Effect of Zinc on Cell Morphogenesis of *Candida dubliniensis*

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Background and objective: *Candida dubliniensis* is a budding yeast that is capable of switching from yeast to hypha to penetrate epithelium and may therefore be an important virulence factor. Yeast and hyphae are benefit to particular situation. Since zinc is found to be one factor that correlates with candidiasis. This provided informative data to our study. Objective of this study is to investigate whether zinc influences on morphogenesis of *C. dubliniensis*.

Method: Germ tube formation in human serum was used as a test method. Thirty isolates of *C. dubliniensis* were incubated in human serum (control), human serum supplemented with zinc (zinc repleted condition), and human serum supplemented with zinc chelator (TPEN) (zinc depleted condition) at 37°C for two hrs. After incubation, germ tube cells were counted under a light microscope in total 20 high power fields for each condition. *C. albicans* was used as positive control.

Result: The result showed that germ tube was normally formed in human serum and human serum supplemented with zinc, no significant difference between both conditions. Conversely, germ tube was significantly inhibited when zinc was depleted. In the body, zinc ion is normally bound to proteins in blood stream to control the growth of harmful pathogen but this situation is also favorable for yeast to spread throughout the body system if it is able to invade the vessel. This result was one useful information showed that zinc influenced on morphogenesis and can be integrated to candidiamia that low zinc in blood stream may provide easier moving for this dimorphic organism causing candidiamia secondary to tissue invasion by yeast-hypha transition.

Conclusion: Germ tube formation was significantly inhibited in zinc depleted condition when compared with in zinc-rich and control conditions.

Key words: *Candida dubliniensis*, morphogenesis, germ tube formation, zinc

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Introduction

Candida dubliniensis normally lives in human as normal flora at many sites of the body [1]. However, the ability to become a powerful pathogen is primarily considered by the immune state of the host, underlying with diabetes, oral contraceptives usage, antibiotics treatment [2] and nutritional deficiency such as zinc deficiency. Virulence factors of *C. dubliniensis* including morphogenesis, phenotypic switching, hydrolytic enzyme production are highly expressed during infection. Moreover, some evidences have shown the correlation of zinc level in serum and vulvovaginal candidiasis (VVC) and acrodermatitis entheropathica [3,4]. Nutritional immunity is an important process that host can limit or prevent the overgrowth of potential harmful microbe including *Candida* and zinc transporter proteins are responsible for this sophisticated mechanism. In case of cell morphogenesis, *Candida* may generate yeast hypha transition when exposed to some stimuli, which

is considered to be important virulence that helps *Candida* successfully switching to pathogenic form to cause disease and this can be investigated by germ tube formation in serum [5]. Evidences demonstrated that true hyphae were predominantly found in patient's lesion. From this point, zinc levels significantly decrease in VVC patients and acrodermatitis entheropathica lead us to investigate whether zinc depleted condition influences the initial step of hypha formation in *C. dubliniensis.*

Objective

The aim of this study is to investigate whether zinc influences on morphogenesis of *C. dubliniensis* by using germ tube formation in human serum as a test method.

Methods

Thirty isolates of *C. dubliniensis* obtained from Srinagarind Hospital were incubated in Yest peptone dextrose (YPD) by shaking at 30°C, 200 rpm. for 18 hrs. The yeast was adjusted to optical density of 0.1 at the wavelength of 530 nm, 500 µl of the yeast suspension was transferred to new eppendorf and centrifuged at 5,000 x g for 15 minutes. After discarding the supernatant, 300 µl of human serum was added, and the experiment was set as zinc rich condition by adding of zinc to final concentration of 100 µM, and zinc depleted conditions by adding zinc chelator (TPEN: N, N, N', N'- tetrakis (2-pyridylmethyl) ethylenediamine) to a final concentration of 300 and 400 µM, then mixed well and incubated at 37°C for 2 hrs before comparing with control which nothing was added. After incubation, yeast-hypha transition or germ tube was counted under the light microscope with 40X objective lens in total 20 high power fields for each condition. *C. albicans* was used as positive control. Percentage of germ tube formation was calculated by the proportion of germ tube positive cell and total yeast cells. The experiment was performed independently with 30 *C. dubliniensis* isolates.

Results

Overnight culture of *C. dubliniensis* isolates were incubated in human serum together with zinc or zinc chelator. Cultures were incubated at 37°C for 2 hrs and washed with PBS two times and stained with Lactophenol cotton blue (LPCB). Germ tube formation was observed (Fig. 1) and the proportion of germ tube positive cell and total number of yeast cell were calculated into percentage of each condition. Control condition showed high distribution of germ tube ranging from 0.59-8%. The ability of *C. dubliniensis* to form germ tube was different depending on isolate (Fig. 2), and the highest germ tube formation was 8 percent which was commonly found for this organism. Percentage of germ tube in zinc supplemented condition was almost similar to control condition ($p>0.05$). Interestingly, when zinc chelators were added, germ tube productions were significantly decreased ($p<0.05$) in both concentrations, 300 and 400 µM TPEN (Fig. 3).

