, which might cause the disease.

**Keywords:** Breeding; *Fusarium oxysporum*; *Musa* sp.; Panama disease; genetic diversity; vascular pathogens; SIX genes

**Abbreviations:** Foc; *Fusarium oxysporum* f. sp. *cubense*; FOSC; *Fusarium oxysporum* Species Complex; FFSC; *Fusarium fujikuroi* Species Complex; TEF 1-α; Translation Elongation Factor 1-α; IGS; Nuclear Ribosomal Intergenic Spacer Region; ITS; rDNA Internal Transcribed Spacer; SIX: Secreted in Xylem.

**INTRODUCTION**

Bananas and plantains (*Musa* spp.) are a main staple food worldwide. Their production represents an important income source in tropical countries where cultivation of those plants is favored [1]. The tropical and subtropical climate conditions also promote the establishment of phytopathogens such as *Fusarium oxysporum* f. sp. *cubense* (Foc), which is the causal agent of *Fusarium* wilt or Panama disease [2]. The Panama disease caused by Foc is considered the utmost factor hampering the global banana production. Foc is an asexual ascomycete, belongs to the *Fusarium oxysporum* species complex (FOSC) that produces macroconidia, microconidia, and the quite resistant chlamydospores, that persist in the soil for years. When such asexual spores germinate, hyphae accede into the plant via the roots. Once inside, the fungus invades xylem vessels and gets dispersed throughout the whole plant, causing yellowing and wilting in old leaves, browning in the vascular tissue and splitting of the pseudostem [3]. Based on the affected *Musa* host plant, Foc is classified into race 1, 2 and 4 distributed in the eight clonal lineages [3]. Foc race 1 (Foc1) causes disease in the Gros Michel subgroup (genotype AAA), and the Silk subgroup (genotype AAB). Foc1 was responsible of the Panama disease epidemic in the 19th century, that devastated the production of bananas, but it is avirulent to the resistant AAA Cavendish cultivar, whose introduction replenished Panama disease in the hosts of Foc1. Because the disease can be present in subtropical or tropical conditions, Foc4 is divided into subtropical race 4 (STR4) and tropical race 4 (TR4) [4]. Since *Musa* spp. are asexually propagated clones, they have reduced genetic variability, which has promoted the emergence and rapid spreading of the TR4. Panama disease is currently a serious threat for worldwide banana production as TR4 has been detected in South East Asia, Australia, Africa, the Middle East, India, Pakistan, Lebanon [5], and recently in Israel [6]. These findings illustrate how TR4 has been dispersing, thus, identification of causal agents in banana plantations should include the monitoring of TR4 as...
a necessary early step to prevent economic losses or even export bans. Furthermore, little is known about the diversity of Foc in Latin America. Foc1 isolates have been detected in diverse banana and plantain cultivars in southern Brazil [7], Minas Gerais at the Southeastern Brazil [8], and recently in Puerto Rico [9]. It is worth to mention that a new lineage within Foc was also identified in Goaia, central Brazil, and it is highly virulent in Latundan bananas (AAA) [10]. To our knowledge, there are no reports of TR4 in Latin America, however, further screenings in diseased plants can broaden our knowledge about the fungal biodiversity causing the Panama disease.

States in southern and southeast Mexico with a coastline on the Pacific Ocean are the major producers of bananas and plantains. More than 2000 tons of bananas and plantains are produced per year [11]. Little is known about the incidence of the causal agents of Fusarium wilt and its corresponding genetic diversity in Mexico. In this work, we present the identification of novel pathogenic fungi isolated from plantains (Musa sp. AAB) growing on plantations on the Pacific coast of Oaxaca, a state of the Southeastern Mexico that contributes to the overall local production of plantains. Isolates were classified based on the sequencing of the known markers Translation Elongation Factor 1-α (TEF 1-α) and the Nuclear Ribosomal Intergenic Spacer Region (IGS) [12]. Analysis of the rDNA Internal Transcribed Spacer 2 (ITS2) indicated that each species complex carries at least one sequence variant of this spacer. Foc and related organisms were identified in this search, and its virulence towards Foc carries at least one sequence variant of this spacer.

