Sexual Life Cycle of *Aspergillus fumigatus*

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= Abstract =

Aspergillus fumigatus, the major etiologic agent of invasive aspergillosis, is a bipolar heterothallic species that produces teleomorphs belonging to the genus *Neosartorya*. Unlike *A. fischeri* and other sexual species phylogenetically related to *A. fumigatus*, the discovery of a sexual state in *A. fumigatus* was unduly delayed due to its requirement of extraordinarily fastidious environmental conditions for the completion of the sexual life cycle compounded with low fertility of the species. A wide range in the degree of fertility and duration required for completion of the sexual life cycle appear to exist among clinical as well as environmental isolates. Discovery and characterization of a pair of opposite mating type strains with high fertility that could produce viable ascospores is urgently needed as a tool of recombinational analysis. **[Kor J Med Mycol 2011; 16(3): 81-85]**

Key Words: *Aspergillus fumigatus*, Heterothallism, Genetic Recombination

Heterothallism in *A. fumigatus*

Aspergillus fumigatus is a ubiquitous mold and humans inhale hundreds of airborne conidia daily[1]. While the inhaled conidia pose no serious threat to normal individuals, they can germinate in the lung and cause life threatening invasive aspergillosis (IA). It is commonly observed in patients with profound neutropenia, such as bone marrow transplant recipients, particularly those with allogeneic stem cell transplants or cancer patients undergoing chemotherapy[2~5]. Patients with Chronic Granulomatous Disease is another high risk group for IA[6]. The incidence of invasive aspergillosis has been increasing throughout the world parallel to the increase in the immunocompromised population.

Genomes of at least two *A. fumigatus* strains have been sequenced and showed 8 chromosomes carrying total of nearly 10,000 genes including the ORFs encoding *MAT-1* or *MAT-2* sequence[7,8]. The equal frequency of *MAT-1* and *MAT-2* strains among environmental as well as clinical origin[7] suggested that *A. fumigatus* is a heterothallic species with a bipolar mating system which is common in pathogenic as well as non-pathogenic fungal species (Table 1). Although the presence of *MAT* genes in the *A. fumigatus* genome suggested the species to be a sexual fungi, effort to discover its sexual state was unsuccessful for decades[9]. This was due to sets of unusually particular environmental parameters that are required for *A. fumigatus*

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Table 1. Recognized Patterns of Sexuality in Fungi

Sexuality	Representative species
Asexual	***Candida glabrata*, *Penicillium marneffei***
Homothallism	***Aspergillus nidulans*, *Pseudallescheria boydii***
Secondary homothallism	*Saccharomyces cerevisiae*, *Neurospora tetrasperma*
Heterothallism	
Bipolar	***Aspergillus fumigatus*, *Histoplasma capsulatum***, *Neurospora crassa*, ***Cryptococcus neoformans***
Tetrapolar	*Schizophyllum commune*, *Ustilago maydis*, *Coprinopsis cinerea*
Dioecism	*Laboulbenia formicarum*