Discussion

Germ tube formation is a critical initial step for hypha formation that helps *Candida* to penetrate host tissue and cause tissue damage[6,7]. According to this experiment, *C. dubliniensis* formed germ tube in zinc rich condition as in human serum control whereas in zinc depleted condition significantly inhibited germ tube formation, and most of them were still in yeast form in both concentrations of zinc chelator. However, *C. dubliniensis* in normal condition or zinc supplemented condition showed germ tube formation but was commonly lower than *C. albicans*. We agreed with Bedell and Soll that only single yeast cell was found in the absence of zinc but budding occurred when micromolar of zinc was added to the medium [8]. This experiment can be further integrated to explain the pathogenesis in *C. dubliniensis.* In blood stream, most of zinc was bound to protein and *Candida* form round yeast cellbeneficial for dissemination. Free zinc could be found on surface and surrounding environment to help in germ tube formation or the yeast- hypha transition which was the initial step of tissue invasion.

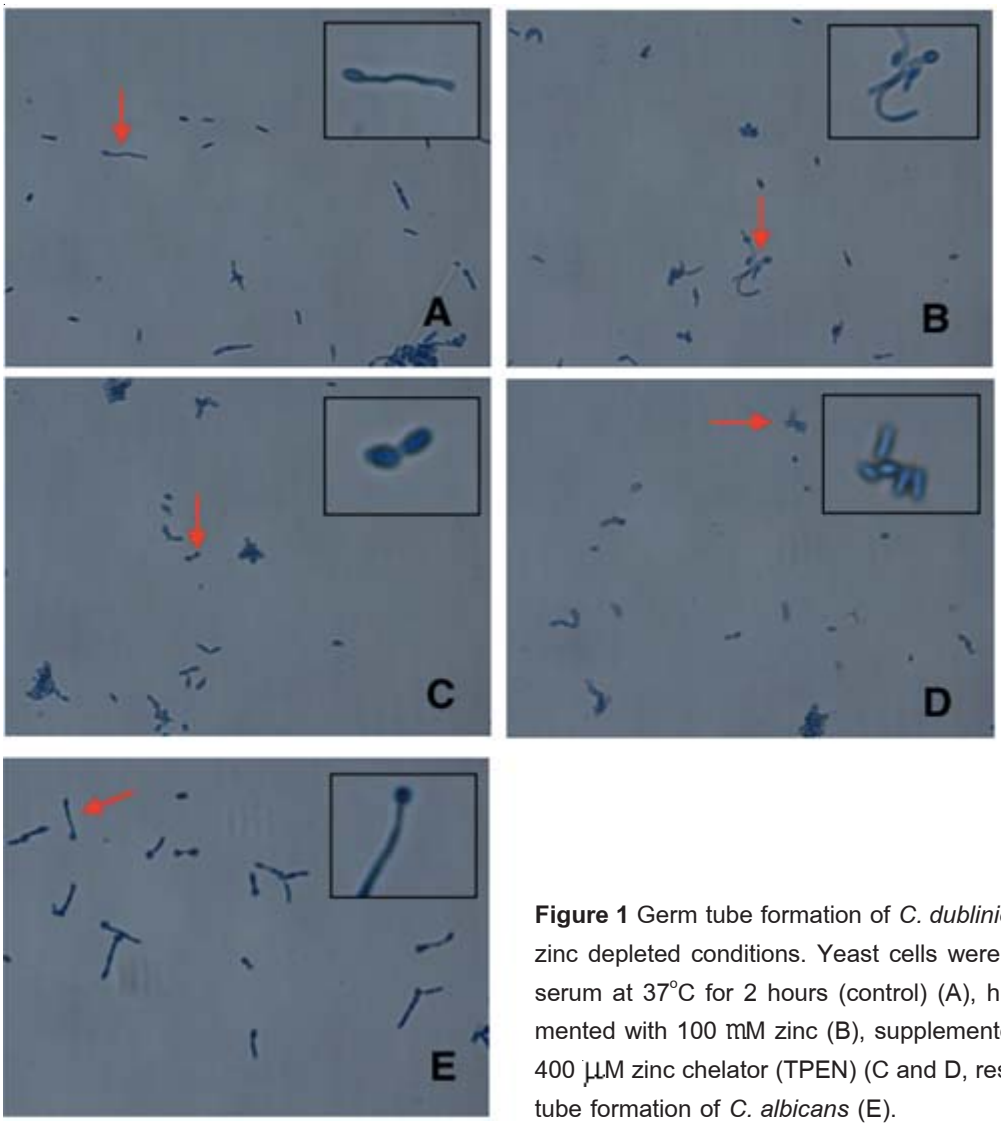

Figure 1 Germ tube formation of *C. dubliniensis* in zinc rich and zinc depleted conditions. Yeast cells were incubated in human serum at 37°C for 2 hours (control) (A), human serum supplemented with 100 mM zinc (B), supplemented with 300 μM and 400 μM zinc chelator (TPEN) (C and D, respectively), and germ tube formation of *C. albicans* (E).

