S9-3

Comparative Study on Hypoxic Stress and CO_2 Sensing Associated with Development in *Aspergillus fumigatus* and *Aspergillus nidulans*

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Aspergillus fumigatus is known as the primary causative agent of aspergillosis, which is an opportunistic infectious fungal disease mainly localized in the respiratory system of human and animals. Although the patients suffered from the invasive aspergillosis as well as the allergic diseases are getting increased, molecular mechanism of *A. fumigatus* infection has not been well elucidated yet. The hypoxic condition is important to *A. fumigatus* because the environment of host cells is usually maintained as hypoxic condition. Currently, *A. fumigatus* has no known sexual development process, while a homothallic fungus *Aspergillus nidulans*, which is a very close relative of *A. fumigatus*, undergo complete sexual development process. Furthermore, in *A. nidulans*, hypoxic condition is one of the most important environmental factor for generating fruit bodies or cleistothecia. To study relationship between hypoxic stress condition and fungal development and virulence in a high-throughput manner, DNA microarray experiments were performed using *A. fumigatus* and *A. nidulans* 70-mer oligo microarray chips which were supported and provided by pathogenic fungi genome resource center (PFGRC). Total RNA samples were prepared in variable time points of the life cycle such as submerged culture (9 and 14 h), asexual induction condition (3, 6, 9, 12, and 24 h after transfer from submerged culture to solid media) and developmental induction after 24 h of hypoxic stress (0, 3, 6, 9, 12 h after hypoxic condition). The gradual hypoxic stress was simply induced by tight sealing of the culture plate with tape or parafilm. In *A. fumigatus*, although no sexual development was observed, developmental process was blocked and no conidia were produced during the 24 h of hypoxic or limited-oxygenic culture condition. Microarray experiment was performed using total RNAs extracted from the 12 samples. Double dye labeling method with cy3 and cy5 was used and mixture of the prepared 12 total RNAs was used as a common reference RNA which acts as control of all microarray experiments.

As a result, we identified 9,607 reliable genes from *A. fumigatus* 9,623 microarray spots (Fig. 1) using

minimum cut off value of 45.67 in 10 out of 12 microarray slides, implying that only 0.2% of microarray spots were not sufficient to be analyzed. For classification of stage-specific co-expressed genes, ANOVA test was applied using $P < 0.05$ parameter. As a result, 702 genes were selected as specific expressed genes (Fig. 2). The selected 702 genes were clustered with 5 groups by K-means analysis (Fig. 3). The K1 and K2 groups were divided by up- and down-regulated gene sets, respectively, in mycelial growth. K3 and K5 represented down- and up-regulated gene sets in aerial culture following hypoxic stress. Furthermore, comparison between *A. nidulans* gene set of induced sexual developmental condition will also provide valuable information of shared and distinct pathway of hypoxic stress response and sexual development process.

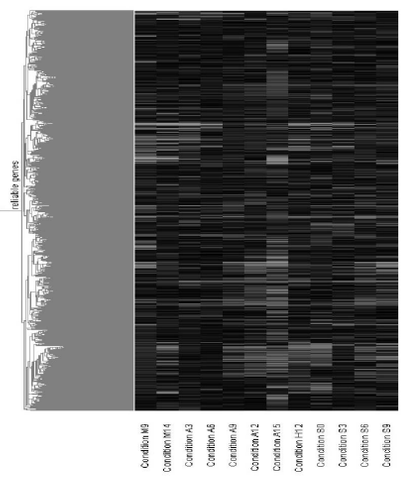

Fig. 1. Analysis of reliable genes of *A. fumigatus* microarray. Among 9623 total spotted genes, 9607 (99.8%) genes were included as reliable genes.

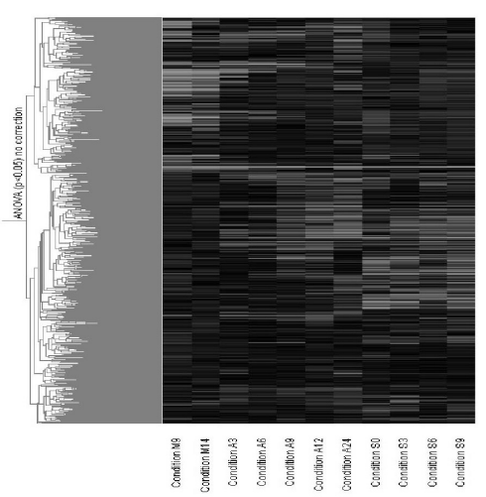

Fig. 2. ANOVA ($P<0.05$) test revealed that 708 genes were specifically expressed with stage specific manner in *A. fumigatus*.

