

First Report of *Fusarium thapsinum* on Imported Banana Fruits into Saudi Arabia

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ABSTRACT

Diseases at the post-harvest stage cause significant yield losses in banana, both quantitatively and qualitatively, reducing its demand in hypermarkets. Two isolates of *Fusarium* recovered from banana fruits were identified as *Fusarium thapsinum*. The resulting cultures were purified and grown on potato-dextrose agar (PDA), malt yeast agar (MYA), and dextrose sabroud agar (SDA) under light for cultural identification. A slowly growing white colony that turned grey-violet in pigmentation on the agar was quite variable on PDA and MYA. On SDA medium bright white mycelia were produced, and macro and microconidia are present but sparse and chlamydospores were absent. The morphology of this species is unique and includes two types of microconidia, mostly pyriform to citriform, 0- or 1-septate forming chains. The pathogenicity of the isolated *Fusarium* was tested on banana fruits. Brownish spots formed 1 week after inoculation of *F. thapsinum* isolates on wounded fruit of green banana. These results suggest that *F. thapsinum*, which has not been reported yet in Saudi Arabia, could be introduced into the country along with imported bananas and may cause diseases on other plant species.

Keywords: *Gibberella fujikuroi*, Fusarium rot, *Musa*, pathogenicity

INTRODUCTION

Post-harvest diseases can cause serious losses of fruits both in terms of quantity and quality. Banana fruits infected with Fusarium rot disease have no market value. *Fusarium thapsinum* (teleomorph: *Gibberella thapsina*) Klittich, Leslie, Nelson and Marasas was first described by Klittich and Leslie (1992). Prior to that it was recognized as *F. moniliforme*. The splitting of *F. thapsinum* was made because of the recognition of *G. thapsina* as a separate mating population and a number of important physiological characteristics, including host preference and toxin production (Klittich *et al.* 1997). *F. thapsinum* causes stalk rot and grain mold of sorghum (Montes-Belmont *et al.* 2003) and was very pathogenic towards sorghum seedlings in an *in vitro* assay system (Leslie *et al.* 2005). In addition to sorghum, *F. thapsinum* can also be recovered from bananas, maize and peanuts (Klittich *et al.* 1997). The degree of pathogenicity is dependent on growth stage (Tarekegn *et al.* 2004) and genetic background of the host (Tesso *et al.* 2004). *F. thapsinum* can show significantly reduced germination (Prom *et al.* 2003). *Fusarium* spp. have been isolated from bananas by several authors in different countries such as India (Peshney and Ghaukar 1984), the Windward Islands (Wallbridge 1981), Panama, Ecuador and the Canary Islands (Jiménez *et al.* 1993, 1997), and Mexico (Hirata *et al.* 2001). Seven strains of *Fusarium* isolated from rotten banana fruits imported into Japan from Mexico were identified as *F. verticillioides* based on morphological and molecular characterization (Hirata *et al.* 2001). *F. proliferatum* was isolated from banana samples collected from 12 localities in Sri Lanka (Anthony *et al.* 2004).

Newly recognized species are isolated in mycogeographical studies, an indication of the degree of diversity in *Fusarium* that remains to be discovered worldwide. Saudi Arabia, a large country is ideally suited to identify new *Fusarium* species from soil and plant hosts as it has a wide range of bioclimatic regions including tropical, arid, and temperate regions. This paper describes morphological and

cultural characteristics of two isolates of *F. thapsinum*. The pathogenicity of both isolates was also confirmed on commercial banana fruits. To date, *F. thapsinum* had never been reported to occur as a contaminating fungus on banana fruits imported into Saudi Arabia from different banana-producing areas.

MATERIALS AND METHODS

Fungal isolates

The fungal isolates used in the current study were recovered from banana fruits (cv. 'Latundan', Philippines) collected from a hypermarket in, Riyadh city, Kingdom of Saudi Arabia. Pieces (0.5 × 0.5 cm) of the banana fruits that showed symptoms were disinfected by dipping into a 10% sodium hypochlorite solution for 4 min followed by rinsing with sterile distilled water three times. Excess water in pieces was eliminated by dabbing on sterile tissue paper. The pieces were then placed on the surface of PDA and incubated at 28°C for 3 days. The fungal mat from each piece was transferred to fresh PDA medium. Cultures were maintained on PDA medium at 4°C and then stored as spore suspensions in 15% glycerol at -80°C.