METHODS

Isolation of fungi

Two samples from plantations of Musa sp. AAB cv. Macho (Subgroup 'Plantain') and Musa sp. AAB cv. Manzano (subgroup 'Silk') were obtained at the municipality of Villa de Tututepec de Melchor Ocampo, in the region Pacific coast region of Oaxaca, Mexico (16° 08'N, 97° 36'W). Both cultivars are susceptible to Panama disease [13]. Pseudostems and leaves from plants showing symptoms of wilting were harvested. Tissue samples were washed with sterile water and cut to obtain square-shaped pieces of approximately 0.5 × 0.5 mm. Squares were subjected to a superficial asepsis with 3% sodium hypochlorite for 1 min, and then, were rinsed with sterile distilled water. They were placed in Potato Dextrose Agar (PDA) plates until morphologically unique and independent isolates were obtained. The name and source of isolates are enlisted in Table 1. Isolates showing mycelium reminiscent to the Fusarium genus were analyzed by lactophenol-cotton blue staining and optical microscopy.

Polymerase chain reaction

Mycelia from the isolates grown in PDA were harvested, frozen in liquid nitrogen and ground with a mortar and pestle. DNA extraction was performed according to a published protocol [14] and the integrity of samples was confirmed by 1% agarose electrophoresis coupled with ethidium bromide staining and UV radiation. DNA from each isolate was diluted and used as template to amplify a fragment of TEF 1-α gene and the complete IGS region according to established PCR conditions [12] by using Recombinant Taq polymerase (Invitrogen). The complete IGS that encompasses the ITS1, 5.8S rDNA and ITS2 was also amplified [15] by using Pfu polymerase (Agilent). The oligonucleotides are enlisted in Table 2.

Cloning of TEF1-α and IGS

Once the PCR products of TEF 1-α and IGS were confirmed by electrophoresis, the amplicons were cloned into pGEM T-Easy (Promega) by following manufacturer’s protocol. Chemically competent E. coli DH10B cells were transformed with the ligation reactions. Positive transformed cells were selected in LB plates supplemented with 100 µg/mL ampicillin and 40 µL of 50mg/mL X-Gal. Cells carrying the expected plasmids were grown overnight in LB medium supplemented with 100 µg/mL ampicillin. Plasmid purification was performed using the alkaline lysis method, and the DNA was purified and sequenced in an ABI PRISM 3730 DNA analyzer at Genecore Inc. (Fuzhou, China) and DNA purification kit at the Mexican Instituto Nacional de Genética. Sequences were deposited in GenBank database of the National Center for Biotechnology Information (NCBI).

Table 1: Fusarium sp. isolates identified in symptomatic plantains in the coast of Oaxaca.

| Name | Source | Part of the Plant |
|------|--------|-------------------|
| M1a  | Musa sp. AAB cv. Macho | Leaf |
| M5   | Musa sp. AAB cv. Manzano | Pseudostem |
| M7   | Musa sp. AAB cv. Macho | Leaf |
| M104 | Musa sp. AAB cv. Manzano | Pseudostem |
| M108 | Musa sp. AAB cv. Manzano | Pseudostem |

Table 2: List of oligonucleotide sequences used on this study.

| Template | Oligonucleotide pairs | Tm (°C) | Amplicon Size (bp) |
|----------|-----------------------|---------|-------------------|
| TEF 1-α  | ef1: 5’-ATGGGTAAAGAGGARAGAACACG-3’ | 63      | ~700          |
|          | ef2: 5’-GGGATGCTAGTATGATGT-3’          |         |                 |
| IGS      | INL11: 5’-AGGCTTCGGCTAACGCTTCTAG-3’ | 62      | ~2200          |
|          | ICNS1: 5’-TTGGCAGTGGAGTGGCAG-3’       |         |                 |
| ITS      | ITS1: 5’-TCCGAGGGATCGGCGGCGG-3’       | 55      | ~540           |
|          | ITS4: 5’-TCTGGCTATGATATGTC-3’         |         |                 |
| SIX1a    | SIX1F: 5’-CCCTCTCTTCTGGGGTTT-3’       | 58      | 153            |
|          | SIX1R: 5’-TAGGTGCTATCAGGCAA-3’        |         |                 |
| SIX6     | SIX6F: 5’-GACMTATGACCCTCCTCCGTYG-3’   | 58      | 197            |
|          | SIX6R: 5’-GGGWMGGTTTCCAGGACAAG-3’     |         |                 |
| SIX9     | SIX9F: 5’-CTCTGGCCAAGCTTCTCCT-3’      | 58      | 164            |
|          | SIX9R: 5’-TTGGAAAGCCAGTGTGAAAG-3’     |         |                 |
| SIX13    | SIX13F: 5’-CGATCGAATATGGGGAAA-3’      | 58      | 196            |
|          | SIX13R: 5’-TTGTAAACTGTCGCGCTG-3’      |         |                 |
successful cloning was confirmed by restriction digestion with EcoRI.

