

INVESTIGATION OF SPORE SURFACE PROTEIN ROLES IN THE OPPORTUNISTIC HUMAN PATHOGEN *MUCOR LUSITANICUS*

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Mucor lusitanicus, a member of the order Mucorales, an opportunistic human pathogen causing mucormycosis in immunocompromised individuals, is the subject of our investigation. Spore surface proteins named CotHs are considered a virulence factor for establishing an infection. To better understand the pathogenicity of *M. lusitanicus* this study aims to investigate the role of these CotH proteins. These proteins are involved in adhesion, host recognition, and evasion of host immune responses in various Mucorales species. In our study, 4 *cotH* mutants have been generated using CRISPR-Cas9 system, namely *cotH13*, *cotH15*, *cotH16*, and *cotH17*. The growth ability of the mutants under various conditions, including exposure to different stressors (Congo Red, Calcofluor White, KCl, NaCl, SDS), and cultivation at different temperatures (18 °C, 25 °C, 37 °C) was investigated. The germination and sporulation abilities of the mutants were examined using light microscopy. Viability subsequent to *cotH* gene disruption was assessed using the colorimetric XTT assay. The survivability of mutant spores was analyzed following interaction with J774.2 macrophages. Simultaneously, pathogenicity of the mutant strains was analyzed in an *in vivo Drosophila melanogaster* infection model. Our results show distinct roles for CotH13, CotH15, and CotH16 protein in growth and adaptation to varying temperature. the MS12+*pyrG*- Δ *cotH17* mutant exhibited heightened sensitivity to the osmotic stressor KCL compared to the MS12+*pyrG* control, while the MS12+*pyrG*- Δ *cotH13* strain demonstrated increased resistance to CFW and SDS. The CotH protein family appears to have a diverse role in the biological processes in Mucorales, particularly in spore wall development and maintenance. The research was supported by the projects NRD K131796, ELKH 2001007 and NRD TKP-2021-EGA-28 and SUPPORTED BY THE ÚNKP-23-4 -SZTE-649 New National Excellence Program of the Ministry for Innovation and Technology from the source of the national research, development, and innovation fund.