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The impact of Mig1 and Orf19.173 genes on *Candida albicans* cell wall biosynthesis in different growth conditions

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ABSTRACT. Transcription factors are proteins encoded in an organism's genome that regulate gene expression and control various biological activities. In fungi, many of these transcription factors are proteins with unidentified functions until now, particularly those that regulate genes associated with *Candida albicans* cell wall biosynthesis. This process is vital for fungal bioactivity and sensitivity to some antifungal agents. This study investigates Mig1 and Orf19.173 transcription factors in response to caspofungin in different environmental conditions as an initial step toward understanding their roles in cell wall biogenesis and drug resistance. A set of *C. albicans* mutant strains were exposed to caspofungin in Yeast Extract-Peptone-Dextrose (YPD) medium at 25°C and in RPMI 1640 medium (supplemented with 10% serum) at 37°C to simulate host conditions. Transmission electron microscopy (TEM) was used to analyze structural changes in the cell wall of *Candida albicans* isolates. The study found that mutant strains (mig1Δ/Δ) and (orf19.173Δ/Δ) were susceptible to Caspofungin in RPMI 1640 medium at 37°C but not on YPD medium at 25°C. Additionally, TEM images revealed that the cell wall of these mutant strains exhibited a noticeable thickness and numerous protrusions when exposed to caspofungin. Based on these findings, it was concluded that the transcription factors Mig1 and Orf19.173 promote cell wall biosynthesis in *C. albicans* when exposed to caspofungin in the host-like environment.

Keywords: Transcription factors; Mutant *Candida albicans;* Mig1 gene; Orf19.173 gene; Caspofungin; Drug resistance.

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Introduction

In recent times, *Candida albicans* has emerged as the most prevalent fungal pathogen capable of causing a wide range of infections in animals and humans (Sardi, Scorzoni, Bernardi, Fusco-Almeida, & Mendes Giannini, 2013; Yapar, 2014); it is also deemed a suitable model for exploring fungal biological activity (Legrand, Jaitly, Feri, d'Enfert, & Sanyal, 2019). The fungal cell wall plays a critical role in supporting fungal survival across diverse environments and facilitating essential biological functions (Durán and Nombela, 2004; Gow and Hube, 2012); it is composed of β-1,3-glucan, along with significant quantities of β-1,6-glucan, chitin, and proteins (Lesage and Bussey, 2006). Moreover, the fungal cell wall is a primary target for antifungal agents, including the Echinocandin group, with caspofungin being particularly noteworthy among them.

Caspofungin is a fungicidal drug that impedes fungal cell wall synthesis by obstructing β-D-glucan synthesis. It is the preferred drug for treating many systemic fungal infections (Hernandez et al., 2004; Denning, 2002). Several studies identify a link between caspofungin sensitivity in the fungal cell wall integrity and the mitogen-activated protein kinase (MAPK) pathway (Reinoso-Martín, Schüller, Schuetzer-Muehlbauer, & Kuchler, 2003; Bruno et al.,2006). Its unique features include the induction of numerous secretory genes in the fungus (Bruno et al.,2006; Rauceo et al., 2008), which makes this antifungal agent a good choice for this study.

Transcription factors are critical in regulating gene expression in all living organisms, controlling the genes involved in biological processes, and are part of signalling pathways. Usually, organisms with larger genomes tend to have more transcription factors per gene (van Nimwegen, 2003). In eukaryotes, many of these transcription factors are not DNA-binding portions but instead form part of the preinitiation complex that binds to promoter regions of the gene that they regulate (Thomas and Chiang, 2006). The biosynthesis of the cell wall is related to a net of gene activity represented mainly by the MAPK signalling cascade is known as the protein kinase C (PKC) cell wall integrity pathway (Lesage and Bussey, 2006;

Correia et al., 2019), this process is connected to fungal pathogenicity and regulated by a group of transcription factors (Zhao, Mehrabi, & Xu, 2007).

Candida albicans possess approximately 240 transcription factors belonging to the Zinc Cluster Family (Zn(II)2Cys6 DNA binding proteins), with many of them having unclear functions (Issi et al., 2017); some members of this group have been shown to play a role in the fungal pathogenicity (MacPherson, Larochelle, & Turcotte, 2006; Fukada and Kambe, 2011). However, the specific functions of several members are still unknown (Issi et al., 2017). This study focuses on the role of Mig1 and Orf19.173 transcription factors in *C albicans* cell wall biosynthesis under stress, targeting the cell wall with caspofungin.

