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TITLE: Insights into *Candida* world: extracellular alcohols

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ABSTRACT

Farnesol has received considerable attention as a *Candida albicans* quorum sensing molecule (QSM). This work aimed to unravel potential *Candida* cell-cell signalling molecules and to get insights into their role in *Candida* spp biology.

First, the role of farnesol (5 to 150 µM) was evaluated concerning the morphology and growth of *C. dubliniensis*, *C. parapsilosis*, and *C. tropicalis* planktonic cultures. Farnesol inhibited *C. dubliniensis* morphological transition from yeast to pseudohyphae, without interfering with growth. It did not induce *C. parapsilosis* and *C. tropicalis* morphological changes but at levels ≥ 50 µM increased cell lag growth phase. In contrast to *C. parapsilosis,* farnesol induced changes in *C. topicalis* viability and cell cycle. This evidences that QSM may have non-signalling roles.

Second, *Candida* spp extracellular alcohols production in 24 h RPMI planktonic cultures were analysed by headspace-solid-phase microextraction and gas chromatography-mass spectrometry. *Candida* spp supernatants contained *E*,*E*-farnesol, 1-dodecanol, 2-phenylethanol and isoamylalcohol. *E*-nerolidol was only produced by *C. albicans* and *C. dubliniensis*. This suggests that other alcohols besides farnesol are produced by *Candida* spp.

Third, the ability of these compounds to regulate *Candida* spp biofilm formation was assessed by adding the alcohols to 3-h adhered cells and 48-h biofilms. After 24 h of contact, biofilm mitochondrial activity and biomass were evaluated. Farnesol affected *C. albicans* and *C. dubliniensis* biofilms. Nerolidol and dodecanol elicited changes in *C. parapsilosis* and *C. tropicalis* biofilm development. Phenylethanol altered *C. tropicalis*

biofilm formation. Isoamylalcohol triggered changes in *C. albicans*, *C. dubliniensis*, and *C. tropicalis* biofilms. This indicates that those alcohols modulate biofilm formation. Fourth, the exogenous administration of a cocktail mimicking *C. albicans* secreted alcohols was evaluated *in vivo*. The cocktail solution delayed mice time of death. On day 3 animals that received the cocktail solution displayed lower renal and brain fungal burden compared to control mice receiving the vehicle solution alone. This suggests that these alcohols play a role during *C. albicans* pathogenesis.

Overall, these results show that cell-cell signalling molecules produced by *Candida* species regulate their physiology and virulence traits.