Short Communication

Pseudohyphae formation in *Candida glabrata* due to CO_2 exposure

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Abstract

Background and Purpose: Formation of pseudohyphae is considered a virulence factor in *Candida* species. Generally, *Candida glabrata* grows as budding yeast cells; however, reports illustrated that *C. glabrata* could form pseudohyphal cells in response to some stimuli. In this study, we provided insight into the ability of *C. glabrata* in forming pseudohyphal cells under different levels of carbon dioxide (CO_2).

Materials and Methods: *Candida glabrata* reference strain (ATCC 90030) was used in this study. Yeast samples were cultured on Sabouraud dextrose broth (SDB) medium and incubated under 3%, 5%, and 10% CO_2 levels for 24, 48 and 72 h. Control cultures were prepared without CO_2 pressure for three days. The possibility of pseudohyphae and mycelium formation in *C. glabrata* was investigated.

Results: The results of this study revealed that the most branching filament-like cells were obtained at high CO_2 pressure (10%) after 72 h. After three days of low CO_2 pressure (3%), only yeast and budding cells were observed without any pseudohyphae formation.

Conclusion: CO_2 could act as a stimulus and induced formation of pseudohyphae in *Candida glabrata* yeast cells.

Keywords: *Candida glabrata*, CO_2 pressure, Pseudohyphae

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Introduction

The members of the genus *Candida* were reported as the most common fungal agents causing nosocomial infections [1, 2]. Over the past decade, the incidence rate of infections caused by non-*albicans Candida* species has been globally on the rise [2]. Among *Candida* species, *Candida glabrata* (*C. glabrata*) is known as the second most common yeast isolated from blood stream infections, particularly in immunocompromised patients [3]. *C. glabrata* is also among the most frequent causes of candidemia with high mortality rate, especially due to natural resistance of this species to antifungal agents [4, 5].

Filamentous growth is deemed as a virulence factor in *Candida* species; filamentation is indicated to play an important role in pathogenesis through evading host defenses [6, 7]. In addition, formation of pseudohyphae could be involved in biofilm formation resulting in drug resistance [7].

Switching to pseudohyphal growth usually occurs in response to some stimuli such as nitrogen starvation [8-10]. Carbon dioxide (CO_2) is a gaseous molecule with important signaling and stimulating roles in some fungal pathogens [11-13]. Besides, CO_2 can act as a stimulus inducing filamentation in *C. albicans* [14].

Filamentous growth of *C. glabrata* in response to diverse physiological conditions and the molecular mechanisms involved in switching still remain inconspicuous. In this study, we sought to evaluate the ability of *C. glabrata* in forming pseudohyphae under various CO_2 pressures.

Materials and Methods

Culture condition

C. glabrata reference strain (ATCC 90030) was employed in this study. Yeast cells were cultured on Sabouraud dextrose agar (SDA; Merck, Germany) and incubated at 37°C for 48 h. The suspension of yeast cells was then prepared using distilled water at final concentration of 1×10^5 CFU/ml [15]. *C. albicans* reference strain was also utilized as positive control for pseudohyphae formation.

Inducing filamentous growth

For examination of filamentous growth, 200 µl

of the final suspension was added to three plates containing SDB (Merck, Germany).The plates were then incubated at 37°C in the presence of 3%, 5%, and 10% CO_2 separately. All the samples under each CO_2 level were then incubated for 24, 48, and 72 h. Control cultures were prepared under the same conditions without any CO_2.

Microscopic examination

Microscopic characteristics of all the samples were investigated to assess pseudohyphae formation, and then they were compared to yeast cells as negative control. In addition, scanning electron microscopy (SEM) examination was conducted based on the standard protocol [16, 17]. Briefly, the *C. glabrata* cells exposed to 3%, 5% and 10% CO_2 were collected separately and mounted on 12 mm slides. The samples were then pre-fixed with 2.5% glutaraldehyde (GA) in 0.1 M cacodylate buffer for 50 min. After washing three times in 0.1 M sodium cacodylate (CAC) buffer, the samples were fixed with 10% osmium tetroxide (OsO_4) in 0.1 M cacodylate buffer followed by washing in cacodylate buffer. Post-fixing of *C. glabrata* cells was carried out with 1% OsO_4. The cells were then washed twice in dH_2O and dehydrated and dried with ethanol [17, 18]. Thereafter, the obtained samples were mounted, coated with gold/palladium, and viewed by DSM-960A scanning electron microscope.

In order to investigate the reversibility of the formed pseudohyphae, *C. glabrata* cells exposed to 10% CO_2 pressure were inoculated in tryptic soy broth (TSB) medium (Merck, Germany) and stored at -20°C for two weeks. The sample was sub-cultured without CO_2 pressure in SDB medium plate and incubated at 37°C. Further formation of pseudohyphae was then evaluated.

Results and Discussion

Observing cell aggregation and chains of more than three branching budding cells of *C. glabrata* were considered as positive result for formation pseudohyphae. Our outcomes indicated no pseudo-hyphal body in the presence of low CO_2 pressure (3%) during 24, 48, and 72 h of incubation. However, few filament-like cells were noted at 5% and 10% CO_2 pressure after 24 h. In addition, the most branching filament-like cells with larger sizes were detected in cultures exposed to 10% CO_2 pressure after 72 h. Generally, high CO_2 pressure diminished bud cells, while it propagated filament-like cells (Figure 1).

According to these results, *C. glabrata* dem-onstrated the ability to undergo morphological changes in response to CO_2 pressure as a stimulus. Figure 2 illustrates SEM results of pseudohyphal growth in *C. glabrata*.

In addition, we investigated reversibility of filamentous growth through sub-culture of the samples after a week in the absence of CO_2 pressure. The obtained results suggested that *C. glabrata* could form pseudohyphal bodies in a similar manner to the prior filamentation.

