

TITLE: PHENOTYPIC SWITCHING IS ASSOCIATED WITH CHANGES IN ULTRASTRUCTURAL ORGANIZATION AND EXTRACELLULAR MATRIX COMPOSITION OF *C. tropicalis* COLONIES.

AUTHORS: SOUZA, C.M¹.; SANTOS, M.M¹.; MORALES, A.T.¹.; PERINI, H.F¹.; PAULO, E. A¹.; SOUZA, N.A.A¹.; FURLANETO-MAIA, L².; FURLANETO, M.C¹.

INSTITUTION: ¹UNIVERSIDADE ESTADUAL DE LONDRINA - UEL (RODOVIA CELSO GARCIA CID, KM 380, S/N - CAMPUS UNIVERSITÁRIO, LONDRINA - PR, CEP: 86057-970). ²UNIVERSIDADE TECNOLÓGICA FEDERAL DO PARANÁ - UTFPR (ESTR. DOS PIONEIROS, 3131 - CENTRO, LONDRINA - PR, CEP: 86020-430).

ABSTRACT:

Phenotypic switching (*PS*) is an event characterized by the occurrence of colonies with altered morphology. A switching system of *Candida tropicalis* denoted 49.07, that comprises smooth colony pattern (clinical isolate 49.07 - parental morphotype) and structured colony pattern (variants morphotypes - crepe and rough) is associated with different virulence traits. The aims of this research were to evaluate the morphogenesis (filamentation) among colonies' cells from parental and variants morphotypes, evaluate the carbohydrate (Carb) and total protein (proT) content to the matrix of these colonies, and expression of genes related to filamentation (*EFG1* and *WOR1*) and matrix production (*BCR1*). The matrix Carb was estimated using glucose as a standard. ProT was measured by BCA kit (Bicinchoninic Acid, Sigma-Aldrich, St Louis, USA), standard by bovine serum albumin (BSA). The matrix compounds were relative to the dry weight of colonies. Filamentation was estimated by counting 1000 cells, at three times of colony development (48, 72 and 96 hours). All tests were repeated thrice in independent assays. For qPCR, Platinum® SYBR® Green qPCR Supermix-UDG (Invitrogen, Carlsbad, CA, USA) was used, and normalized by equation $2^{-(\Delta-\Delta Ct)}$. The extracellular matrix was observed in all colony's patterns, however, the Carb and proT matrix contents to the parental (isolate 49.07) (Carb 13.75 ± 11.05 mg^{carbohydrate/g^{colony}}; proT $1, 1 \pm 0.26$ mg^{protein/g^{colony}}) were significantly lower than observed by rough variant (Carb 33.74 ± 12.47 mg^{carbohydrate/g^{colony}}; proT 3.47 ± 1.42 mg^{protein/g^{colony}}) ($p < 0.05$). qPCR showed that *BCR1* levels were higher in the rough colonies (4.6 ± 0.51) compared to parental colonies (1 ± 0) ($p < 0.05$). In addition, Carb production was correlated with *BCR1* expression (Pearson Correlation $r^2 = 0.9885$, $p = 0.0483$). Hyphae percentage among colonies' cells was maintained over different incubation times. The morphotypes with structured pattern, rough ($7.12 \pm 0.91\%$) and crepe ($3.57 \pm 0.51\%$), showed higher hyphae percentage than parental morphotype ($0.64 \pm 0.27\%$) ($p < 0.05$). Colony filamentation was associated with up-regulation of *EFG1* and *WOR1* genes ($r^2 = 0.7708$, $p < 0.05$). This study demonstrated that switching alters composition of matrix of *Ct* switched colonies, with filamentation playing a structural role on these colonies. Furthermore, filamentation was associated with expression of *EFG1* and *WOR1* genes in structured morphotypes.

Keywords: *Candida tropicalis*, colony structure, phenotypic switching.

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