

Full Length Research Paper

# The first report of *Fusarium proliferatum* causing garlic bulb rots in Mexico

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Accepted 13 February, 2013

**Garlic bulbs were collected in the North of Aguascalientes, Mexico. The plant pathogens were isolated, purified and identified down to its species, based on taxonomic keys. Their identity was confirmed by Internal Transcribed Spacer (ITS) and pathogenicity tests were conducted under laboratory conditions. *Fusarium proliferatum* was identified by the pathogenicity tests. The fungus was isolated from the bulb and stem parts presenting purple and reddish lesions, obtaining *F. proliferatum* consistently. This is the first report *F. proliferatum* in garlic in Mexico; although it had already been reported for garlic in Spain, Serbia, USA and Germany.**

**Key words:** Identification, rot of bulbs, *Allium sativum*.

## INTRODUCTION

Garlic is considered one of the most profitable crops in Mexico, with a planted surface area of 5,654 ha (FAO, 2009) and a domestic production in 2009 of 56,088 ton. 87% of garlic production is concentrated in the states of: Zacatecas, Guanajuato, Baja California, Aguascalientes and Sonora (INEGI, 2010). In these regions, fungal diseases are the main cause of huge economic losses, including white rot, *Sclerotium cepivorum* Berk, and diseases such as *Botrytis* spp., *Sclerotium rolfsii*, *Penicillium* spp. and *Fusarium* spp. (Velásquez and Medina, 2004a). *Fusarium* spp. has shown stronger presence in the last eight years, after Velásquez and Medina (2004b) of a new symptomatology called garlic bulb rot in cultivars at Aguascalientes and Zacatecas, caused by *Fusarium* spp. Rot of bulbs by *Fusarium* spp.

has become a limiting factor to garlic and onion production in different growing areas, including not only in Mexico, but also countries like Argentina, considered the 4<sup>th</sup> largest garlic producer in the world (Kiehr and Delhey, 2005). *Fusarium* spp. has a broad range of hosts. In alliaceae, *Fusarium* ssp. damages onion seedlings with damping-off, besides attacking garlic under storage conditions. *Fusarium* spp. is widely distributed in the world and is among soil-borne fungi that attack garlic and onion causing rot of onion bulbs (*Fusarium oxysporum* f sp *cepae*) and in garlic (*Fusarium culmorum*). Damage caused by this pathogen can produce 45% less yield (Schwartz and Mohan, 1995). Recently, *Fusarium proliferatum* developing internal rot, white mycelium, bulbs and reddish stems has been found to be the cause of garlic bulb rot, in Spain, Serbia, USA and Germany (De Cara et al., 2010; Stankovic et al., 2007; Dugan et al., 2003; Seefeldter et al., 2002). Considering the importance of the disease in garlic growing regions, this work aimed to confirm the presence of *F. proliferatum* in Aguascalientes,

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Mexico as the cause of garlic bulb rot.

## MATERIALS AND METHODS

### Sampling

Garlic plots were sampled in the North of Aguascalientes during February to May, as well as in August to September 2011. 12 plants presenting symptoms of the disease and 3 apparently healthy plants were sampled at random from ten plots. Bulbs were collected during sampling process in August and September.

### Fungus isolation from bulbs and plants

The isolation process began by cleaning the tissue of diseased plants with an alcohol-impregnated cotton ball at 70%. Small incisions were cut at the disease leading edge, where the pathogen was developing actively. Sample tissues were disinfected with a hypochlorite sodium solution at 1% for 3 min, before being rinsed with sterilized water and placed in Petri dishes with Potato Dextrose Agar (PDA) medium, auditioned with streptomycin (100 ppm). The dishes were incubated at  $25\pm 2^\circ\text{C}$  for 7 days until obtaining pure cultures.

### Identification

After seven days, mycelia and spores were mixed in a preparation that was observed under the microscope to identify the gender using Barnett and Hunter (1998) keys. *Fusarium* species were identified using the keys of Domsch et al. (1980), Nelson et al. (1983), Leslie and Summerell (2006).

### Species

A small fragment of the colony was taken from monoconidial cultures and was transferred to the center of Petri dishes, containing PDA and Spezieller-Nährstoffarmer agar (SNA). The inoculated dishes were kept at  $26^\circ\text{C}$  for 15 days. After 10 days, the diameter of the colonies was measured in PDA and after 15 days the SNA inoculated dishes were opened to observe the microscopic characteristics of the colonies. The microscopic analysis was done using a Leica microscope (ATC 2000) enlarged at 60X, 150X and 600X. The identification was mainly based on the structure, composition and ultra-structure of conidial cells, conidiophores and chlamydospores, using the keys of Domsch et al. (1980), Nelson et al. (1983) and the laboratory handbook of Leslie and Summerell (2006).

