

Alternative respiration mechanism produced by soil humic acid in *Aspergillus nidulans*

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Purpose: Filamentous fungi respire by using two terminal oxidase. Cytochrome c oxidase (COX) is a ubiquitous respiratory oxidase in mitochondria, and alternative oxidase (AOX) is considered to be an assistant role for COX. This study investigates the fungal AOX-dependent respiration activity in the presence of humic acid (HA), which is a major organic matter in soil.

Methods: The fungus *Aspergillus nidulans* was cultured in the medium containing HA, and its physiological, biochemical, and genetic responses were analyzed.

Results: We found that adding HA to liquid culture increased AOX activity in *A. nidulans*. Sterile soil increased the fungal AOX activity, too. We also found that HA increased cellular superoxide dismutase and catalase activity, and decreased reactive oxygen species (ROS) level, which agrees to predicted HA's role in ROS reduction. Growth rate of the fungus was higher in the presence of HA. These results indicated that HA switches respiration mechanism from COX-dependent to AOX-dependent one, and diminishes ROS stress. Gene disruptants of *acuK* and *acuM* encoding transcription factors for acetate utilization produced little AOX activity and its transcript in the HA-exposed cells, indicating that they are required for transcription activation by HA. Adding HA partially inhibited a typical autolysis phenomenon observed in the late-stage of the culture, suggesting that HA contributes to maintain the cells in stationary growth phase. Fifteen filamentous fungal species produced more AOX activity in the presence of HA.

Conclusions: Filamentous fungi respond and adapt to HA, and probably to soil environment, by regulating their metabolic mechanisms.