

Spermine Modulate *Yarrowia lipolytica* Morphogenesis by an Auxin-Dependent Pathway and P-type H⁺-ATPase Activation

Cogo, A.J.D.^{1,2}, Dutra, K.R.¹, Almeida Jr, P.S.G.³, Silva, M.G.³, Okorokov, L.A.¹, Façanha, A.R.², Okorokova-Façanha, A.L.¹

¹Laboratório de Fisiologia e Bioquímica de Microrganismos e ²Laboratório de Biologia Celular e Tecidual - Centro de Biociências e Biotecnologia; ³Laboratório de Ciências Físicas - Centro de Ciência e Tecnologia, Universidade Estadual do Norte Fluminense Darcy Ribeiro, Av. Alberto Lamago, 2000, Pq. Califórnia, CEP:28013-602. Campos dos Goytacazes-RJ

Introduction and Objectives: The mechanisms underlying the morphogenesis in fungi by polyamines remains unclear. Here, we investigate whether the hyphal growth of *Yarrowia lipolytica* modulated by spermine (Spm) occurs through activation of P-type plasma membrane H⁺-ATPase and the recruitment of auxin-dependent pathways. **Material and Methods:** A role for Spm and auxin in hyphal induction was investigated in culturing cells with or without Spm, polyamines biosynthesis inhibitor (CHA), indole-3-acetic acid (IAA), auxin transport inhibitor (TIBA) and auxin signaling inhibitor (PCIB). The H⁺ pumping of P-type H⁺-ATPase was analyzed in vitro fluorometrically and in vivo by scanning ion-selective electrode technique. **Results and Discussion:** Spm and IAA increased significantly the number of hyphal cells, whereas the growth with the inhibitors prevented morphogenetic transition by 80-90%. Growth with Spm resulted in wrinkled colonies surrounded by longer hyphal fringes, whereas the presence of the inhibitors produced less convoluted colonies with short fringes. The addition of IAA reversed the inhibitory effect of CHA, whereas the effects of TIBA and PCIB were not reversed by Spm. At yeast-hypha transition point (18h of growth), the P-type H⁺-ATPase increased its activity, and at 20h the enzyme obtained the greatest stimulation by Spm for H⁺ pumping (~2.3-fold), V_o (~2.0-fold), ATP hydrolysis (~1.5-fold) and Pma1p expression (~1.5-fold) when compared to control. The stimulatory effect of Spm on H⁺-pumping and ATPase activity was significantly abolished by TIBA and PCIB. Spm and IAA markedly enhanced extracellular alkalization by *Y. lipolytica* cells, whereas TIBA, PCIB and vanadate inhibited it. This alkalization was followed by increased release of ammonia in culture medium. **Conclusions:** The data provide a new mechanistic insight on an integrative role of polyamines and auxins as key signaling molecules of the yeast-to-hypha transition and mycelial morphogenesis through an 'Acid Growth Mechanism', quite similar to that previously described in plants.

Acknowledgements: CNPq, FAPERJ, UENF.

Key words: polyamines, H⁺-ATPases, morphogenetic transition.