

RESEARCH ARTICLE

Morphological Changes of Conidiogenesis in Two *Aspergillus* Species

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Abstract

Identification of aspergilli based on comparative of their diagnostic features in different growth media is a common manner between most of mycologists. Asexual sporulation in *Aspergillus* species is very complex processes which include numerous morphologically distinct stages. In the present study, asexual reproductive structures of *Aspergillus terreus* and *Aspergillus versicolor* have been tracked and the development of conidiophores have been compared in two types of growth media MEA (Malt Extract Agar) and YES (Yeast Extract Sucrose) which considered the most common growth media in identification of aspergilli. Interestingly, YES agar medium induce *A. terreus* to retard the asexual sporulation process in a distinctive manner. Unusually, the early conidiophore of *A. terreus* distends to form infertile (immature) vesicle which then produces a second conidiophore. The later conidiophore expands to form fertile (mature) vesicle which differentiates into conidial head. The development of one or more conidiophores from the immature vesicles of *A. terreus* instead the foot cells or areal hyphae remain mostly uncharacterized by many researchers. On the other hand, the asexual sporulation in both *Aspergillus* species was dramatically decreased on YES agar medium when compared by MEA medium. Moreover, the accessory conidia in *A. terreus* and reduced Penicillium-like structures that produce another type of phialidic conidia in *A. versicolor* were mostly not observed on YES agar medium.

Keywords: *A. terreus*, *A. versicolor*, asexual sporulation, scanning electron microscope.

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INTRODUCTION

Aspergillus species (collectively called aspergilli) are the most common saprophytic and/or parasitic fungi that found in various climates worldwide. Most aspergilli reproduce asexually by forming conidiospores via a process called conidiogenesis (Axelrod, Gealt, & Pastushok, 1973). Basically, conidia and conidial head structure are the most important morphological features that used by mycologists to identify and classify *Aspergillus* species into genera or distinguish closely related species. Although genetic approach have been interpreted in fungal taxonomy, phenotypic concepts are still necessary (Hyde, Abd-Elsalam, & Cai, 2011).

Asexual reproductive cycle in aspergilli can be divided into two stages vegetative growth and sporulation development. The growth phase includes germination of a conidiospores and form a network of interconnected hyphae known as a mycelium. When the environmental factors changed, some of hyphae stop growing and initiate asexual reproduction by forming conidiophore and conidia (Adams, Wieser, & Yu, 1998).

Moreover, the conidiogenesis process is influenced by environmental factors including carbon and nitrogen source, pH, temperature, incubation period, and water potential (SITTON, 2004). One of the most important factors strikingly affecting morphological criteria in *Aspergillus* species is a composition of culture media (Khalil, 2016; Kim et al., 2005; Saha, Mandal, Dasgupta, & Saha, 2008; Saxena, Sangeetha, Vohra, Gupta, & Gulati, 2001). Malt extract agar and potato dextrose agar were the stander identification growth media for *Aspergillus* identification (Pitt, Hocking, & Beuchat, 1998).

Furthermore, genetic regulations of the induction of conidia in fungi have been properly studied by many authors (Roncal & Ugalde, 2003; Sun et al., 2012). However, several genes have been found to be involved in this process and controlled asexual sporulation (Roncal & Ugalde, 2003; Sun et al., 2012; J.-W. Xu, Zhao, Xu, & Zhong, 2012; Zhou, Wang, Qiu, & Feng, 2012). Expectedly, environmental stresses have been found to act as regulatory factors for these genes (Chi & Craven, 2016).

Accordingly, definite the effective environmental factors that induce asexual

sporulation process in aspergilli considered the first step towards making an accurate identification. This paper present the morphological properties of *Aspergillus terreus* and *Aspergillus versicolor* including the conidiogenesis process when cultured on malt extract agar and yeast extract agar medium as a common medium used for identification of aspergilli.

MATERIALS AND METHODS

Microorganisms

Aspergillus terreus (JCM 10227) and *Aspergillus versicolor* (NRRL 238) were kindly obtained from Culture Collection of school of Pharmacy and Bio-molecular science, Liverpool John Moores University, United Kingdom.