Morphological identification and characterization

For cultural characterization, colonies of the two *F. thapsinum* isolates were grown on PDA, malt yeast agar (MYA) and Sabroud dextrose agar (SDA). Cultural and microscopical methods described by Nirenberg and O'Donnell (1998) were used to confirm *Fusarium* species. Cultures were examined microscopically under low magnification (100-200X) to study morphological features of the aerial mycelia. When sporulation was observed in the cultures, agar blocks containing conidial structures were mounted on a microscopic slide with a drop of sterile water and examined at 400X.

Pathogenicity test

To determine the pathogenic characters of *F. thapsinum*, inoculation tests were carried out in duplicate to test the isolates' pathogenicity to healthy banana fruit (same cultivar). Additionally, the surface of the fruit was first wound with a bundle of 20 sterilized sewing needles (ca. 2 mm in total diameter). These had been kept immersed in isopropyl alcohol, dried and briefly sterilized by careful heating over a lit match or flame until its tip was yellow was cooled at room temperature. Mycelial mats on PDA (ca. 5 x 5 mm) were placed onto wounded parts. As a negative control, uninoculated PDA blocks were also placed on the wounded fruit. The inoculated fruits were placed into plastic boxes and incubated at 25°C for 1 week.

RESULTS

Isolation, cultural and morphological studies

Post-harvest rot of banana fruits were surveyed in four major hypermarkets in Riyadh city. *Fusarium* and anthracnose rots were detected as the main cause of banana rots. Two isolates of *Fusarium* recovered from banana fruits were identified as *F. thapsinum*. Among the three culture media tested, SDA was the best for mycelial growth of *F. thapsinum* isolates. *F. thapsinum* forms an abundant white mycelium which may darken (violet pigments) with age on PDA medium (Fig. 1). Colonies on SDA were bright white aerial mycelium, becoming grayish-violet on MYA. Pig-

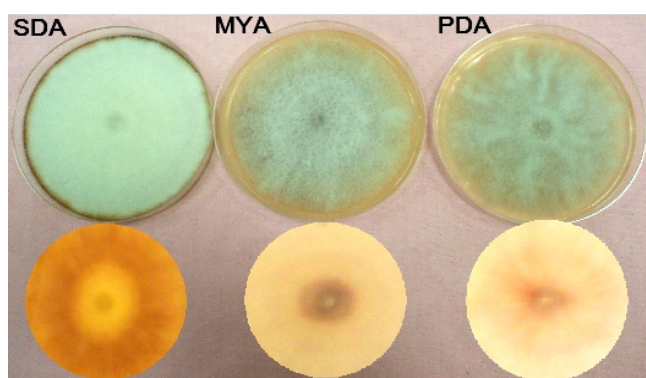


Fig. 1 Cultural characteristics of *Fusarium thapsinum* (teleomorph: *Gibberella thapsina*). Colonies on SDA are bright white aerial mycelia (top row), but become grayish-violet on MYA and PDA. Pigment on the reverse side of the culture (bottom row) is pale orange on SDA, and grayish-violet on MYA and PDA.

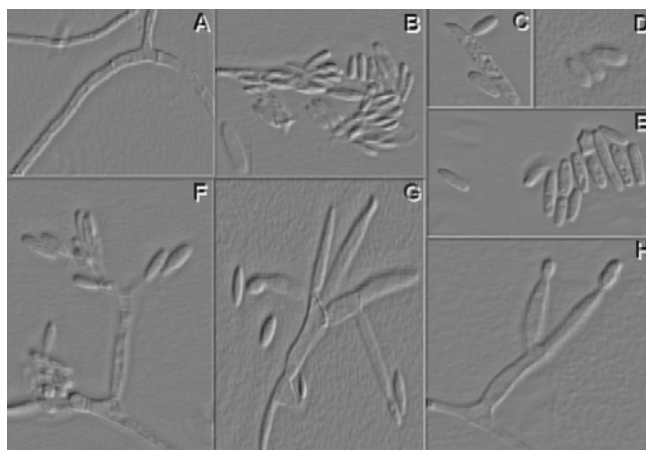


Fig. 2 Morphological characters of *Fusarium thapsinum* (teleomorph: *Gibberella thapsina*). Septated mycelium (A). Microconidia in long chains (B). Macroconidia (C). Microconidia mostly pyriform to citriform with 0- or 1-septum (D, E) Verticillately branched conidiophores produced on aerial mycelium (F). Polyphialides (G) Conidiophores with verticillate phialides (H). Scale bars = 10 µm.



