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Full Length Research Paper

A basic procedure for single spore isolation of *Fusarium verticillioides* and *Fusarium subglutinans*

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An easy single spore isolation technique for *Fusarium verticillioides* and *Fusarium subglutinans* is described and illustrated. The technique depends only on, reused basic equipments, time saving and enables poorly funded laboratories to carry it out with no need to a great deal of skills to be performed.

Key words: single spore isolation, pure cultures, *Fusarium verticillioides, Fusarium subglutinans.*

INTRODUCTION

Fusarium verticillioides (syn. *F. moniliforme*) and *F. subglutinans* belonging to the Liseola section of *Fusarium* (Nelson et al., 1983), are well known pathogens of corn, causing stalk and ear rot world-wide (Nelson et al., 1981; Leslie et al., 1990; Logrieco et al., 1993; Bottalico, 1998). These pathogens have the ability to produce and accumulate mycotoxins in infected tissues that may have dangerous effects on humans and animals with carcinogenic potential such as fumonisins (Nelson et al., 1993; Asran and Buchenauer, 2002; Aboul-Nasr and Obied-Allah, 2013), fusaric acid (Bacon et al., 1996), and moniliformin (Marasas et al., 1984). However, there is no evidence that *Fusarium* mycelia growing out of plant tissues or from soil particles necessarily belong to a single species which may lead to misidentification (Booth, 1977). Also fungi growing on the natural substrates are not entirely free of other organisms and therefore direct transfer of their spores will carry contaminants. Moreover, slow-growing fungi will be overgrown by highly sporulating fungi. Another complication is that certain bacteria strongly inhibit the growth of fungi and thus the isolation of fungi would be unsuccessful and dilution of spore masses as well as different antibiotics [e.g. penicillin, 0.5 g/L and streptomycin, 0.5 g/L] can be used to avoid such bacterial and yeast contaminations (Smith, 1969). Methods based on spore-dilution principle works well with hyphomycetes that have large colored conidia, such as *Alternaria*, *Corynespora*, and *Helminthosporium*, but it is difficult to be used with species of *Aspergillus* and *Penicillium* (Hansen, 1926).

The conventional method of isolating well separated spores streaked on the agar medium under a stereoscopic microscope was used by Tuite (1969). It is also worthy to mention that not all spores germinate on artificial media, and other techniques may be required for successful isolation of these fungi. On the other hand, fungal cultures stored in a high nutrient medium, such as potato dextrose agar [PDA] may lose their ability to produce enzymes or metabolites, or perform other functions and care is therefore needed in selecting a storage medium (Smith and Onions, 1994). Some other sophisticating single spore isolation technique using micromanipulators and a hand maid glass needle were described by Matsushima (1975), Teik-Khiang (1999). Also preparing *Fusarium* cultures for identification was held by the single spore technique, devised by Hansen (1926) and Toussoun and Nelson (1976). To ensure isolates are pure, working with single spore isolation is strongly recommended. In fact fungal isolates grown from single spores demonstrate species characteristics and uniform growth more clear than those of mass transfer of

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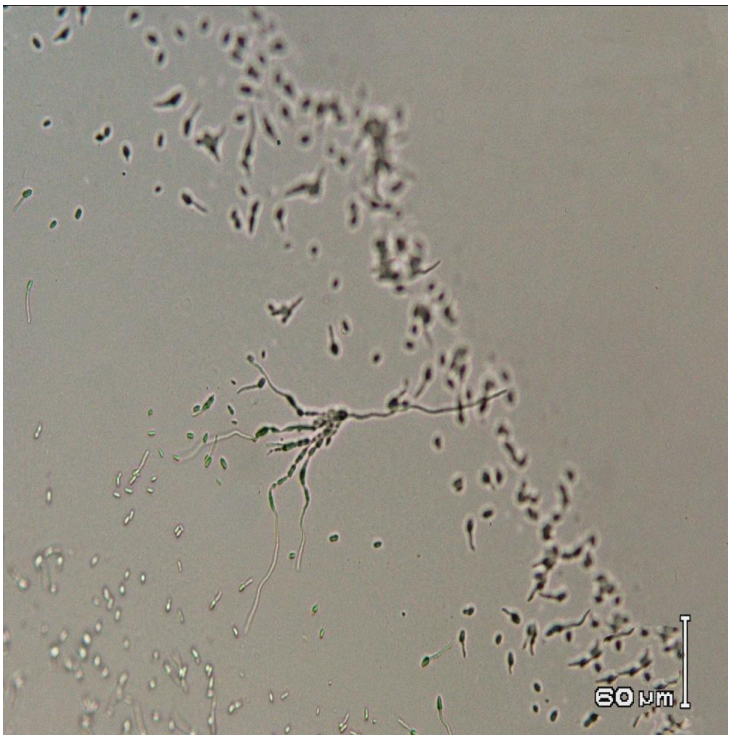

