
IN VIVO PASSAGE OF CANDIDA ALBICANS RESULTS IN PHENOTYPIC VARIANTS WITH DIFFERING VIRULENCE POTENTIAL

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We previously reported on 5 patients who relapsed with candidemia at least 1 month after receiving apparently successful treatment. In each case, molecular typing and antifungal susceptibility testing confirmed that the original and recurrent strains were identical. Nevertheless, 4/5 patients died, leading us to hypothesize that the strains might have become more virulent with persistent exposure to the host environment. Indeed, serial passage of bacteria and viruses in animal hosts leads to adaptive responses that increase replicative capacity and virulence. To investigate this possibility in *C. albicans*, we infected ICR mice intravenously with 1×10^5 CFU of SC5314. Organisms (designated KidneyPassage1) were recovered from the kidneys of moribund mice by growing onto SDA plates with antibiotics, and inoculated into a new set of mice. The organisms were again recovered from the kidney (KidneyPassage2), and four additional passages were performed (KidneyPassages3-6). Similarly, organisms from the liver and spleen were recovered from the original infection with SC5314 and serially passed (LiverPassages1-6 and SpleenPassages1-6, respectively). Passage of *C. albicans* through the kidney and liver resulted in heightened virulence: mice infected with KidneyPassages4-6 and LiverPassages4-6 survived a median of 7 days, compared to 18 days for mice infected with SC5314, KidneyPassages1-3 and LiverPassages1-3 ($p < 0.003$). In contrast, mice infected with SpleenPassage2-6 showed no mortality at day 28, suggesting that passage attenuated virulence. These observations appeared to correlate with differences in colony morphologies. SC5314, KidneyPassages1-6, LiverPassages1-4 and SpleenPassages1-2 grew as homogeneous white, smooth colonies on SDA. LiverPassages4-6 and SpleenPassage3, on the other hand, existed as heterogeneous populations of the smooth, white colonies and wrinkled, yellow colonies. SpleenPassages4-6 grew solely as wrinkled, yellow colonies. The yellow colonies exhibited dense mats of hyphae on SDA, whereas the white colonies were exclusively yeasts. In hyphal-inducing liquid media, however, the white colonies formed normal hyphae, whereas the yellow colonies did not form hyphae; rather, they grew as bloated yeasts with short hyphal stems. In addition to being non-pathogenic in mice, the yellow colonies showed significantly longer doubling times than the white colonies, less adherence to epithelial cells, and less phagocytosis and killing by PMNs. Comparative genomic hybridization of strains using whole genome microarrays showed no significant differences, implying that the morphologic and virulence differences were not caused by gene rearrangements or duplications/deletions. The strains are currently being characterized further using additional *in vitro* assays and transcriptional profiling.