Species in bold are human pathogens

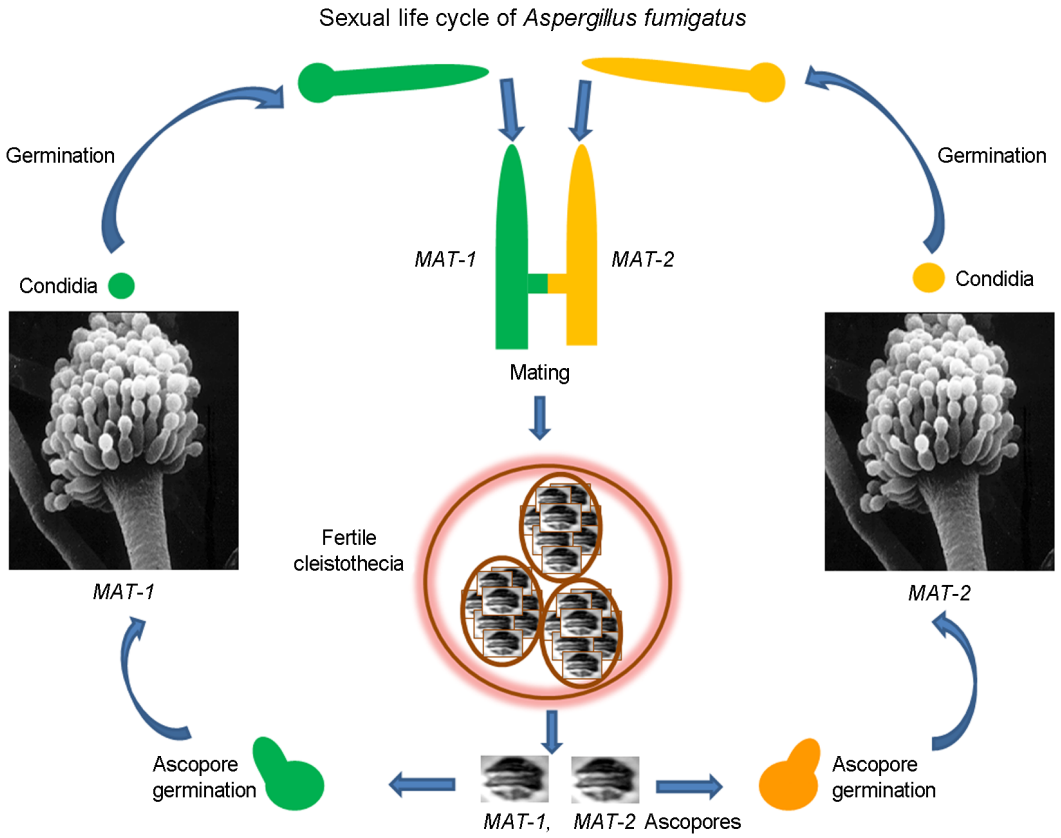

Fig. 1. Heterothallic life cycle of *A. fumigatus*

to undergo sexual reproduction. O'Gorman et al. first reported the sexual state of *A. fumigatus* by mating *MAT-1* and *MAT-2* strains isolated from air samples obtained in Dublin, Ireland in 2009[10]. *MAT-1* crossed with *MAT-2* strains produced cleistothecia only on oatmeal agar plates sealed with ParaFilm and incubated at 30℃ for 6 months in the dark[10] (Fig. 1). No other species producing

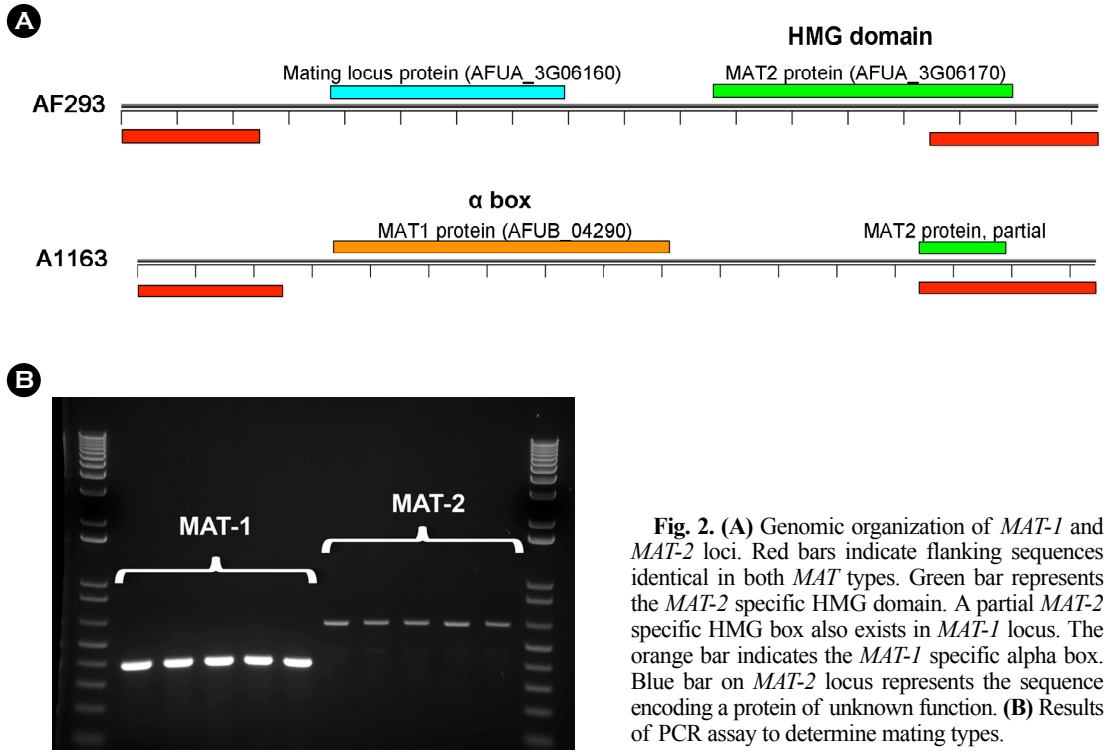

Fig. 2. (A) Genomic organization of *MAT-1* and *MAT-2* loci. Red bars indicate flanking sequences identical in both *MAT* types. Green bar represents the *MAT-2* specific HMG domain. A partial *MAT-2* specific HMG box also exists in *MAT-1* locus. The orange bar indicates the *MAT-1* specific alpha box. Blue bar on *MAT-2* locus represents the sequence encoding a protein of unknown function. **(B)** Results of PCR assay to determine mating types.