Material and methods

This study used a set of mutant strains of *C. albicans*; strains (orf19.173Δ/Δ and mig1Δ/Δ) were used to test the study hypothesis, while *C. albicans* mutant strain (cas5Δ/Δ) was used as a sensitive strain to caspofungin (negative control strain), and *C. albicans* (DAY286) as a wild-type strain. The subinhibitory concentration of caspofungin was spotted by the gel diffusion method on Mueller-Hinton agar using a MIC strip kit from (Liofilchem, Inc. USA). The study strains were grown for 24 hours in two different conditions: on Yeast Extract-Peptone-Dextrose (YPD) medium with and without caspofungin at 25°C and on RPMI1640 medium (supplemented with 10% serum) at 37°C with and without caspofungin, to be a host environment-mimicking médium (Siller et al., 2008).

A stock yeast suspension equal to OD600=0.1 was prepared from overnight-grown strains on YPD broth. A two-fold serial dilution of the stock suspension was performed, and all dilutions were cultured under the two growing conditions mentioned earlier to investigate the morphological alterations in the cell walls of the study strains. The TEM technique (ZEISS EM technologies, Germany) was followed to investigate the morphological changes in the cell wall caused by exposure to caspofungin in different environmental growth conditions.

Results

The data showed that the caspofungin's minimum inhibitory concentration for the wild-type *C. albicans* was 125 ng mL^{-1} (Figure 1), and this is aligned with previously available data about the sensitivity of *C. albicans* to caspofungin (Pfaller et al., 2006; Yang et al., 2017), and this concentration was later used to induce cell wall damage in the study strains (Pfaller et al., 2003; Hernandez et al., 2004; Pfaller et al., 2011; Yang et al., 2017)

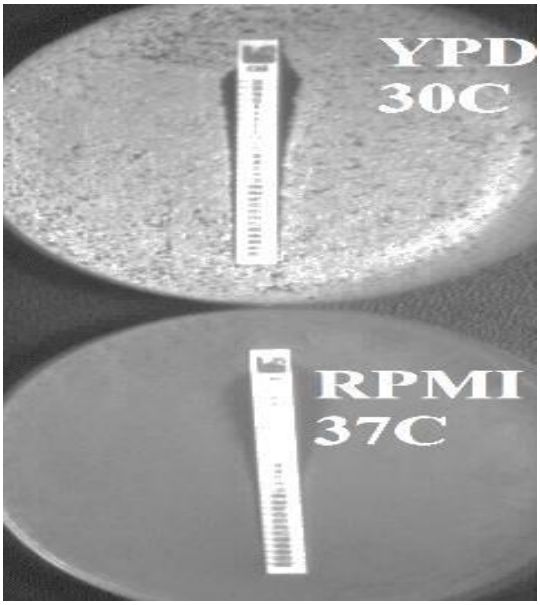

Figure 1. The MIC of caspofungin for the wild-type *Candida albicans* (DAY286) in YPD at 30°C and RPMI1640 at 37°C.

Growing of study mutant strains in the different growing conditions showed that (mig1Δ/Δ and orf19.173Δ/Δ) in addition to the cas5Δ/Δ, are sensitive to caspofungin on RPMI1640 medium with caspofungin (Figure 2).

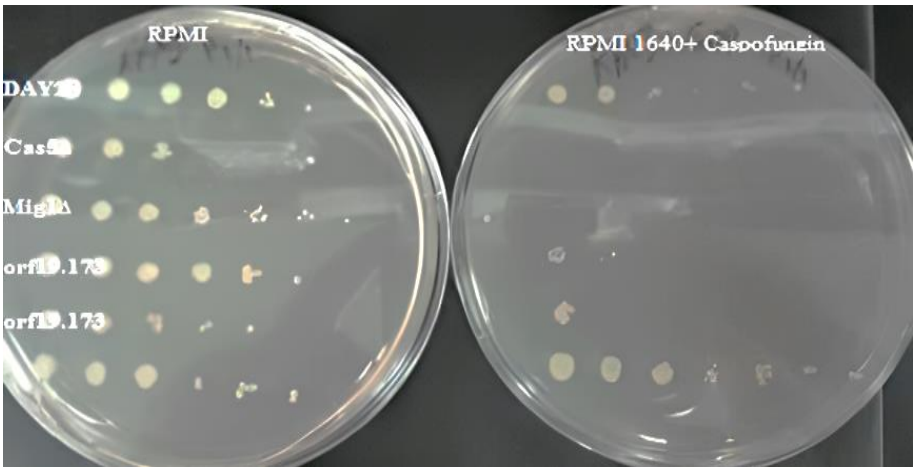

Figure 2. *Candida albicans* mig1Δ/Δ and orf19.123Δ/Δ strains on RPMI1640 with and without caspofungin showing the sensitivity of these strains to caspofungin, DAY286 strain as wild-type controls and Cas5Δ/Δ as susceptible control strain.

