
WHAT MAKES CRYPTOCOCCOUS NEOFORMANS VIRULENT?

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Growth rate at 37°C, polysaccharide capsule, melanin production and extracellular phospholipase B (PLB) are putative virulence factors for *Cryptococcus neoformans*. Previous studies on virulence have assessed these factors independently, thus the relative importance of each factor in pathogenicity cannot be assessed. We evaluated these virulence factors for 18 cryptococcal isolates recovered from HIV-infected patients. The pathogenicity was determined in an ICR murine model of cryptococcal meningitis using LD₅₀ at 90 days and quantitative brain burden at 7 days. The isolates displayed a wide range of LD₅₀: LD₅₀ was <100 CFU for 22%, 100 to 1,000 CFU for 22%, 1,000 to 15,000 CFU for 17%, and >20,000 CFU for 39%. When mice were infected with equal inocula of each isolate, there was an inverse association between brain burden at 7 days and LD₅₀ (p=0.04). The isolates were compared for differences in polysaccharide capsule size, *in vitro* growth rates, melanin production, and extracellular PLB activity. Capsule size was measured *in vitro* after an overnight growth in enriched medium at 37°C as well as directly in homogenates of infected brains. Melanin production was assessed by assay of phenoloxidase activity, and extracellular PLB by egg yolk containing medium. All isolates possessed a capsule, and had phenoloxidase and extracellular PLB activities. Although the size of capsule was not significantly different between strains when measured *in vitro*, it was significantly different when measured *in vivo* in infected brain homogenates; the capsule size was always larger *in vivo* than *in vitro*. By univariate analyses, the size of capsule measured *in vivo* (p=0.0001), the *in vitro* growth rate (p=0.04) at 37°C, and the amount of PLB production (p=0.001) inversely correlated with LD₅₀ by univariate analyses. There was no correlation between the amount of phenoloxidase activity and LD₅₀. Multivariate analyses using factors proven significant by univariate analyses identified only capsule size (p=0.001) as an independent factor correlating with LD₅₀. In conclusion, our findings suggest that the presence of capsule, phenoloxidase and PLB activities are necessary for *C. neoformans* to infect humans. Within the host, *C. neoformans* might induce genes to trigger the enlargement of capsule size, which in turn will enhance its pathogenicity.