Culture characteristics

Culture characteristics of *Aspergillus* species were studied on two differential media, malt extract agar (MEA) HIMEDIA and yeast extract sucrose agar (YES) medium (2% yeast extract (HIMEDIA), 15% sucrose (HIMEDIA), and 1.5% agar (Sigma Aldrich)). Morphological characteristics including the diagnostic macroscopic features for identification such as colony diameter after ten days, color (conidia and reverse), exudates and colony texture were studied. Microscopic characteristics for the identification were conidial heads, stipes, color and length vesicles shape and seriation, metula covering, conidial size and shape were investigated.

Bright field microscope

Microscopic examination was carried out by tearing small apart of mycelium in a droplet of 0.1 % lactophenol blue or by cover slip culture technique which designated by (Nugent, Sangvichen, Sihanonth, Ruchikachorn, & Whalley, 2006) and the preparation were examined by bright field microscopy built in with camera using ×40 and ×60.

Scanning electron microscope

Small pieces from the fresh colony of *Aspergillus* culture were fixed in 2.5% glutaraldehyde for 20 minutes to provide a rapid inter and intra-cellular penetration. Fixed specimens were dehydrated by series concentrations of ethanol, ending with 100% dehydrating liquid in highest purity. Typically, concentrations are steps of 10, 20, 30, 50, 70, 90, 95, 100% at 10 minutes for each, with 3 changes at

100%. Acetone was used as the intermediate fluid because it is miscible with carbon dioxide. Critical point drying (CPD) was utilized to avoid collapse in SEM. This step is carried out by substituting acetone with liquid CO₂ then the liquid CO₂ is kept at a critical temperature and pressure (34.5°C and 1200 psi (pounds per square inch)). Eventually, samples were coated with gold using an Emitech K550X coating machine. The specimens were then loaded into FEI (Quanta 200) ESEM (Environmental Scanning Electron Microscopy, 2008) and observed over a range of magnifications. Images were got by using an image capture system (Oxford Instruments, INCA system, Oxford, UK).

Counting of fungal spores

Counting of fungal spores was achieved by taking 9mm disc of fungal cultures into 10 ml test tube filled with 5 ml of distilled water. Test tubes were shaken vigorously by vortex mixer for 5 min. Ten micrometer of fungal spores suspension was transferred into a petroffhausser counting chamber and then spores was counted. Consequently, the number of fungal spores in 5 ml was calculated.

RESULTS AND DISCUSSION

Colonies of *A. terreus* on MEA reaching 50 mm in diameter after incubating for ten days

at 25°C. Surface colors are likely brownish to buff and reverse dark yellow to brown (Fig. 1). Conidial heads on MEA densely columnar, 70-150 µm in length 40-50 µm in diameter. Conidiophore smooth to slightly rough, hyaline, 150-250µm in length and 5-6µm wide. Vesicle biserial, hemispherical to globose, 15-20µm in diameter (Table 1). Metulae flask shape, covering upper 2/3 of the vesicle, 4.5-7.5×2.0-3.5µm; phialides 5.5-8.0×1.5-3µm. Conidia globose to subglobose, 2-3µm in diameter (Figs. 1&3). Usually, growth of *A. terreus* on MEA follows the same way of other *Aspergillus* species. The vegetative growth initiated by the germination of conidiospores which lead to the formation of tubular hyphae that grow and branch to form mycelium. The mycelium expands and grows until environmental conditions are divers. At this time, aerial hyphae are rising up in the center of the colony and some of them successively differentiate into conidiophores (Adams et al., 1998).

On the other hand, colonies of *A. terreus* on YES agar plate reach 70 mm in diameter after incubating for ten days at 25°C. Surface colors be likely whitish and when it's get old changing to yellow in the center of colony while the reverse color pale yellow (Fig.1). Two conidiophores are formed, one from the foot cells and the other