DNA sequencing

DNA was sequenced by capillary electrophoresis at Macrogen Inc, Korea (https://dna.macrogen.com/eng/). Plasmids carrying the TEF 1-α and IGS clones were sequenced using the T7 and SP6 universal primers. Primer walking was necessary to obtain the complete sequences of IGS. The complete ITS PCR products were sequenced by using the oligonucleotides used for amplification. Electropherograms were analyzed in Chromas Version 2.6.5, and sequences were deposited in GenBank (https://www.ncbi.nlm.nih.gov/nucleotide/). List of the accession numbers of TEF 1-α and IGS are included in the Table 3. The ITS sequences are deposited in GenBank with the accession numbers MK250065 to MK250069.

Alignment and phylogenetic analysis:

Sequences of TEF 1-α and IGS from the isolates reported here, and reference sequences retrieved from GenBank and Fusarium-16 [16] (ESM1) were aligned separately by using the ClustalW algorithm. Phylogeny of concatenated alignments were subject to Maximum Likelihood (ML) method with 1000 bootstrap replicates by using PhyML 3.0 [17]. The phylogenetic tree was built in MEGA6 [18]. The Clustal W algorithm was also used to align the ITS2 sequences of all isolates together with previously reported sequences of *F. oxysporum* NRRL22902 (U34566.1), *F. subglutinans* NRRL22034 (GQ167235.1). These reference sequences displayed 100% identity with the sequences of the isolates. The ITS2 of *F. fujikuroi* NRRL13566 (AY249382.1) was included as reference of ITS2 Type II [15].

**In vitro propagation of plants**

Micropropagation of *Musa* sp. AAB cv. Manzano plants was established by following reported procedures [19,20]. Briefly, axenic plants were produced after surface disinfection of flower buds incubated in MS medium [21] supplemented with 5 mg/L Benzyl amino purine (BAP) and 5 g/L Phytagel as gelling agent. Direct organogenesis was induced after 8 weeks of incubation. The shoots were then transferred into solid MS supplemented with 0.5 mg/L BAP and Phytagel. Newly emerging shoots were excised and transferred into MS medium supplemented with 0.1 mg/L of Indole-3-acetic acid (IAA) to induce rooting. After 4 weeks, rooted plantlets were transferred into plastic containers with vermiculite rinsed with 25% MS solution without sucrose. Under these conditions, plants were grown and acclimatized until reach a 6-leaf stage, then, they are harvested and used for infection experiments. All procedures of plant tissue culture were run in a growth chamber at 26°C with a 16 h light and 8 h darkness photoperiod.

Pathogenicity assays

The experimental design was based on the aggressiveness test previously reported [10], it was performed twice. Healthy plants generated as described above were harvested, and their roots were thoroughly washed with distilled water. Every *Fusarium* isolate was grown in PDA at 28°C for 1 week. Spores were then collected with sterile distilled water and adjusted to a concentration of 10⁶ spores/ml. Inoculation with every *Fusarium* isolate was performed by submerging the roots of five independent plants in the spore solutions for 30 seconds. After inoculations, plants were planted in plastic bags containing a mixture of peat-vermiculite 1:1. Symptom solutions for 30 seconds. After inoculations, plants were planted in plastic bags containing a mixture of peat-vermiculite 1:1. Symptom development and severity in the leaves was monitored and scored based on previous reported criteria [22].