Candida species are considered as important fungal pathogens causing a wide spectrum of diseases ranging from superficial to life-threatening infections [19]. Infections due to non-*C. albicans* species such as *C. glabrata* increased over the past decade. Generally, various virulence factors are postulated

Figure 1. Microscopic examination of filamentous growth in *C. glabrata* (ATCC 90030); the most cell aggregation and branching pseudohyphal cells at 10% CO_2 pressure after 72 h

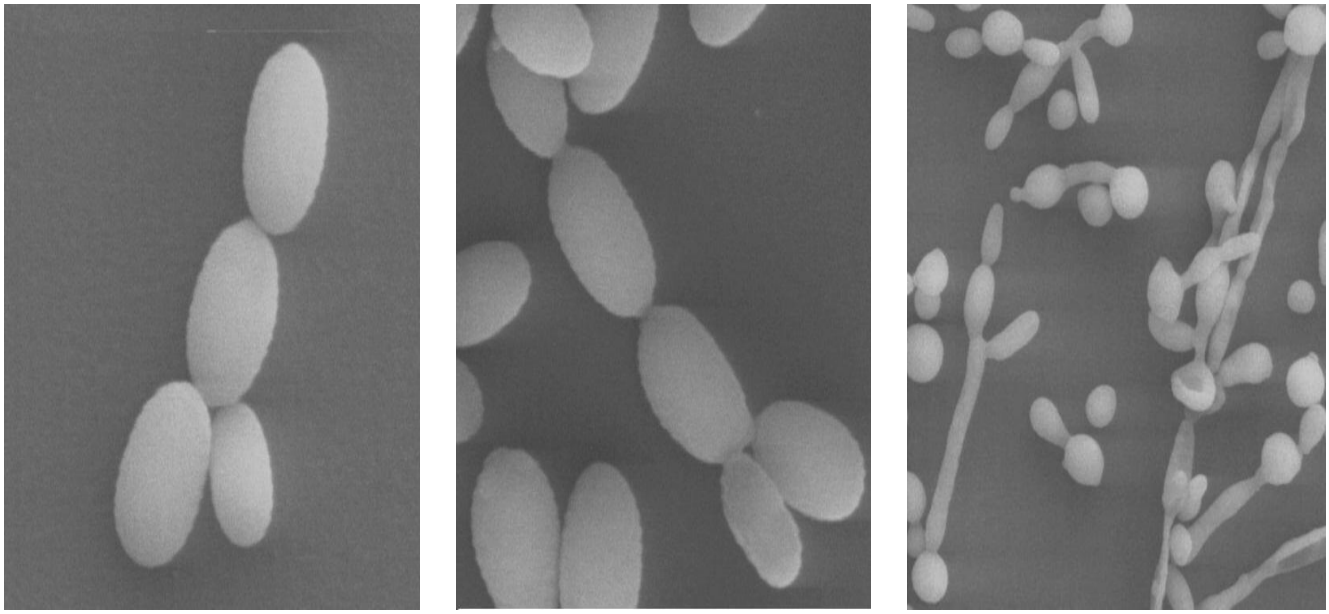

Figure 2. Scanning electron microscopy examination of *C. glabrata* ATCC 90030 filamentous growth; (A) few branching budding cells were observed at 5% CO_2 pressure after 72 h; (B) the most branching pseudohyphal cells were observed in cultures exposed to 10% CO_2 pressure after 72 h; (C) pseudohyphae formation in *Candida albicans* standard strain

for pathogenicity of *Candida* species including morphological switching, biofilm formation, and production of hydrolytic enzymes [6, 7].

Although *C. glabrata* usually grows as budding yeast cells, reports indicated that this species could form pseudohyphal cells during nitrogen starvation [10]. In addition, changes in oxygen and carbon dioxide levels influence cell activity of *Saccharomyces cerevisiae*, and high-pressure oxygen, as well as CO_2 lead to cell inactivation [20].

In the present study, we evaluated the effect of CO_2 on formation of pseudohyphae in *C. glabrata.* The obtained results indicated that high-pressure CO_2 could induce pseudohyphal switching of the *C. glabrata* cells. As went before, a great number of filament-like cells were observed under 10% CO_2 pressure after 72 h. Interestingly, the morphology of the yeast cells seems to be linked with physiological conditions since the most branching filament-like cells were obtained at higher CO_2 level.

CO_2 is applied for antimicrobial activity, especially for the preservation of foodstuffs, as it can affect microorganisms by inactivation of the cells [21]. Shimoda et al. reported that temperature and CO_2 pressure independently contributed to inactivation of *Saccharomyces cerevisiae* cells [22].

Assessment of *C. glabrata* morphological alterations under different CO_2 pressures described in this study revealed that the highest rate of pseudohyphae formation was attained upon prolonging the incubation time and enhancing CO_2 pressure. High-pressure CO_2 was associated with further switching of yeast cells to branched filamentous cells. Moreover, cultures exposed to low-pressure CO_2 (3%) exhibited more yeast and bud cells without any pseudohyphae formation. Different signaling pathways and transcription factor genes are essential for morphological changes in yeast cells. The STE11, STE12, and STE20 proteins are indicated to be at play in morphological changes of *S. cerevisiae* and adaptation of *C. glabrata* to hypertonic stress [23-26]. To determine whether *C. glabrata* STE genes are responsible for these morphological changes, future studies are required.

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Author's contribution

S. R. and S. K. designed and managed the project. E. S. performed the tests. S. AKA. was project partner and wrote the first draft of the manuscript. S. R. edited the final manuscript. S. D. was project partner.

Conflicts of interest

None declared.

Financial disclosure

No financial interests related to the material of

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