

---

## AN ANTIBODY-BASED SCREENING STRATEGY IDENTIFIES SIX *C. ALBICANS* GENES EXPRESSED DURING HUMAN CANDIDIASIS THAT CONTRIBUTE TO VIRULENCE

Clancy CJ, Cheng S, Nguyen MH  
University of Florida College of Medicine and the VA Medical Center, Gainesville, USA

Our lab is interested in identifying *C. albicans* genes expressed during human candidiasis as a screening strategy for novel virulence factors. Toward this end, we used sera from HIV-infected patients with oropharyngeal candidiasis (OPC) that had been adsorbed against candidal antigens *in vitro* to probe a *C. albicans* genomic expression library. We identified 59 genes encoding immunogenic antigens and confirmed expression within human OPC tissues by RT-PCR and/or immunohistochemistry. Forty-seven *in vivo* expressed genes have known or putative functions, including transcriptional regulation (16), nutrient metabolism (8), cytoskeleton/cell wall (7), stress response (7), transport (4), and miscellaneous (5). Seven genes encode known *C. albicans* virulence factors. To prioritize previously unstudied genes for further investigation, we focused on four groups: 1) candidate morphogenesis genes identified by putative function or differential expression; 2) genes encoding proteins homologous to well-established bacterial virulence factors; 3) genes not previously studied in *C. albicans* that contain recognized functional domains; and 4) genes with no homologues in *S. cerevisiae*. We used the *ura*-blaster method followed by reinsertion of *URA3* to its native locus to create disruption mutants of representative genes in each group. In group 1, *NOT5* and IPF8663 were required for normal morphogenesis in liquid hyphal-inducing media and under microaerophilic conditions of embedded growth, respectively. Both genes were necessary for morphogenesis on solid media, complete virulence during murine disseminated candidiasis (DC) and adherence to oral epithelial cells *in vitro*. *NOT5* made significant contributions to virulence during murine OPC, but could not be implicated during murine vaginal candidiasis or GI colonization and dissemination. Of further note, total immunoglobulin levels against purified Not5p among patients with OPC (10) and DC (11) were significantly higher than levels among healthy controls (11) (p-values < 0.05). In group 2, *LPD1* encodes a protein homologous to the *S. pneumoniae* and *M. tuberculosis* virulence factor dihydrolipoamide dehydrogenase. Disruption of *C. albicans LPD1* caused *in vitro* growth defects in YPD and other media. *LPD1* did not contribute to morphogenesis and was not required for adherence to oral epithelial cells. A null mutant strain caused no mortality during murine DC, likely due to a growth defect *in vivo*. In group 3, *BUR2* contains a cyclin domain at the N-terminus; cyclins are eukaryotic proteins that interact with cyclin-dependent kinases to control cell division and regulate transcription or cell cycle progression. *KEL1* contains a kelch repeat domain; kelch domains have a variety of functions in eukaryotes and prokaryotes, including actin cross-linking, galactose oxidation and localization to areas of polarized cell growth. Neither gene contributed to morphogenesis in liquid media or adherence to oral epithelial cells. *KEL1* was required for normal hyphal formation on solid media, whereas *BUR2* was not. Despite the lack of striking phenotypes *in vitro*, both genes made significant contributions to virulence during murine DC. In group 4, IPF15632 did not contribute to morphogenesis, but was required for normal flocculation and cell wall integrity. Furthermore, IPF15632 did not contribute to virulence during murine DC, but was required for complete virulence during murine OPC and adherence to oral epithelial cells. In conclusion, our screening strategy identified *C. albicans* genes of diverse functions that are expressed during human candidiasis. We demonstrated that six *in vivo* induced genes encode previously unrecognized virulence factors. Interestingly, some genes were associated with virulence at both deep tissue and selected mucosal sites. Other genes appear specific for virulence at mucosal sites, although mucosal-specific factors may not contribute at all mucosal sites. Finally, antibody responses against selected *in vivo* induced antigens might discriminate between patients suffering from invasive candidiasis and uninfected controls.