Figure 1. Five µL drop of conidial suspension under compound microscope at the edge of a marked circle on Petri dish, where intense germinating conidia appear.

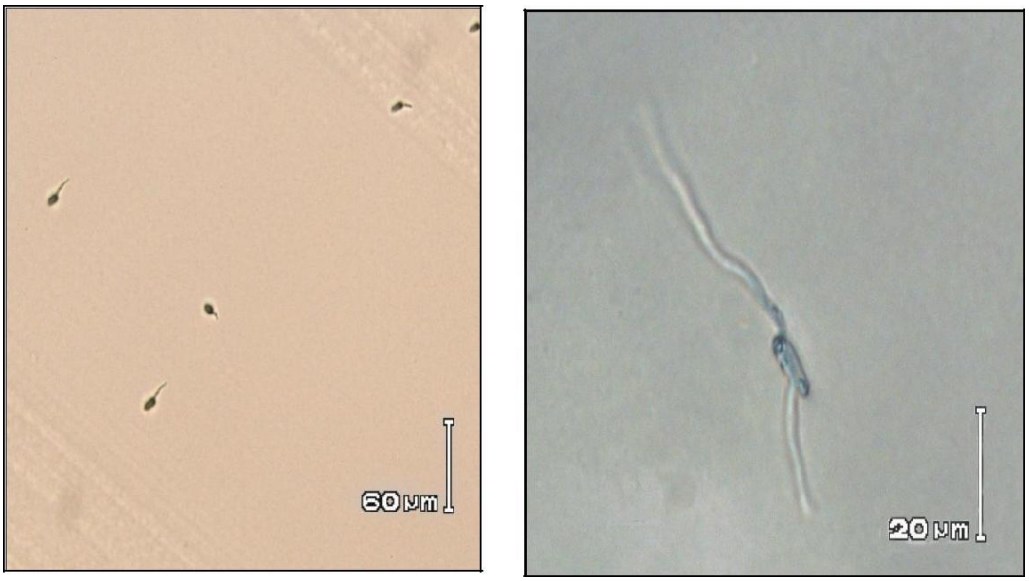

Figure 2. Germinating microconidia of *Fusarium verticillioides* on water agar medium after 12 hrs of incubation at 25ºC.

inoculums (Leslie and Summerell, 2006). In addition, there are many other methods to obtain pure fungal culture by single spore isolations or hyphal tip cultures as described by Hansen (1926), Hildebrand (1938), Smith and Onions (1994) and Wang-Chig and Wen-Hsiung (1997). The simplest and most economical way of obtaining single spore cultures was described by Booth (1977).