Conclusion

Germ tube formations of all 30 isolates of *C. dubliniensis* were significantly inhibited in zinc depleted condition. Germ tube formations in zinc-rich and control condition were not significantly difference. Thus, zinc had an effect on cell morphogenesis of *C. dubliniensis*. Zinc depletion caused *C. dubliniernsis* remained in yeast form.

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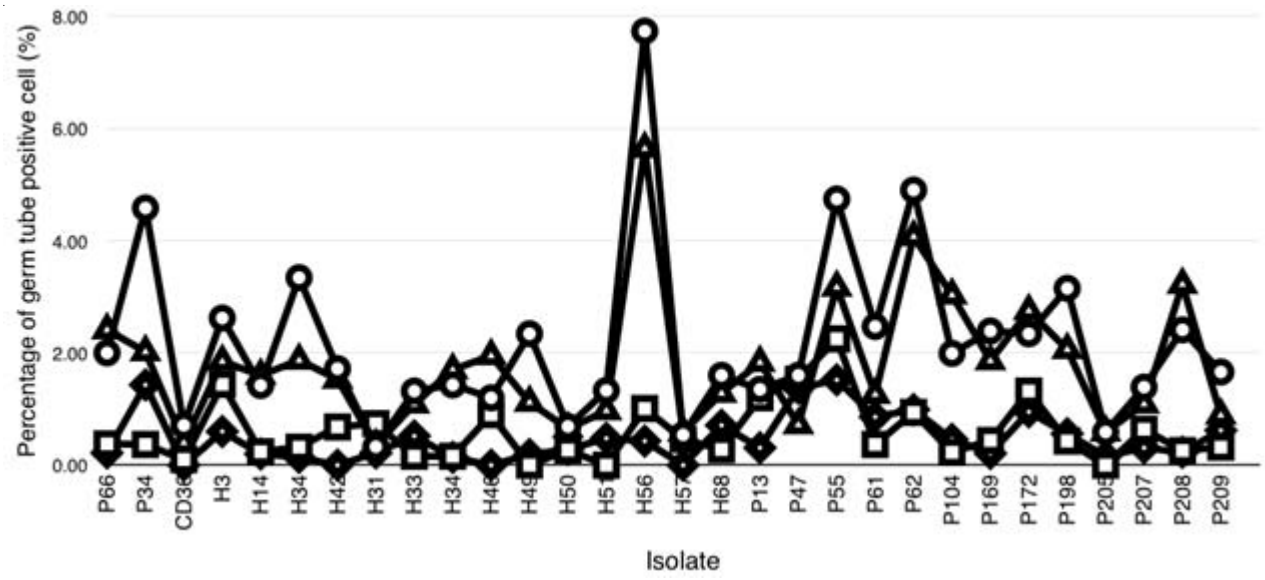

Figure 2 Percentage of germ tube positive cells of *C. dubliniensis* isolates. In human serum (o), human serum supplemented with 100 µM zinc (△), and supplemented with 300 µM (□) and 400µM zinc chelator (◇)

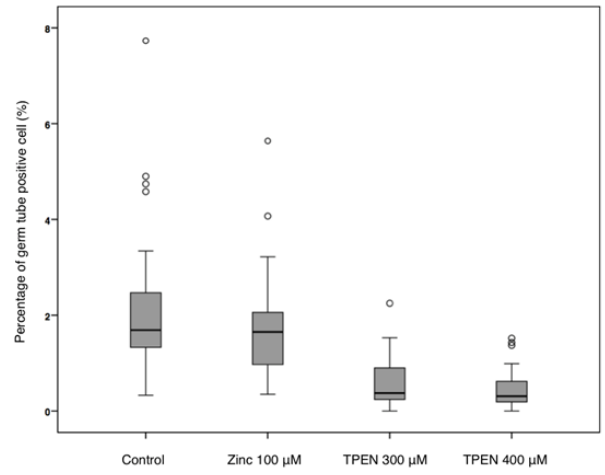

Figure 3 Percentage of germ tube positive cells of *C. dubliniensis* in zinc rich and zinc depleted conditions. Total of 30 isolates were incubated in human serum (control), 100 µM zinc, 300 and 400 µM zinc chelator (TPEN) adding, respectively, for 2 hours at 37°C.

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