Figure 3 displays Transmission Electron Microscope (TEM) images of the (mig1Δ/Δ and orf19.173Δ/Δ) mutant strains that were grown overnight on a medium containing a subinhibitory concentration of caspofungin; this image indicates that the mutant strains had displayed specific characteristics not observed in the wild-type strain (DAY286) grown under similar conditions. In particular, the mutant strains had a thicker cell wall layer containing glucan and chitin and an external fibrillar structure extending from the cells.

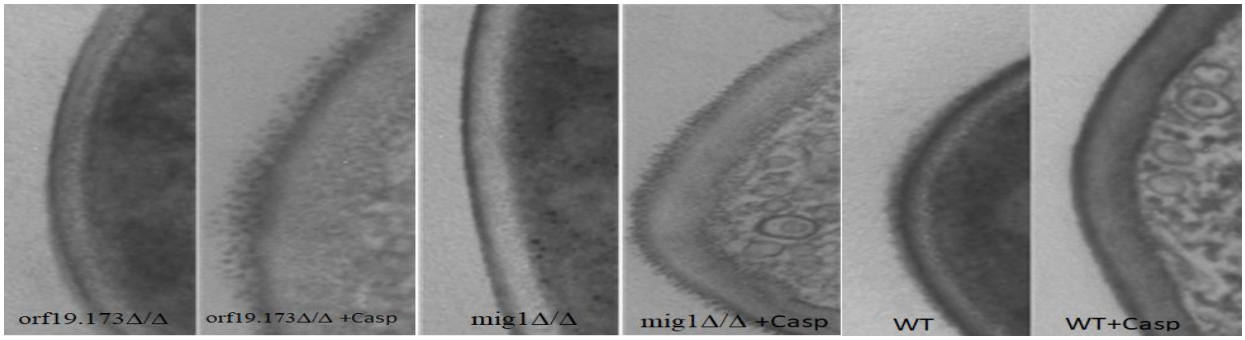

Figure 3. TEM image for mig1Δ/Δ and orf19.173Δ/Δ strains exposed to caspofungin showing an apparent thickening in the cell wall compared to the type wild-type (DAY286) strain

Discussion

According to the results of this study, the (mig1Δ/Δ and orf19.173Δ/Δ) mutant strains of *C. albicans* are susceptible to caspofungin in host environmental conditions, which indicates that these genes play a part in cell wall biosynthesis. Previous studies have demonstrated that the (mig1) gene is involved in the regulation of carbon source metabolism and transport, as well as glucose repression and plays a role in the expression of several pathogenicity genes (Zaragoza, Rodríguez, & Gancedo, 2000; Shashkova, Welkenhuysen, & Hohmann, 2015; Nurcholis, Murata, Limtong, Kosaka, & Yamada, 2019; Lagree et al., 2020), as well as negatively regulating cell wall synthesis (Rippert, 2016). There is no direct reference to the role of (orf19.173) in *C. albicans*; evidence shows that this gene maintains the mitochondrial genome in *S. cerevisiae*, but its role in *C albicans* remains ambiguous (Uppuluri and Chaffin, 2007).

The cas5Δ/Δ mutant strain is expected to be sensitive to caspofungin due to the role of the cas5 transcription factor in mediating the cell wall stress signalling caused by caspofungin and leads to increased susceptibility to various cell wall and plasma membrane stresses and interferes with pathogenicity in vivo (Bruno et al*.,* 2006; Xie et al*.,* 2017).

TEM images have shown that the cell wall of *C. albicans* overgrows and develops protrusions when exposed to caspofungin, resulting in a significant increase in mannan content (Walker and Munro, 2020). This study suggests that certain transcription factors in *C. albicans*, including mig1, orf17164, and cas5, play a critical role in response to caspofungin, depending on the growth environment and the biosynthesis of *C albicans* cell wall is affected by these factors, and it is affected by caspofungin when growing at 37°C, but not at 30°C.

Conclusion

This study provides evidence of the roles of transcription factors mig1, orf19.173, and cas5 in Candida albicans' response to caspofungin, particularly in cell wall biosynthesis. The findings suggest these factors regulate cell wall integrity and stress response, especially at 37°C. The differential reaction at various temperatures and the cell wall overgrowth upon caspofungin exposure highlights the complexity of cell wall regulation in C. albicans.

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