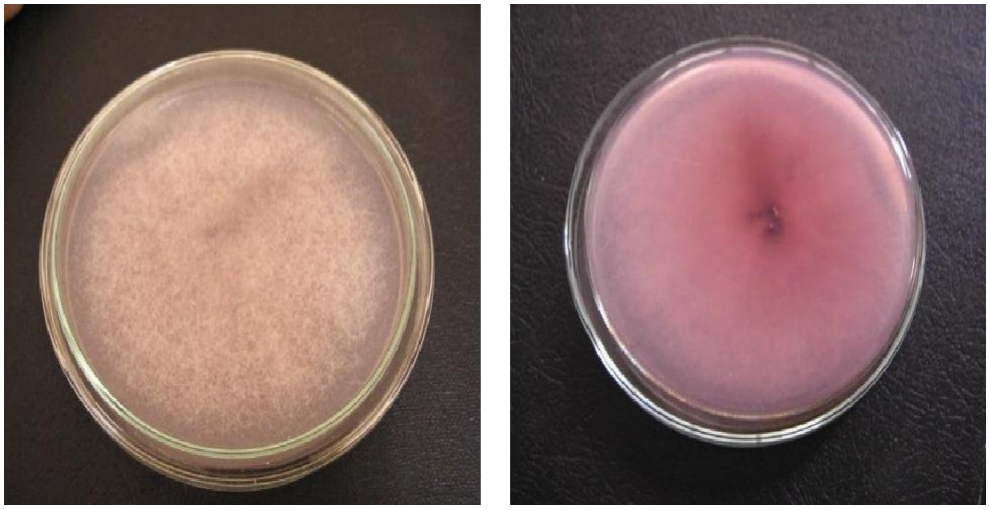

Figure 3. Colony and reverse color of *Fusarium verticillioides* developed after single conidium transffered on to PSA and incubated for 7 days at 25°C.

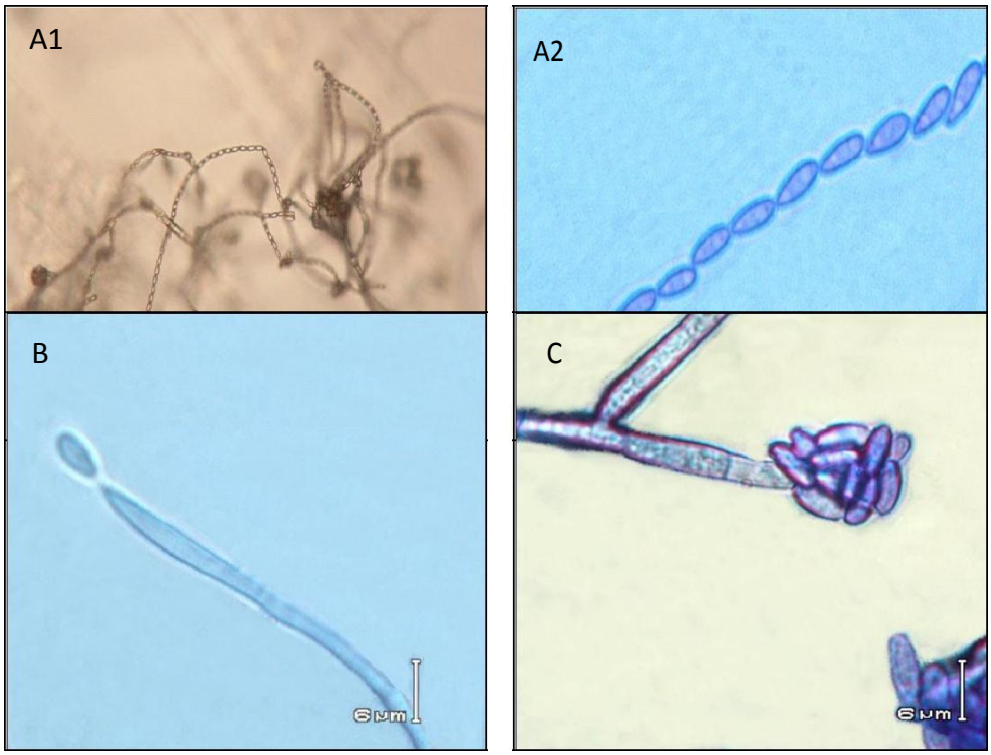

Figure 4. *Fusarium verticillioides* after single spore subculture, showing the: A1 and A2- Chains of microconidia, B- Monophialides, C- Clusters.

