1006 Hemolysis assay and hemolysin gene (HLP) expression in switched phenotypes of Candida glabrata

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Objectives: Phenotypic switching is believed to contribute to the virulence of Candida spp.. Recently it has been shown that phenotypic switching does occur in Candida glabrata (Cg), the second most frequently isolated Candida species. However, the relationship between phenotypic switching and virulence of Cg is unknown. Our recent works showed that production of hemolysis is an important virulence factor of Candida species (Luo et al, J Clin Microbiol, 2001). Hence, the aim of this study was to evaluate the differential hemolytic activity and HLP expression in "switched" strains of Cg.

Methods: Switched strains of Cg were obtained by using the method suggested by Lachke et al (Infect Immun, 2000) with minor modifications. The hemolysin production was evaluated using a modification of the plate assay described by Luo et al (vide supra), by spot-inoculating yeast suspension on a sugar enriched sheep blood agar medium. The expression of HLP gene was assessed using a standard RT-PCR procedure, with the housekeeping gene Act1 serving as an internal control (Zhao et al, Microb Pathol, 1998).

Results: After 7 days' incubation, four major phenotypes termed white (Wh), light brown (LB), dark brown (DB) and very dark brown (vDB) were obtained. Hemolytic assay showed that phenotypes vDB, DB and LB had significantly higher hemolytic activity than the Wh phenotype. When the levels of HLP transcript in the four switch phenotypes of Cg was assessed by RT-PCR analysis, the levels of transcript were lowest in Wh cells, intermediate in LB cells, and highest in DB and vDB cells, the relative ratios being 1:7:15:17, respectively.

Conclusions: These results indicate that the expression of HLP gene and hemolytic activity of C. glabrata are likely to be associated with the switched phenotypes of the yeast. This is the first report of a distinct correlation between the colony phenotype and a virulent attribute in Cg.

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