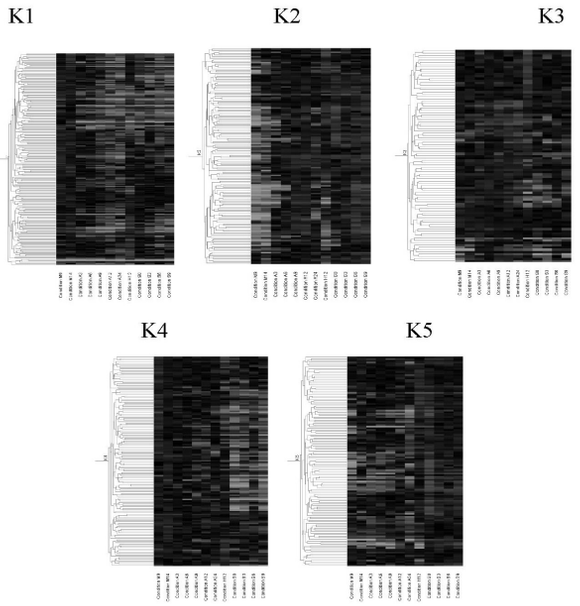

Fig. 3. Classification of K-means pattern. K5 group indicates hypoxic stress

By using the microarray analyses, we identified that the genes encoding carbonic anhydrase (CA), which mediate reversible interconvertion between gaseous CO_2 and bicarbonate ions (HCO_3^-) for maintaining cellular homeostasis, are up-regulated in hypoxic condition. Like vertebrates, carbon dioxide (CO_2) plays an important role in respiration of various microorganisms including fungi. Furthermore, differential CO_2 concentration can act as a signaling cue. Since mammals maintain high CO_2 concentration (~5%) compared to natural environment (~0.036%), animal fungal pathogens must adapt to high CO_2 levels for their infection.

CAs are ubiquitous and grouped into four general classes that are α-, β-, γ-, and δ-CA. Since *A. fumigatus* is a major fungal pathogen causing invasive aspergillosis in immunocompromised patients, adaptation of different CO_2 concentration, probably mediated by CAs, could be important. Genome sequence of *A. fumigatus* allowed us to identify three conserved β-CAs designated as *cafA* (CA in *A. fumigatus*), *cafB* and *cafC*, respectively (Fig. 4). Microarray and RT-PCR experiments demonstrated that all three CAs are expressed during the life cycle. Similarly, *A. nidulans* genome also contains three CA orthologs which are named as *canA* (CA in *A. nidulans*), *canB* and *canC*. However, *canC* may not be functional because a fungal transposon was integrated into the 3' end of *canB* locus. Interestingly, plate sealing caused up-regulation of *cafA* and *canA* expression, suggesting that the limited aeration leads to the shortage of CO_2 which promotes up-regulation of the CA. Characterization of these genes associated with the plate sealing or hypoxic stress will give an important clue of CO_2 sensing and developmental progresses in filamentous fungi. This work was supported by grant from KOSEF (R1-2006-000-11204-0) and KRF (KRF-2005-070-C00123).

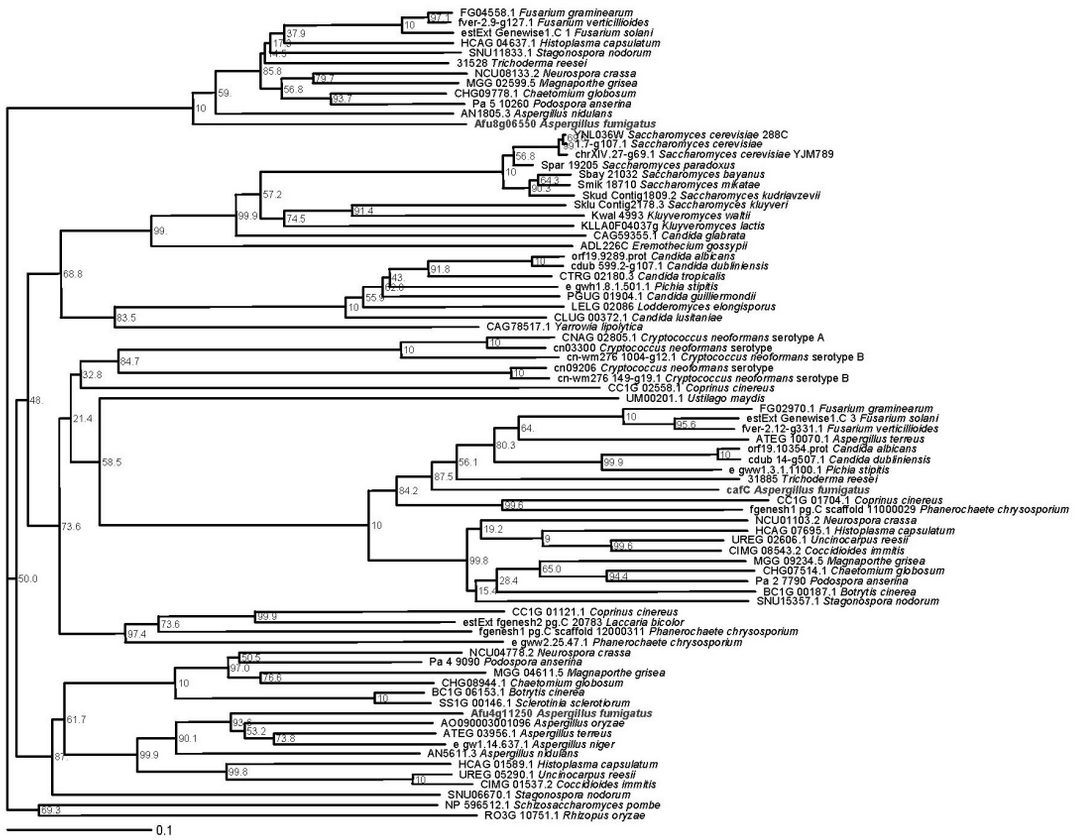

Fig. 4. Phylogenetic analysis of carbonic anhydrases in various fungi. Four clades were identified and *A. fumigatus* contains three carbonic anhydrases in the genome.