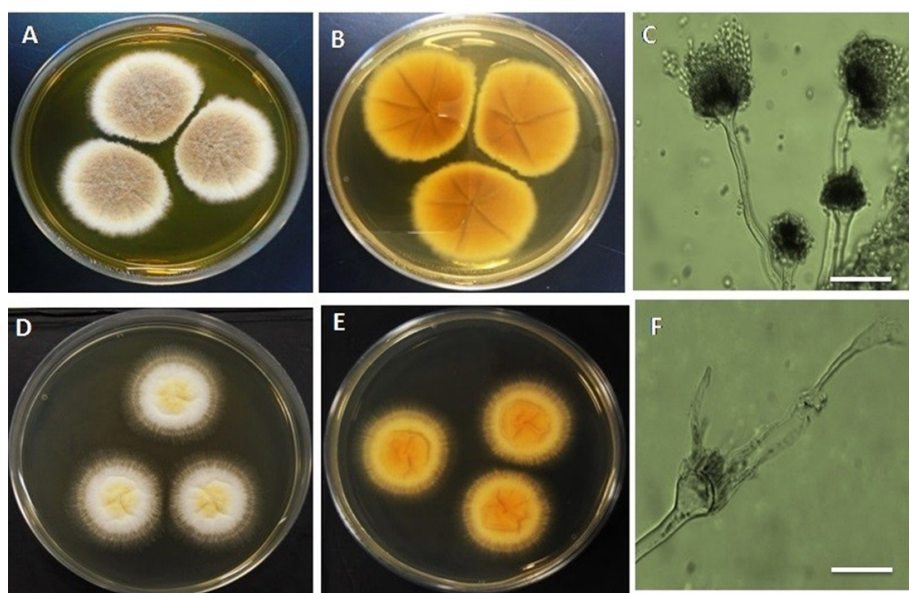


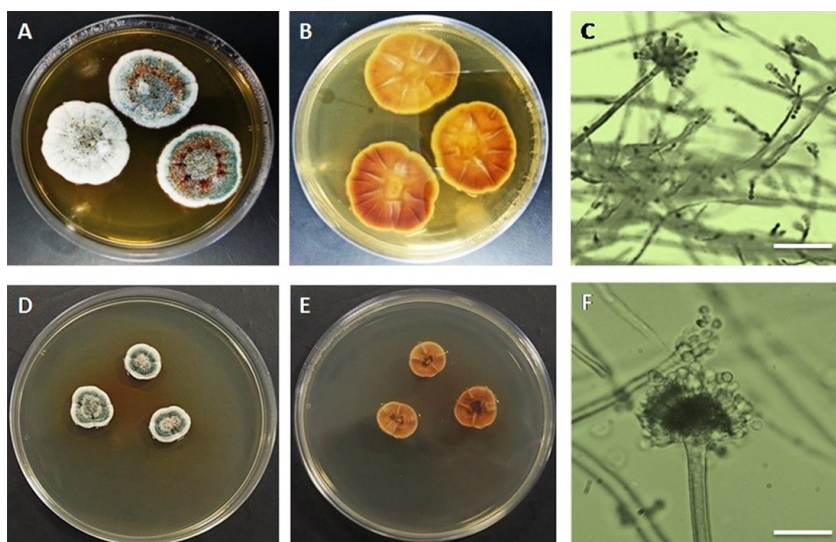
Fig. 1. *Aspergillus terreus* A&B. Surface and reverse on MEA C. conidiophore with conidial head D&E. Surface and reverse on YES agar F. Conidiophores with immature vesicle (Bar in C & F = 100µm).

Table 1. Culture characteristics of *A. terreus* in two common growth media MEA and YES agar

Characters	Examination on MEA	Examination on CYA
Culture examination		
Growth characteristics	Colonies grow moderate to rapidly reaching 2.0-5.0 cm diameter in 10 days at 25°C, cinnamon to light brown, buff colonies. Reverse light brown.	Colonies grow moderate to rapidly reaching 3.0-7.0 cm diameter in 10 days at 25°C, white colonies with yellow pigment in center. Reverse light brown.
Microscopic examination:		
Conidial heads	Columnar.	Hemisphere
Conidiophore	5.0-6.0 µm in diameter.	4.0-6.0 µm in diameter. Two conidiophores have the same size
Vesicle	Globose to subglobose shape. 15.0-20.0 µm.	Both vesicles are globose to subglobose. fertile one 10.0-15.0µm. infertile vesicle 7.0-10.0µm
Matula and phialides	In two series (biseriate)	In two series (biseriate)
Conidia	Conidia, spherical, 2.0-3.0 µm.	Conidia, spherical, 2.0-3.0 µm.
Sporulation No. of spores/cm ² (×10 ⁵)	75	40

come out of the vesicle. Both conidiophores are mostly same in the length approximately 100-120µm. The first immature vesicle is globose and smaller than the second one 7-10µm (Table 1). The second vesicle is mature and produces matula and phialides exactly as this in MEA (Figs. 1&3).

