Determination of chitin content in fungal cell wall: an alternative flow cytometric method

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Abstract

The conventional methods used to evaluate chitin content in fungi, such as biochemical assessment of glucosamine release after acid hydrolysis or epifluorescence microscopy, are low throughput, laborious, time-consuming, and cannot evaluate a large number of cells. We developed a flow cytometric assay, efficient, and fast, based on Calcofluor White staining to measure chitin content in yeast cells. A staining index was defined, its value was directly related to chitin amount and taking into consideration the different levels of autofluorecence. Twenty-two *Candida* spp. and four *Cryptococcus neoformans* clinical isolates with distinct susceptibility profiles to caspofungin were evaluated. *Candida albicans* clinical isolate SC5314, and isogenic strains with deletions in chitin synthase 3 (*chs3Δ*/*chs3Δ*) and genes encoding predicted GlycosylPhosphatidylInositol (GPI)—anchored proteins (*pga31*Δ/Δ and *pga62*Δ/Δ), were used as controls. As expected, the wild-type strain displayed a significant higher chitin content ($P < 0.001$) than *chs3Δ/chs3Δ* and *pga31*Δ/Δ especially in the presence of caspofungin. *Ca. parapsilosis*, *Ca. tropicalis*, and *Ca. albicans* showed higher cell wall chitin content.

Although no relationship between chitin content and antifungal drug susceptibility phenotype was found, an association was established between the paradoxical growth effect in the presence of high caspofungin concentrations and the chitin content. This novel flow cytometry protocol revealed to be a simple and reliable assay to estimate cell wall chitin content of fungi.