Detection of SIX genes

DNA from fungal isolates was used as a template to amplifying SIX genes commonly present in *Foc* [23]. Oligonucleotides were

| ID/NRRL | Host | TEF | IGS |
|---------|------|-----|-----|
| M1a     | *Musa* sp. AAB Macho | MG018806 | MG193546 |
| M5      | *Musa* sp. AAB Manzano | MG018805 | MG193545 |
| M7      | *Musa* sp. AAB Macho | MG018802 | MG193542 |
| M104    | *Musa* sp. AAB Manzano | MG018803 | MG193543 |
| M108    | *Musa* sp. AAB Manzano | MG018804 | MG193544 |
| NRRL22172 | Maize | AF160262.1 | FO_0185_IGS-2733 |
| V101096 | Barley | AJ543567.1 | AY250995.1 |
| V101087 | Bread wheat | AJ543570.1 | AY253668.1 |
| NRRL13999 | Sugarcane | AF160278.1 | FO_0170_IGS-2733 |
| NRRL22016 | Corn | AF160289.1 | FO_0160_IGS-2733 |
| NRRL25226 | Mango | AF160281.1 | GU737449.1 |
| NRRL4743 | Mango | GU737416.1 | GU737473.1 |
| NRRL36266 | Cyclamen | FJ985339.1 | FJ985572.1 |
| NRRL26033 | Tomato | AF008507.1 | FJ985484.1 |
| NRRL26203 | Tomato | AF008501.1 | FJ985487.1 |
| NRRL25603 | *Musa* sp. AAA Cavendish | AF008487.1 | FJ985480.1 |
| NRRL25607 | *Musa* sp. ABB Bluggoe | AF008489.1 | FJ985469.1 |
| NRRL25609 | *Musa* sp. ABB Harare | AF008490.1 | FJ985481.1 |
| NRRL26022 | *Musa* sp. ABB Pisang Awak | AF008491.1 | FJ985482.1 |
| NRRL26029 | *Musa* sp. ABB Silk | AF008493.1 | FJ985483.1 |
| NRRL36114 | *Musa* sp. ABB Pisang Manunung | FJ985328.1 | FJ985561.1 |
| NRRL36118 | *Musa* sp. ABB Pisang Awak | FJ985330.1 | FJ985563.1 |
| NRRL36149 | *Musa* sp. | FD_01770_IGS-2733 |
| TR2 | *Musa* sp. ABB Bluggoe | KC889020.1 | KC869389.1 |
| NRRL26406 | Muskmelon | AF008504.1 | FD_01193_IGS-2733 |
| NRRL26906 | Carnation | AF246839.1 | FJ985514.1 |
| NRRL38302 | Pinus | GU170559.1 | FJ985679.1 |
| NRRL25375 | Human | AY527521.1 | AY527718.1 |
designed according to SIX gene sequences available in GeneBank (Table 2). PCR was performed by using the qARTA Taq polymerase following the manufacturer’s recommendations. All PCR reactions started with a 5 min denaturation at 95°C step, followed by 35 cycles of 94°C for 30 s, 58°C for 30 s and 72°C for 30 s. After amplification, reactions were analyzed by electrophoresis in 2.5% agarose gels stained with ethidium bromide under UV illumination. Amplification of the TEF 1α was used as a positive control.

RESULTS

Fusarium isolates from the coast of Oaxaca belong to two distinct species complexes