Colonies of *A. versicolor* on MEA reaching 30-35 mm in diameter after incubating for ten days at 25°C. Colony surface was light to dark greenish to bluish green color while reverse was light brown to orange. Moreover, colonies of *A. versicolor* on YES agar medium reaching 20-25

**Fig. 2.** *Aspergillus vericolor* A & B. Surface and reverse on MEA C. Two pattern of conidiophores (aspergillate and penicillate structures) D&E. Surface and reverse on YES agar F. Conidiophore with conidial head (Bar in C & F = 400µm).

mm in diameter after incubating for ten days at 25°C (Fig. 2). Colony surface was green to greenish while the reverse was reddish brown in color. In both growth media, conidiophores elongate and swell to form fertile vesicle with smooth walled stipes 200–700 5–7.0µm. Vesicles are spherical to sub-spherical, or pyriform to spatulate, 8-15µm in diameter. Conidial heads biserial, Phialides 5–8.0µm borne on metulae 4.5–7.5µm (Table 2). Both phialidic conidia which produced from conidial head and penicillium like structure are globose to sub globose with roughened wall (Figs. 2, 4 & 5).

Consequently, the nutrient level of YES agar medium is much sufficient for *A. terreus* than that in MEA. Thus, many researchers stated YES to be the best media for hyphal growth (Maheswari, Singh, & Sahu, 1999; Saha et al., 2008; Suzuki & Iwahashi, 2016; S. Xu, Yuan, & Chen, 1984).

Whereas, sucrose is one of the most obvious different between YES and MEA, it plays an important role in sporulation process (Latha, Prakasam, Jonathan, Samiyappan, & Natarajan, 2013). Fungi on glucose free growth medium produce the high number of conidia. This is probably due to low sugar concentration. Moreover, vegetative growth in some fungal species was stimulated in vitro by the restoration

of the medium with sugar (Latha et al., 2013).

In fact, when sufficient nutrients are available and environmental conditions are favorable *A. terreus* sustained in vegetative growth. However, when conditions diverse *A. terreus* tends to initiate sporulation process through forming aerial hypha which then differentiate to conidiophore and terminate by vesicle. Continuously, on MEA vesicle produces simultaneous matula and phialide (all matula on the surface of vesicle developed in the same time). Eventually, Phialides sprout chains of conidiospores which germinate when nutrient is available to produce germ tube and vegetative hyphae once again. Moreover, some researchers recorded that, *A. terreus* has ability to produce microconidia borne directly on a short conidiophores which arise laterally from vegetative hyphae (Walsh et al., 2003).

Interestingly, when *A. terreus* cultured on high rich sucrose medium such as YES agar the first conidiophore that differentiate directly from areal hyphae produce infertile vesicle. After a short of time, this vesicle is simultaneously fully covered by matula like structure. One or two of them are differentiated into conidiophores which expand to form the second fertile vesicle.

Table 2. Culture characteristics of *A. versicolor* in two common growth media MEA and YES agar

Characters	Examination on MEA	Examination on CYA
Culture examination		
Growth characteristics	Colonies grow rapidly in 10 days at 25°C, greenish to reaching 3.0-3.5 cm diameter 2.5 cm diameter in 10 blue green colonies. Reverse light brown to orange.	Colonies grow moderate to rapidly reaching 2.0- days at 25°C, greenish to blue green colonies. Reverse light brown to reddish
Microscopic examination		
Conidial heads	pyriform to spatulate	pyriform to spatulate
Conidiophore	5.0-7.0 µm in diameter.	5.0-7.0 µm in diameter.
Vesicle	Globose to subglobose shape. 8.0-15.0 µm	8.0-15.0 µm. Globose subglobose shape.
Matula and phialides	In two series (biseriate)	In two series (biseriate)
Conidia	Roughglobose to sub-globose, 2.0-3.0 µm.	Roughglobose to sub-globose, 2.0-3.0 µm
Sporulation No. of spores/cm ² (×10 ⁵)	85	70