In the present work, we developed an easy single spore isolation technique for *F. verticillioides* and *F. subglutinans* which depends only on basic equipments that can be reused, saves time, enables poorly funded laboratories to carry it out.

***Fusarium verticillioides* and *F. subglutinans* isolates collection**

A total of 76 *Fusarium verticillioides* and 10 *F. subglutinans* isolated from 80 corn samples were

Figure 5. Colony and reverse color of *Fusarium subglutinans* developed after single conidium transffered on to PSA and incubated for 7 days at 25°C.

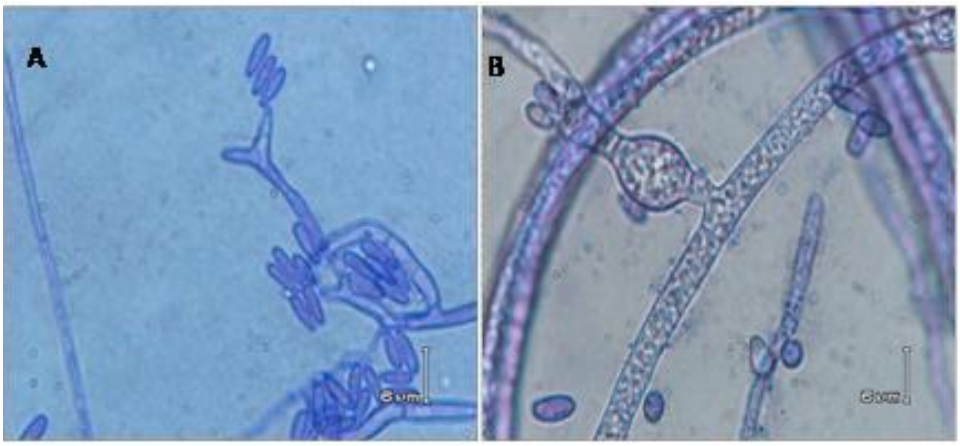

Figure 6. *Fusarium subglutinans* after single spore subculture, showing the: A- Branched phialides, B- Chlamydospoers.

collected from different regions of Sohag Governorate using two conventional methods, direct-plate (Pitt et al., 1992) and surface disinfection plate (Müllenborn et al., 2008) and recovered on dichloran-Rose-Bengal Chloramphenicol agar medium (Tournas et al., 2006). *Fusarim* isolates were identified morphologically based on macro and microscopic characteristics (Booth, 1977; Nelson et al., 1993; Leslie and Summerell, 2006).

Single spore technique for *F. verticillioides* and *F. subglutinans* isolates

a) Three milliliter of 2% water agar Poured into unscratched Petri dish and allowed agar to solidify.

b) A suspension of conidia either from a sporpdochium or from aerial mycelium was prepared in 5 mL sterile water in a sterile vial.

c) A micro pipette was used to transfer 5 µL of conidial suspension aseptically on a drawn circle with a permanent marker on the back side of a Petri dish containing water agar medium.

d) The conidial suspension located on the drawn circle is touched by an L-shape inoculating needle several times and streaked across the water agar plate.

e) The plate was incubated for 12 to 16 h at 25°C, thereafter was examined under a dissecting microscope. A lot of germinating conidia appeared on the site of inoculation and by following the streaked lines using the low power of the microscope, single germinating conidia could be observed (Figure 1).

g) Suitably positioned germinated conidia (Figure 2) were removed using presterilized surgical blade [Wuxi Xinda Medical Device Co., Ltd. Sterilized by GAMMA radiation].

h) Single spores were cultured on potato sucrose agar medium [PSA] (Booth, 1971; Booth, 1977).

i) Finally, identification of *F. verticillioides* and *F. subglutinans* cultures was accomplished (Figures 3, 4, 5 and 6).

CONCLUSION

In conclusion, this method involves simple procedures, inexpensive, requires only basic equipments and does not need expert laboratory technicians to carry it out.

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