Neosartorya state of teleomorph requires such particular set of environmental conditions[9]. There are 23 *Aspergillus* species that produce *Neosartorya* teleomorph and they can all complete sexual cycle in 2~3 weeks at 25℃ on conventional mycological media such as malt extract agar[9,11,12]. In addition, fertility of *A. fumigatus* appeared lower compared to other heterothallic as well as homothallic species of *Neosartorya*[9]. All these *Neosartorya* species produce conidial state belonging to Section Fumigati and many of their conidial state is indistinguishable from that of *A. fumigatus*[11,12].

The structural organization of *MAT* loci among *Neosartorya* species has been characterized in *A. fumigatus* and *A. fischeri* (*N. fischeri*). As in other heterothallic filamentous ascomycetes, the genes found at the *MAT-1* and *MAT-2* loci of *A. fumigatus* are highly divergent in sequence and hence, they have been defined as "idiomorphs" rather than alleles. The *MAT-1* locus contains a characteristic alpha box while *MAT-2* locus contains a characteristic high mobility group (HMG) gene (Fig. 2A)[7]. Determination of mating type in each *A. fumigatus* strain is readily accomplished by PCR amplification (Fig. 2B) of *MAT-1* specific alpha box sequence and *MAT-2* specific HMG sequence using available primers[7]. The molecular determination of mating type always concurs with the biological mating determined by crossing each strain with *MAT-1* or *MAT-2* tester strains. However, there are strains that fail to mate with *MAT-1* or *MAT-2* tester strains and the degree of fertility varies widely among strains originated from both environmental and clinical sources[9,10]. According to our study, there are strains that can complete sexual cycle and produce abundant cleistothecia containing viable ascospores only in four weeks[13] while other strains require six months[10]. Cleistothecia produced in *A. fumigatus* contain ascopores that are morphologically indistinguishable from

various other *Neosartorya* species unless examined by scanning electron microscopy (SEM). The delicate markings on the ascospore surface are subtle but distinct in each species[12]. Interestingly, however, the SEM of *A. fumigatus* ascospores showed the morphology of ascospores indistinguishable from those produced in *N. assulata*[10,12].

Heterothallism as a tool for recombinational analysis in *A. fumigatus*

Recent description of heterothallism in *A. fumigatus* has evoked expectations that rapid progress in genetic study is forthcoming[9,10]. Although the strains of *MAT-1* and *MAT-2* thus far characterized were useful in recombination analysis of certain genetic markers attached to strains of either environmental or clinical origin[9,14], low fertility combined with extra long incubation period posed a serious hurdle for progress in *A. fumigatus* genetic study. The ascoposres (meiotic spores) are always contaminated with asexual spores (conidia). However, ascoposres can readily be purified from contaminating conidia by heat treatment since exposure of spore suspension to 70℃ for 30 min can inactivate 100% of contaminating conidia. The urgent issue regarding the heterothallism of *A. fumigatus* as a tool for genetic study is to find the *MAT-1* and *MAT-2* mating pairs that can undergo sexual reproduction in a significantly shorter period than 6 months. We screened 50 *A. fumigatus* strains isolated either from clinical or environmental source and identified some pairs of strains that can produce viable ascospores in four weeks instead of six months. Significant variation in the degree of fertility was found among 50 strains but no association was found between fertility or duration required for cleistothecial formation and the source of isolation[13]. Viability of ascospores in these rapid maters was lower than 50% at 4 weeks but the per cent viability increased upon prolonged incubation. The *MAT-1* and *MAT-2* strains that completes sexual cycle in four weeks were as virulent as the strain B-5233 which has been extensively used in our laboratory for pathobiological study of *A. fumigatus*[15]. These findings indicated that the pairs of strains which we discovered to complete the sexual cycle can serve as an invaluable tool for genetic analysis in *A. fumigatus*. Our laboratory is currently focusing on the detailed characterization of these rapid maters.

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