To this end, the fertile vesicle produce matula and phialide and then conidia are formed in chains. Moreover, the accessory conidia in *A. terreus* have been observed on MEA medium while it is mostly disappeared on YES agar medium (Fig. 5). Probably, accessory conidia in *A. terreus* appeared to be more susceptible to high sugar concentrations in growth media (Deak, Wilson, White, Carr, & Balajee, 2009).

Based on our knowledge, no one has been reported vesicle of *A. terreus* or any other *Aspergillus* species can sprout one or more conidiophores instead matula or phialides. Even mutant strains of *A. nidulans* and *A. fumigatus* which designated to inhibit sporulation process and carry on vegetative reproduction, they only produce abnormal elongated conidiophore with or without conidia from its vesicle (Adams et

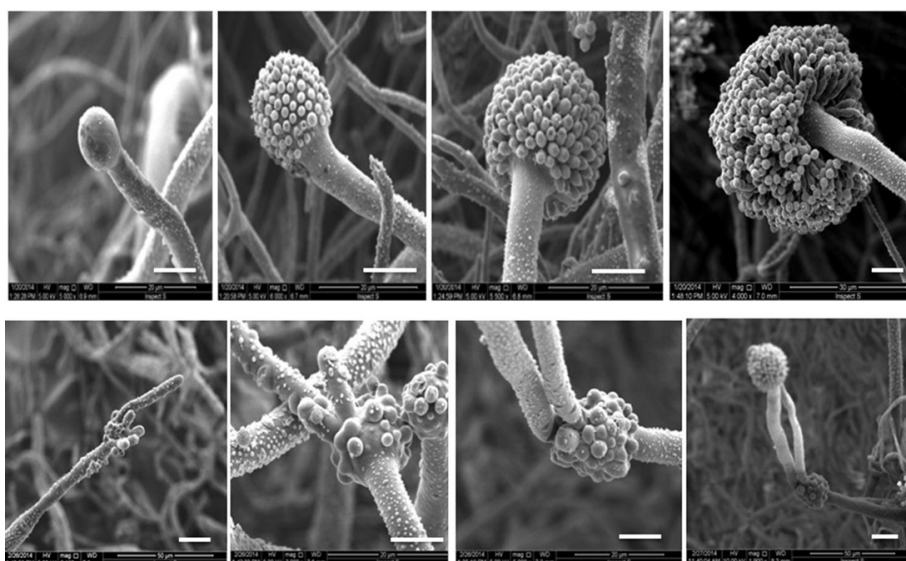


Fig. 3. Scanning electron micrographs of *Aspergillus terreus*. The top row shows the formation of conidiophore and conidial head on MEA medium. The bottom row shows the formation of conidiophores and the fertile and infertile vesicles on YES agar medium (Bars = 10µm).