### Molecular

DNA's extraction was done using Doyle and Doyle method (1990). The extraction product was analyzed in agarose gel at 1% through electrophoresis before amplifying transcribed ITS1 and ITS4 internal regions by polymerase chain reaction (PCR), among ribosomal genes (rDNA) 18S to 5.8S and 5.8S to 28S, using the pair of sequence elicitors: ITS1 (TCCGTAGGTGAACCTGCGG)/ITS4 (TCCTCCGCTTATTGATATGC) adding to each sample an enzyme buffer, at 1x, 1  $\mu\text{l}$ ; dNTP's at 0.2 mM, 1  $\mu\text{l}$ ; MgCl at 2 mM, 0.4  $\mu\text{l}$ ; ITS1 at 1 pM, 0.5  $\mu\text{l}$ ; ITS4 at 1 pM, 0.5  $\mu\text{l}$ ; 0.5 UDO of Taq polymerase, 0.1  $\mu\text{l}$ ; DNA adjusted problem at 50 ng, 1  $\mu\text{l}$ ; and 5.5  $\mu\text{l}$  of ultra-pure sterile water to adjust the final volume at 10  $\mu\text{l}$ . PCR reaction conditions were the following: 1 cycle of initial

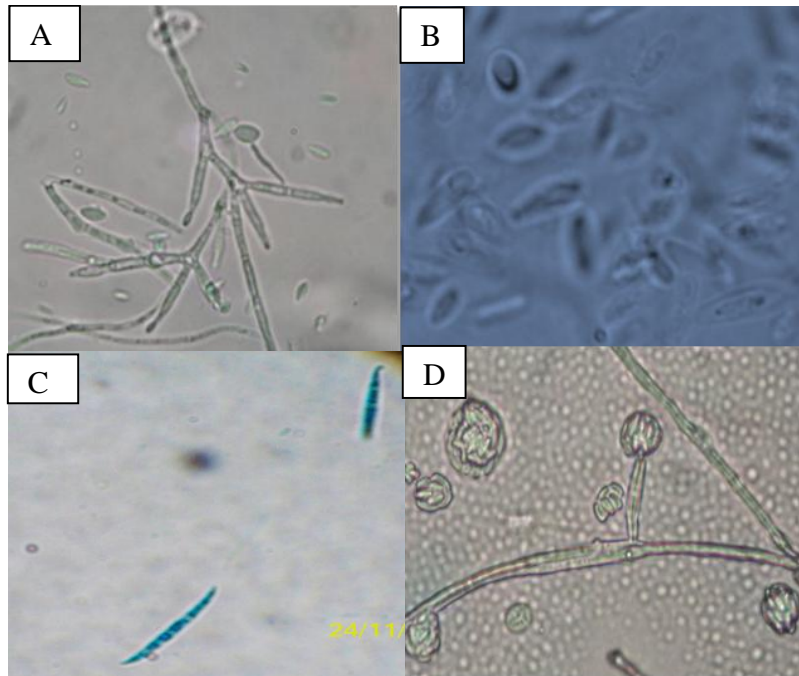
denaturalization at  $94^\circ\text{C}$  for 5 min, 30 cycles of de-naturalization at  $95^\circ\text{C}$  for 10 s, 30 cycles of alignment at  $57^\circ\text{C}$  for 30 s, 30 cycles of extension at  $72^\circ\text{C}$  for 2 min. and 1 cycle of final extension at  $72^\circ\text{C}$  for 5 min. The amplification was analyzed in agarose gel at 1% through electrophoresis. PCR resulting product was purified using Invitrogen PureLink® Quick Gel Extraction and PCR Purification Combo Kit, sequenced in two directions (5' to 3' and 3' to 5') with automatic sequencer. Base pairs obtained were compared with the sequences reported in the data base of NCBI's gens bank (National Center for Biotechnology Information, www.ncbi.nih.gov).

