Effect of *Candida albicans* and *Candida dubliniensis* planktonic/biofilm quorum sensing molecules on yeast morphogenesis

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ABSTRACT

One of the aims of this work was to study the effect of farnesol, a quorum sensing molecule for *Candida albicans*, on morphologic inhibition of *Candida dubliniensis*. The second goal of this work was to confirm if *Candida dubliniensis* also excreted quorum sensing molecules, on both planktonic and biofilm forms.
The results clearly demonstrate that *Candida dubliniensis* undergoes morphological alterations triggered by farnesol. It was also found that supernatants of *Candida dubliniensis* and *Candida albicans* grown in both planktonic or biofilm forms contain molecules that are capable of suppressing pseudohyphae formation on *Candida dubliniensis* cells grown in RPMI 1640.
It can be concluded that both *Candida dubliniensis* and *Candida albicans* produces quorum sensing molecules either in planktonic or biofilm forms, which regulates *Candida dubliniensis* morphology.

KEYWORDS: quorum sensing molecules, *Candida dubliniensis*, *Candida albicans*, biofilms

RUNNING TITLE: Quorum sensing phenomena in *Candida* spp.

INTRODUCTION

Candida dubliniensis has been recovered primarily from the oral cavity of immunosupressed patients (Sullivan *et al.* 1995) and is an opportunistic yeast, responsible for severe Candidiasis. One of its phenotypic characteristics is the capacity to switch from yeast to hyphal morphology. This behaviour is remarkably similar to *Candida albicans* and induced the mismatch of these two species for years (Sullivan *et al.* 2004). The capacity to undergo a morphologic switch has been related with virulence (Calderone and Fonzi, 2001) and several factors are responsible for it. Among these are quorum sensing molecules (QSM), which are cell-cell signalling molecules and are responsible for community genetic regulation mechanisms, controlling microbiological functions. These molecules are metabolites released by planktonic and biofilm cells. Bacterial quorum sensing has been largely studied, but concerning yeasts, namely, *Candida* species, little is known. Regarding *Candida albicans*, only few studies have been done in this field mostly reporting the effect of farnesol (Ramage *et al.*, 2002; Hornby *et al.*, 2001) or tyrosol (Chen *et al.*, 2004) as quorum sensing molecules, able to modulate the morphogenesis.
So, the main aim of this work was to confirm if QSM are released by *Candida dubliniensis* and the effect of these molecules, namely in the yeast morphology.

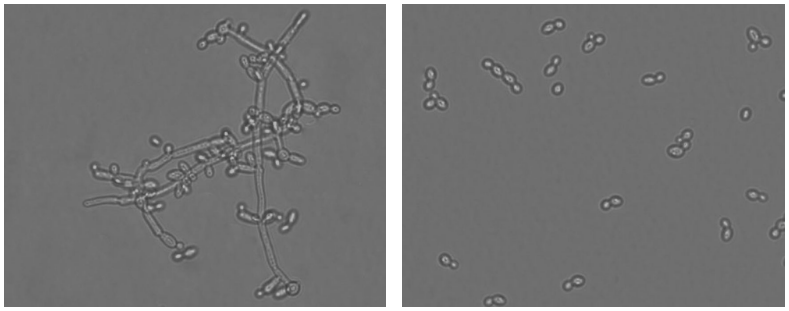
METHODS

One strain of each *Candida* species was used: *Candida dubliniensis* 7987 (CBS) and *Candida albicans* 1472 (CECT). Before each assay cells were grown in Sabouraud Dextrose broth at 37ºC, for 18h and 130 rpm.
In order to evaluate the effect of farnesol on *Candida dubliniensis* morphology, 1×10^6 cells/ml were grown 12h at 37ºC and 130 rpm in RPMI 1640 and in RPMI 1640 supplemented with 300 µM E,E-trans-farnesol (Sigma). Stationary cells were observed under contrast phase microscopy. Morphological evaluation was performed classifying and enumerating yeast forms and pseudohyphae. The percentage of inhibition of pseudohyphae formation was determined according to Chiller *et al.* (2000).

The effect of QSM of both planktonic and biofilm *Candida dubliniensis* and *Candida albicans* cells was assessed on planktonic *Candida dubliniensis* cellular morphology. For that, planktonic and sessile growth of both *Candida* species was performed as described by Ramage *et al.* (2002). Briefly, after 24h of growth supernatants from planktonic or biofilm cells were recovered and diluted 1:1 with 2× RPMI 1640. In order to standardize the assays the dry weight of cells grown in biofilm and planktonic forms was determined. A 1×10^6 cells/ml suspension was prepared in supernatant medium and *Candida dubliniensis* cells were grown for 12h, at 37ºC and 130 rpm. The evaluation of cell morphology and percentage of inhibition were carried out as described above.
All experiments were carried out in duplicate in three independent assays. In microscopic analysis 15 fields of each sample were observed.