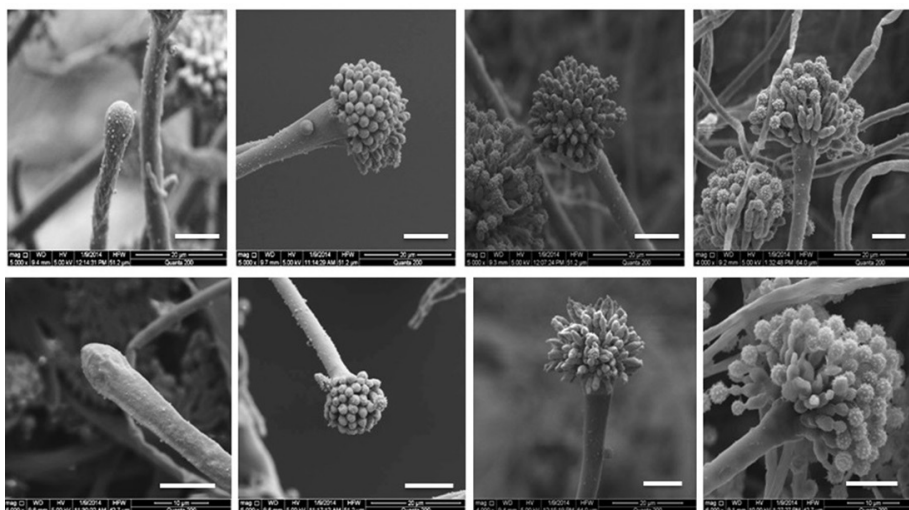


Fig. 4. Scanning electron micrographs of *Aspergillus versicolor*. The top row shows the formation of conidiophore and conidial head on MEA medium. The bottom row shows the formation of conidiophore and conidial head on YES agar medium (Bars = 10µm).

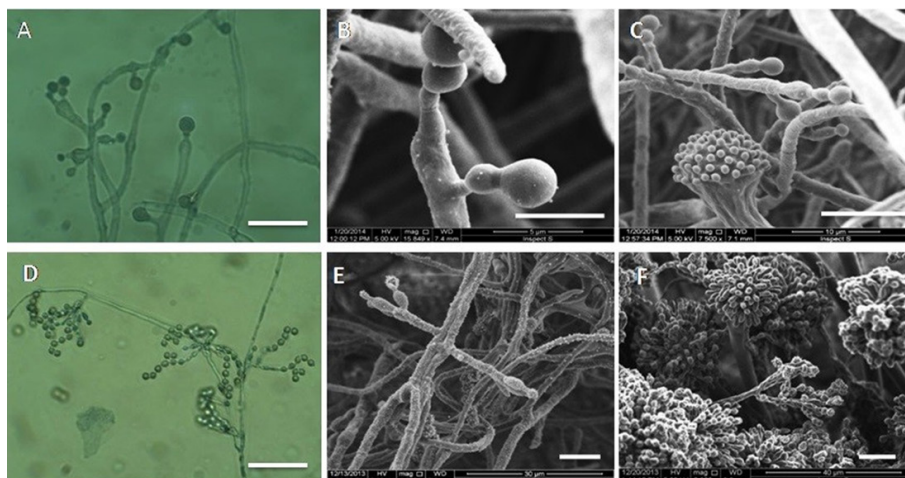


Fig. 5. Bright field and scanning electron micrographs. Top row shows the accessory conidia in *Aspergillus terreus* grown on MEA. Bottom row shows the phialidic conidia on penicillium like structure in *Aspergillus versicolor* on MEA (Bars: A & D = 400μm; B =5μm; C,E,&F = 10μm).

al., 1998; Krijgsheld et al., 2013; Ni, Gao, Kwon, Shin, & Yu, 2010). It believes that, some genes induced by high concentration of sugar to increase the vegetative growth and delay sporulation process (Krijgsheld et al., 2013). In this case, suppression of sporulation process occur when fungus sustaining the mycelium growth while aerial hyphae if it is formed do not differentiate into conidiophore and conidial head (Adams et al., 1998).

In our point of view, *A. terreus* might be found another or more than one way to delay the sporulation process. In addition to the previous way, the aerial hypha differentiates into conidiophores and immature vesicle which subsequently produce one or more additional conidiophores and vesicles before starting in actual sporulation process.

Obviously, the vegetative growth of *A. versicolor* on MEA was much faster and greater than that on YES agar. Contrary, asexual sporulation on MEA was much higher than that on YES agar. However, conidiogenous structures of *A. terreus* showed no variations on two growth media except the absence of penicillium like structures on YES agar medium. It has also been assumed that the phialidic conidia that produced by penicillium like structures might be inhibited by sucrose in YES agar medium (Khalil, 2016).

In conclusion, the taxonomic criteria should be taken in concern when culturing and

identifying *A. terreus* on any growth medium contain high sugar concentration specially YES agar medium. Moreover, it is useful to use high rich sugar medium in biotechnology where sporulation processes undesired during this application. Furthermore, SEM represents a precious investigative tool to examine and characterize any newly morphological changes that occur to fungi as environmental conditions alter.

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CONFLICT OF INTEREST

The author report no conflicts of interest. The author alone is responsible for the content and writing of the paper.

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