### Pathogenicity *in vitro* essays

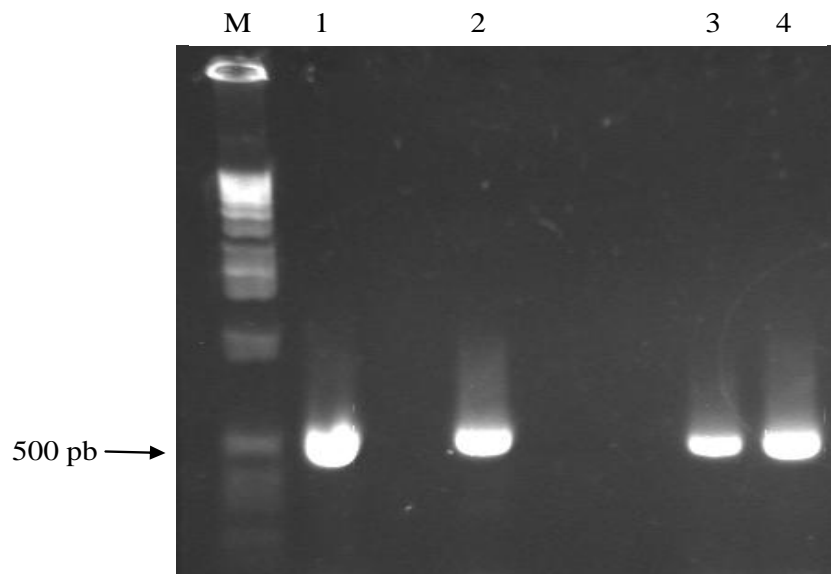
Twelve *in vitro* grown plants in (MS medium) with three leaves were injected with treatment around the basal area using a syringe (where the color changes from white to green) with a spores concentration of  $10^6$  conidia/  $\text{ml}^{-1}$  sterile distilled water, while the control were inoculated only with sterile distilled water. The plants were grown and incubated at  $25^\circ\text{C}$  with 12 h light conditions. After the appearance of the symptoms, the tissue was dissected 21 days later in order to recover the inoculated fungus.

## RESULTS AND DISCUSSION

The presence of *F. proliferatum* was determined, forming abundant single-cell microconidia, rod-shaped with flat base, sometimes resembling a pear. These microconidia formed chains and false heads. Some of them had three to five relatively thin septae, with thin walls and curved apical cells, as well as monophialides and polyphialides (Figure 1). These observations coincide with the reports of Nelson et al. (1983) and Leslie and Summerell (2006), who mentioned that *F. proliferatum* forms chains of flat-base single-cell hyaline microconidia with polyphialides and monophialides; curved macroconidia with three to five thin septae, slightly curved, with curved apical cell and little developed basal cell. The identity of the cells was confirmed through Internal Transcribed Spacer-Polymerase Chain Reaction (ITS-PCR) technique, where the amplified products yielded around 500 pb (Figure 2). The sequences were aligned according to Gen Bank data base of the National Center for Biotechnology Information (NCBI) from USA (www.ncbi.nlm.nih.gov). From the values produced, only the sequences with the highest value for comparison purposes were lowered. The presence of *F. proliferatum* was determined, with Access number NCBI HQ113948.1 and a similarity ratio of 99 to 100%, from Chinese origin. A week after the artificial inoculation, the plants presented pink to brown basal lesions that turned gray-brown and finally necrotic. Fungi from plants presenting herein aforementioned symptomatology were re-isolated, obtaining *F. proliferatum* consistently. The control check did not show any symptoms. *F. proliferatum* is an important pathogen producing huge losses in agriculture, due to its broad range of hosts. This plant pathogen has economic importance since it affects crops such as rice, corn, banana, sorghum, asparagus, pine trees, palm trees, etc (Leslie and Summerell, 2006), onion (Galván et al., 2008)



**Figure 1.** Morphological characters of *Fusarium proliferatum*. A, Phialids and microconidia; B, microconidia; C, macroconidium; D, monophialid with false head.



**Figure 2.** PCR amplification of DNA, from *F. proliferatum* isolates using primers ITS1/ITS4. Lane M, Molecular weight marker; Lanes 1, 2, 3 and 4, isolates from *F. proliferatum*

and garlic (Dugan et al., 2003). *F. oxysporum* and *F. proliferatum* caused meaningful losses during 2005 and 2006 in USA's National Germplasm Bank (Dugan et al., 2007). In Europe, *F. proliferatum* was isolated from onion seeds (Mannerucci et al., 1987) and was reported as the main plant pathogenic agent affecting garlic bulbs in

Hungary (Simey, 1990). This fungus has also been reported in Spain, Serbia, USA and Germany in garlic bulbs, as the cause of garlic bulb rots (De Cara et al., 2010; Stanovic et al., 2007; Dugan et al., 2003; Seefelder et al., 2002). This is the first report of rot of garlic bulbs caused by *F. proliferatum* in Mexico.

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