TITLE: ACTION OF FUNGAL ENZYMES ON ERGOSTEROL OF *CANDIDA ALBICANS* CELL WALL

AUTHORS: QUEIROZ-FENANDES, G.M; SORRECHIA, R.; PIETRO, R.C.L.R.

INSTITUTION: DEPARTAMENTO DE FÁRMACOS E MEDICAMENTOS, FACULDADE DE CIÊNCIAS FARMACÊUTICAS DE ARARAQUARA- UNESP (RODOVIA ARARAQUARA JAÚ, KM 01-S/N BAIRRO: CAMPOS VILLE 14800-903 - ARARAQUARA, SP, BRASIL)

ABSTRACT:

Studies have shown that molecules of the ergosterol biosynthesis pathway are important targets of various classes of antifungal agents used to treat *Candida albicans* infections. Polyenes such as amphotericin B act at the ergosterol level, binding tightly to this molecule. The aim of this study was investigate the action of fungal enzymes on ergosterol of *C. albicans* cell wall. A previous growth of *C. albicans* in YNB for 16 hours at 30 °C and 200 rpm was performed. After, 0.5 mL of this culture was added to 50 mL of YNB (OD<0.2) and incubated for 16 hours in the same conditions. Before the end of incubation, amylase and lipase fungal, produced respectively by *Aspergillus oryzae* (CCF-OIC3919) and *Aspergillus niger* (industrial strain), in submerged fermentation in a orbital shaker were added to the flasks and left in contact for 30 min (amylase and lipase). An untreated control was also performed. The cells were centrifuged at 3000 g for 5 min and the pellet submitted to the lysis process with solid-glass beads. After, 3 mL of 25% KOH alcohol solution was added, shaken vigorously for 1 min, and heated in a bath at 90°C for 1 hour. After cooling (room temperature) the ergosterol was removed by partition with 1 mL of water and 3 mL of hexane. The tubes were maintained in freezer at -20 °C for 72 hours and after, the upper portion, unfrozen, was submitted to a spectrophotometer reading between 200 and 300 nm. The presence of ergosterol was verified by the visualization of peaks corresponding to the sterols and the concentration was calculated based on initial weight of the cells and the absorbances. The tests were performed in triplicates. The evaluation showed spectrophotometric peaks characteristic of sterols and when cells were treated with amylase there was a decrease in concentrations of ergosterol (0.121 mg) whereas cells treated with lipase showed an increase in concentration (0.176 mg) as compared to untreated cells (0.150 mg). It can be suggested that amylase may have affinities for molecules involved in ergosterol biosynthesis while lipase can have promoted the release of ergosterol from the *C. albicans* cell wall, because this enzyme acts on ergosterol anchorage lipids in the plasma membrane, leading to higher concentrations.

Keywords: ergosterol, amylase, lipase, fungi, *Candida albicans*.
Support: Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) e PADC-UNESP.