Inspection of fungal diversity coming from symptomatic plants resulted in the isolation of five independent morphotypes (Table 1). Phylogenetic analysis of the markers TEF 1α and IGS was performed to examine the genetic diversity of such isolates (Figure 1). A ML tree revealed that isolates M1a and M5 were closely related to Foc NRRL25609, an isolate from Musa sp. ABB classified as Foc2. It belonged to the clonal lineage VIII and vegetative compatibility group (VCG) 01214 [24,25]. In accordance with this finding, most of the isolates recently identified in Puerto Rico also belonged to the VCG 0124 [9]. The isolate M104 was not related to the known races of Foc, instead, it was more related to the pathogens of tomato NRRL26033 and NRRL26203 [26]. All members of FOSC in this analysis are clustered together, including F. foetens, which were included as a reference of another member of FOSC. This phylogenetic analysis revealed that F. sacchari and F. verticillioides were species assigned to M7 and M108, respectively. Both are members of the F. fujikuroi species complex (FFSC). M7 is quite similar to NRRL31649, also isolated form Musa sp., and F. sacchari NRRL13999 isolated from sugar cane. The phylogenetic analysis of M108 reveals that F. verticillioides NRRL22172 is the most related species. Due to evident polymorphisms in the sequences of ITS2, that intervenes the 5.8S and 18S ribosomal genes, the ITS2 sequences are grouped into two divergent types in the Fusarium genus. Members of FOSC primarily carry variants of the ITS2 Type I, although some members FFSC have also copies of this Type, which might be consequence of interspecies hybridization or gene duplication events [15]. The complete ITS of the isolates was sequenced and aligned together with reference sequences of ITS2 Type I and Type II. All the isolates presented here carried at least one copy of the ITS2 Type I (Figure 2). It is worth to mention that none of the identified isolates was related to the TR4. Phylogenetic analysis presented here is supported by lactophenol cotton blue staining and optical microscopy observations, which revealed the production of characteristic structures such as chlamydospores and false heads in the isolates from FOSC. The FFSC isolate M7 produced septate mesoconidia, the isolate M108 produced chains of microconidia reminiscent of F. verticillioides (Supplementary Information). Taken together, morphology and phylogenetic analysis illustrate that fungi from FOSC and FFSC were found in the organs of plantains displaying symptoms of Fusarium wilt.

Fusarium isolates are virulent in Musa sp. AAB cv. Manzano

A pathogenicity assay was performed in order to confirm whether the identified Fusarium spp. caused symptoms related to Panama disease. Acclimatized plantains obtained by a micropropagation method were infected by submerging their roots in a spore solution.
Figure 2: Partial alignment of ITS2 sequences reveals the presence of ITS2 Type I in the Fusarium isolates from the coast of Oaxaca. The sequences of *F. oxysporum* NRRL22092 and *Fusarium subglutinans* NRRL22034 are used as a reference for the ITS2 Type I sequences. The sequence of *F. fujikuroi* NRRL13566 was included as a reference for ITS2 Type II.

**Supplementary information.** Representative optical microscopy observations. Slightly falcate macroconidia generally of 3 to 5 septa with curved and conical apical cells (a) and microconidia (b) from the isolate M1a. Chlamydospores (c) and microconidia (d) form the isolate M5. Chlamydospores (e) and microconidia grouped in false heads (f) from the isolate M104. Macroconidia with one or two septa (g) and microconidia (h) from isolate the M7. Macroconidia emerging in chains (i) and forming small aggregates in the isolate M108 (j). a, c, e, f, h scale bar = 20 µm; b, d, g, i, j scale bar = 10 µm.

Figure 3: Plant-pathogen assays exhibited different degrees of virulence. a) Emergence of Fusarium wilt in leaves was detected in plants of *Musa* sp. AAB cv. Manzano 2 months after inoculation. White arrows indicate diseased tissue. A cut cornc of the inoculated plant is placed at the right. b) Monitoring of the symptoms 24 and 36 days post inoculation reveals the severity of the disease caused by each isolates. The colour code is shown next to the graphic.
Five plants were used per each treatment, and the experiment was performed twice. Every isolate caused symptoms both in leaves and corms of inoculated plants (Figure 3a); however, the emergence of wilting was dependent on the isolate, and the M5 was one of the FOSC that produced symptoms in leaves sooner, and provoked more severe disease by the end of the experiments (Figure 3b). Yellowness and necrosis caused by isolates of FFFC were weaker, and leaf deformation was detectable.

