

Title: *Candida* spp. extracellular alcohols: investigation of their production and role in sessile cells

Topic: Fungal Biofilms

Authors: Margarida Martins¹, Mariana Henriques¹, Joana Azeredo¹, Sílvia M. Rocha², Manuel A. Coimbra², Rosário Oliveira¹

Affiliations: ¹Institute for Biotechnology and Bioengineering, Centre of Biological Engineering, Universidade do Minho, Campus de Gualtar, 4710-057 Braga, Portugal

²Departamento de Química, Universidade de Aveiro, 3810-193 Aveiro, Portugal

Cell-cell signaling alcohol molecules (farnesol, nerolidol, 1-dodecanol, 2-phenylethanol and isoamyl alcohol) were recently identified in *Candida albicans* and *Candida dubliniensis* supernatants. To date, it was not known whether these molecules are produced by other *Candida* species and their role in biofilm formation is still not fully clarified.

AIMS: The aims of this study were to evaluate (1) the extracellular alcohols composition of *Candida parapsilosis* and *Candida tropicalis* supernatants and (2) the role of *Candida* excreted alcohols in *C. albicans*, *C. dubliniensis*, *C. parapsilosis* and *C. tropicalis* biofilm development.

METHODS: *C. parapsilosis* and *C. tropicalis* extracellular alcohols production by planktonic cultures was analysed by headspace-solid-phase microextraction and gas chromatography-mass spectrometry. The ability of these compounds to regulate *Candida* species biofilm formation was assessed by adding the alcohols (as pure

compounds) after 3 h of adhesion and on 48-h biofilms. After 24 h of contact, biofilms were analysed in terms of mitochondrial activity, by the reduction of a tetrazolium salt (XTT), and total biomass, by crystal violet staining.

RESULTS: The analyses of culture supernatants showed that *C. parapsilosis* and *C. tropicalis* extracellular media contained *E,E*-farnesol, 1-dodecanol, 2-phenylethanol and isoamyl alcohol but not *E*-nerolidol.

The effect of these compounds was variable across the different fungal biofilms. *E,E*-Farnesol affected *C. albicans* and *C. dubliniensis*. *E*-Nerolidol and 2-phenylethanol elicited *C. parapsilosis* and *C. tropicalis* changes. One-Dodecanol effects were detected for *C. parapsilosis* and *C. dubliniensis*. Isoamyl alcohol triggered changes in sessile cells of *C. albicans*, *C. dubliniensis* and *C. tropicalis*.

CONCLUSIONS: Due to the important repercussions of *Candida* biofilms, it is of utmost importance to understand factors that affect their formation. Physiological and high levels of these alcohols play a role in biofilm formation. The effects are *Candida* species' and biofilm development stage dependent, with different involvements in biofilm biomass and mitochondrial cellular activity.