

Summary

Doctoral (Ph.D.) dissertation: Physiological and morphological characterization of *tert*-butylhydroperoxide tolerant *Candida albicans* mutants

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We tested the hypothesis that adaptation of *Candida albicans* to chronic oxidative stress, which occurs when this pathogen is exposed to immune system cells, would inhibit formation of hyphae and reduce pathogenicity. To reach this goal, *C. albicans* cells were exposed to increasing concentrations of *tert*-hydroperoxide (*t*BOOH), a lipid-peroxidation-accelerating agent, and mutant strains with heritable *t*BOOH-tolerance were isolated and characterized. The hypha-formation of the mutants was negligible on Spider agar clearly indicating that the development of oxidative stress tolerance hindered *Candida* cells to undergo dimorphic switches. One of the isolated mutants, *C. albicans* AF06, was five times less pathogenic in mice than its parental strain, due to its reduced germ tube, pseudohypha and hypha-forming capability and decreased phospholipase secretion. An increased oxidative stress tolerance may therefore be disadvantageous when this pathogen leaves blood vessels and invades deep organs. The AF06 mutant was characterized with high intracellular concentrations of endogenous oxidants, reduced mono- and polyunsaturated fatty acid contents, the continuous induction of the antioxidative defence system, a significantly decreased cytochrome C-dependent respiration and a significantly increased cyanide-resistant alternative respiration. The mutation did not influence cell size, cell surface, cellular ultrastructures including mitochondria and recognition by human polymorphonuclear leukocytes.

*t*BOOH tolerant *C. albicans* mutants were developed from clinical isolates, and all of them were characterized with an increased tolerance of the oxidative stress generating agents *t*BOOH and H₂O₂, continuous induction of the antioxidative defence system, reduced pseudohypha and hypha-forming capability, decreased phospholipase secretion and delayed growth in Sabouraud dextrose agar and broth media. Changes in antimycotic (fluconazole, voriconazole, amphotericin B, 5-fluorocytosine) tolerances as well as in total and cytochrome c-dependent respirations showed versatile patterns. The natural selection of oxidative stress tolerant mutants is unlikely and, not surprisingly, a screening study failed to detect any *C. albicans* strains with increased oxidative stress tolerance among 46 clinical isolates.

We hypothesize that the development and selection of oxidative stress tolerant respiratory *Candida* mutants may also occur *in vivo*, e.g. when a reduced respiration is beneficial for the fungus to cope with antimycotic agents.

Key words: *Candida albicans*, oxidative stress, *tert*-butylhydroperoxide