Amplification of SIX genes commonly found in the causal agents of Panama disease

A critical event during colonization the xylem vessels and establishment of the Fusarium wilt is the secretion of small and cysteine-rich proteins known as ‘Secreted in Xylem’ (SIX), which were initially identified in the sap of tomato plants infected with F. oxysporum f. sp. lycopersici (Fol) [22]. So far, there have been 14 SIX genes identified in F. oxysporum. Nevertheless, every forma specialis has a specific combination of such genes, which contributes to disease establishment across a narrow host range [27]. SIX1a, SIX6, SIX9 and SIX13 are often found in isolates of the three Foc races. Additional copies of SIX1 (SIX1b and SIX1c), SIX2 and SIX8 are specific to the race 4 [23]. To get initial molecular evidences regarding the pathogenicity of these isolates, oligonucleotides were designed to amplify the known SIX genes present in Foc. The amplification products are presented in the Figure 4. The SIX1a amplicon was detected in all the isolates regardless the species complex to belong. The SIX6 amplicon is detected in M1a, M5, M104 and M108. SIX9 was detected in isolate M104 and M108, and SIX13 was amplified in isolates M1a, M5 and M104, which were classified as F. oxysporum. M1a and M5 are phylogenetically related, and shared the same combination of the SIX amplicons. However, M5 was the most virulent in our conditions; thus, virulence did not correlate with the presence or lack of these genes. Despite the SIX genes are found in virulent isolates of F. oxysporum, we did amplify selected SIX genes in isolates M7 and M108, both of them belong to the FFFC. We also designed oligonucleotides to amplify SIX2 and SIX8, which are typically detected in the race 4. However, there was no amplification detected in any isolate examined in this research (data not shown).

DISCUSSION

The FOSC encompasses saprophyte constituents of the rhizosphere and soil borne phytopathogenic fungi, including Foc [28]. The lack of sexual reproduction in F. oxysporum might be compensated by anastomosis which enables horizontal gene transfer (HGT) processes, that could originate over 70 formae especiales distributed in many clonal lineages [29]. In the case of Foc, 23 clonal lineages have been reported, they could emerge from events of HGT events that carried over the genetic information required to parasitize Musa spp.

Although there are reports of Foc in Latin America, we barely know about outbreaks of this pathogen on plantations in Mexico. In order to investigate the genetic diversity of the causal agents of Panama disease, we sampled symptomatic plantains on the coast of Oaxaca, Mexico. After this focused analysis, we demonstrated that the isolates M1a and M5 are two Foc individuals related to NRRL25609, a reference Foc2 strain isolated from Musa sp. ABB. In this context, the genome B of plantains such as Musa cv. Manzano might encode for factors that facilitate the colonization of Foc2. In spite of the close phylogenetic relationship between M5 and M1a, wilting symptoms produced by both isolates are contrasting, being the M5 the most virulent. Variations in the content and regulation of virulence genes might explain the difference in the symptoms caused by M1a and M5. The ML phylogeny of concatenated TEF 1-α and IGS inferred a close relationship between the virulent isolate M104 and the tomato pathogens NRRL26033, NRRL26023, that belong to a lineage unrelated to Foc [24]. Recently, a wide analysis of the diversity of Foc in Indonesia –the centre of origin of banana- demonstrated that Fusarium wilt is not limited to FOSC, since isolates form FFSC and other species complexes are virulent against Gros Michel and Cavendish [30]. F. verticillioides is a well-known pathogen of maize, but some isolates have been identified in rotten banana fruits produced in Mexico [31]. In this work, we identified isolates F. verticillioides M108 and F. sacchari M7, both of which are members of the FFFC. Leaf symptoms caused by the isolates of M108 and M7 are minimal, but it demonstrates that individuals of the FFSC are part of the diversity of Fusarium in plantains AAB in the coast of Oaxaca, Mexico. Their presence in symptomatic plants might be facilitated by virulent organisms such as Foc that suppress the plant immune system to permit the growth of weak pathogens. Fusarium sacchari was also recovered form symptomatic banana plants in Puerto Rico [9], but no symptoms were produced.

Whole genome sequencing of the F. oxysporum model strains has revealed the presence of core and lineage specific (LS) chromosomes [32,33]. LS chromosomes are also known as conditionally dispensable (CD) chromosomes or pathogenicity chromosomes, they are unique to each virulent F. oxysporum isolate and were acquired by HGT [34]. LS chromosomes carry genes encoding virulence factors such as the SIX genes, their own transcriptional regulators and the majority of transposable elements (TE) in the whole genome [33,35,36]. Each LS chromosome was likely outcome from “copy-paste” or “cut-paste” mechanisms promoted...
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