RESULTS AND DISCUSSION

Once farnesol is one of *Candida albicans* quorum sensing molecules that affect the formation of hyphae (Hornby *et al.*, 2001), the first aim of this work was to determine if farnesol had a similar effect on *Candida dubliniensis* cells and in what extent. *Candida dubliniensis* cells were observed under contrast phase microscopy (Figure 1) after growing in the absence and presence of farnesol 300 µM.



(a) (b)

Figure 1 – Contrast phase images of *Candida dubliniensis* grown in RPMI 1640 (a) and in RPMI 1640 supplemented with 300 µM of farnesol (b).

The effect of farnesol on the suppression of pseudohyphae formation was notorious (Figure 1), since only yeast forms were observed after growth in medium supplemented with farnesol. Moreover, this dose of farnesol (300 µM) induced 100% of inhibition of pseudohyphae formation. The results obtained for *Candida dubliniensis* are similar to the reported in literature for *Candida albicans* (Ramage *et al.*, 2002; Hornby *et al.*, 2001). In fact, the present results show that in defined medium farnesol is involved in the morphological switch of *Candida dubliniensis*

Farnesol is among the QSM that were identified in *Candida albicans* planktonic and biofilms supernatants. *Candida albicans* biofilms supernatants were proved to affect its morphology (Ramage *et al.*, 2002). Thus, excreted metabolites from planktonic and biofilm cultured cells of *Candida albicans* and *Candida dubliniensis* were used to grow *Candida dubliniensis* planktonic cells. The effect of supernatants on cellular morphology was quantified and is presented in Figure 2.

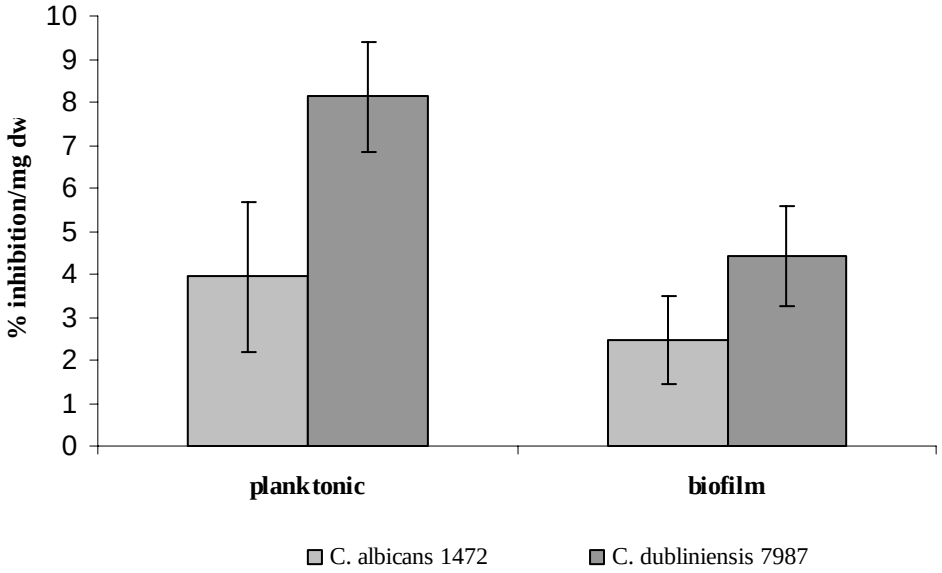


Figure 2 – Values of the percentage of inhibition of *Candida dubliniensis* pseudohyphae formation treated with 24h supernatants of *Candida dubliniensis* and of *Candida albicans* planktonic or biofilm cells per mg of dry weight (dw).

In order to standardize the values obtained from cells grown in suspension or sessile form the percentage of inhibition was expressed as a function of cell dry weight.
The first main observation is that supernatants, not only from *Candida albicans* but also from *Candida dubliniensis* contain molecule(s) that affect *Candida dubliniensis* morphology (Figure 2)

and the effect on morphology is identical to the one observed with farnesol. However, the values of the percentage of inhibition cannot be directly compared because they are expressed per dry weight. In the same way, these values cannot be compared with the values found by other authors (Ramage *et al.,* 2002) for *Candida albicans*.

As illustrated in Figure 2, metabolites secreted by planktonic cells, of both *Candida* species, seem to affect in a higher extent the morphology of *Candida dubliniensis* than by biofilm ones. Moreover, when evaluating the results obtained with supernatants from the two species, it can be observed that molecules present in *Candida dubliniensis* supernatant have a higher average effect on their own cells than supernatants recovered from *Candida albicans*.

CONCLUSIONS

Results presented herein show for the first time that *Candida dubliniensis* morphology is regulated by farnesol, with 100% of inhibition for a 300 µM farnesol dose. It can also be pointed out that these metabolites excreted by planktonic and biofilm *Candida* cells not only affect its own species, but also have cross reactivity inter species. In fact this could be an important fact for the control of mixed biofilms through QSM.

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