Fig. 3 Symptoms of *Fusarium* fruit rot of banana: whitish hyphae and conidia of *Fusarium thapsinum* are observed on the surface of infected fruit.



Fig. 4 *F. thapsinum* pathogenicity on banana fruit. T1, T2, and T3: treatments inoculated with mycelial agar plugs; C: inoculated with agar plugs.

ment on the reverse side of the culture was pale orange on SDA, but grayish-violet on MYA and PDA. *F. thapsinum* mycelium was septated (Fig. 2A). Microconidia in long chains and chlamydospores were absent (Fig. 2A). One- or 0-septate microconidia are usually club-shaped with a flattened base, but may occasionally be pyriform (Fig. 2 B-E). Microconidia are produced on monophialides usually in chains that can be quite long, but occasionally also may be found in false heads (Fig. 2F). *F. thapsinum* produced polyphialides on all tested media (Fig. 2G). Microconidia may be arranged on the phialides singly (Fig. 2H).

Pathogenicity test

Many dark brownish spots were observed on the epidermis. Whitish mycelia were also frequently observed on the larger spots (Fig. 3). Some larger brown spots, especially on the wounded parts, had whitish mycelial colonies and were often surrounded by halos (Fig. 4). The inoculated fungus was re-isolated from diseased banana.

DISCUSSION

Banana (*Musa* spp.) is cultivated in numerous tropical countries throughout the world, and in many of these countries, its cultivation and marketing play very important roles, both economically and socially (Santos *et al.* 2005). For international trading, banana fruits are usually harvested before ripening, and stored at relatively low temperature during transportation and market processes. Long-distance transport and extended storage period in the market may make banana sensitive to disease incidence (Thompson and Burden 1995). It is likely that *F. thapsinum* is mainly associated with monocotyledonous plants such as grain sorghum, maize, banana and more recently, a prairie grass, *Andropogon gerardii* (Leslie *et al.* 2004). These isolates' identification in this study were based on morphological and cultural criteria. Two isolates were tested rigorously for morphological features on three media which promoted the formation of microconidia. Profuse mycelial growth and pro-

minent lines spread from the center of the actively growing culture on MYA and PDA. Pigment on the reverse side was pale orange on SDA but grayish-violet on MYA and PDA, although pigments are not usually diagnostic for a species, with the yellow pigment produced by cultures of *F. thapsinum* being a prominent exception (Klittich *et al.* 1997). With respect to morphological characteristics, *F. nygamai* is intermediate between *F. verticillioides*, *F. thapsinum* and *F. oxysporum*, all of which include representatives that produce violet pigmentation in colonies on PDA. Pyriform or citriform microconidia with 0-1 septa were present in long chains, while chlamydospores were not produced by *F. thapsinum* isolates. Conidiogenous cells were mostly monophialidic and occasionally polyphialidic. *F. thapsinum* and *F. proliferatum* produced both monophialides and polyphialides. *F. thapsinum* was first recognized as a distinct mating population within *F. moniliforme* (Klittich *et al.* 1992), and then later elevated to species rank (Klittich *et al.* 1997). Phylogenetically, *F. nygamai* is most closely related to *F. thapsinum* (O'Donnell *et al.* 1998), but *F. nygamai* also may be a species complex that is in need of further resolution (Leslie *et al.* 2005), which could make it difficult to position accurately on a phylogenetic tree. *F. thapsinum* is also similar to *F. andiyazi* but can be differentiated by the presence of pseudochlamydospores in *F. andiyazi* (Marasas *et al.* 2001). The pathogenicity of two isolates of *F. thapsinum* was studied by inoculation of banana fruits; both isolates were pathogenic to wounded fruits. Inoculations of banana fruits with *F. thapsinum* produced a soft rotten circular area with brown colour. *F. verticillioides* and *F. oxysporum* are serious pathogens affecting only wounded fruits (Alvindhia *et al.* 2002). This is the first reported occurrence of *F. thapsinum* from commercial banana imported into Saudi Arabia.

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