Microscopic Morphologies of Different Switched-phenotypes in *Candida dubliniensis*

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Background and Objective: *Candida dubliniensis* is an opportunistic pathogen originally isolated from AIDS patients and has ability to invade and cause systemic disease in immunocompromised patients as *Candida albicans* does. Phenotypic switching phenomenon, one of the essential virulence factor, has been firstly found in *Candida albicans*. It seems to play a role in the switching of *Candida* from normal flora to pathogenic form. This study has focused on morphologies of different phenotypes during phenotypic switching in *Candida dubliniensis*.

Method: Total 36 isolates of *Candida dubliniensis* were tested on an indicator medium containing 1 mM $CuSO_4$. Cellular morphologies of variant switched-phenotypes were observed under the microscope (400X) by lactophenol cotton blue staining.

Result : It was found that the phenotypic switching system in *Candida dubliniensis* included white (Wh), light brown (LB), dark brown (DB), wrinkle (Wr), fuzzy light brown (Fz) and brain-like appearance (Br) phenotypes. When cell morphologies in each switched phenotypes were observed under the microscope, it was found that white phenotype showed much hyphae production, while other types showed budding yeast form.

Conclusion : This study has suggested that filamentous form is more penetrating capable than budding yeast form, thus white phenotype is possible to be a pathogenic form of *Candida dubliniensis*. However, for more understanding of the pathogenesis, the virulence in each phenotype other than morphogenesis have been further studied.

Key words: *Candida dubliniensis*, phenotypic switching, morphology

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Proceeding

Introduction

Candida dubliniensis is a pathogenic yeast species, first identified as a distinct taxon in 1995. *Candida dubliniensis* is a recently described opportunistic pathogen originally isolated from AIDS patients and is also occasionally isolated from immunocompetent individuals[1]. *Candida dubliniensis* has ability to invade and to cause systemic disease in immunocompromised patients. Phenotypic switching phenomenon has been firstly found in *candida albicans*. It is not only be classified by colonial morphology change, it seems to play a role in the switching of *candida* from normal flora to pathogenic form. High-frequency phenotypic transition is a defining feature of the pathogenic *Candida albicans*. The ability of switching between different morphological forms in response to environmental cues is widely thought to be

associated with virulence. Moreover, phenotypic switching was not only found in *Candida albicans* but also in non-albicans *Candida* species such as *Candida glabrata*, when growing on indicator medium, the different switched phenotypes were obtained. Since the incidence of infection caused by non-albicans *Candida* species is increasing[2], this study has focused on morphologies of different phenotypes during phenotypic switching in *Candida dubliniensis*.

Objective

The aim of this study was to investigate the morphologies of each switched phenotype in *Candida dubliniensis*.

Methods

We obtained samples totally 36 isolates of *Candida dubliniensis* from Srinagarind hospital, faculty of Medicine, Khon Kaen university. All clones were stored at room temperature on SDA slants (2% glucose, 1% bacto peptone, 2% agar) until studied. Each isolate of *Candida dubliniensis* was tested on an indicator medium containing 1 mM $CuSO_4$ and types of phenotypic switching were determined after seven days of incubation. Cells expressing the different switched phenotypes were stored in glycerol at -70 °C. The cell morphologies of colonies exhibiting variant phenotypes were observed under the microscope (400X) by lactophenol cotton blue staining.

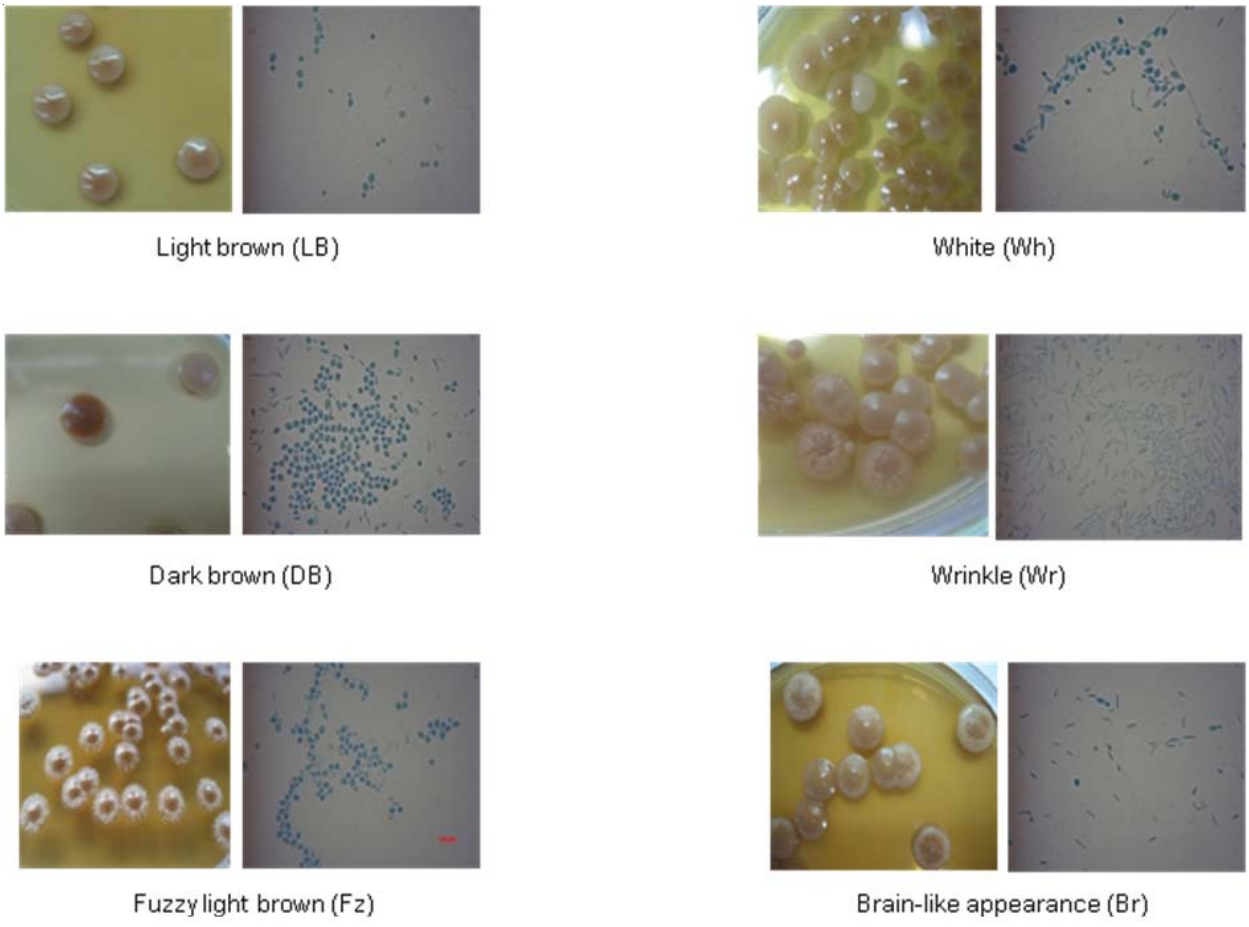

Figure 1 Types of phenotypic switching in *Candida dubliniensis*; light brown, white, dark brown, wrinkle, fuzzy light brown and brain-like appearance phenotypes. The colony morphologies are on the left and the microscopic morphologies (400X) are on the right side of each panel.

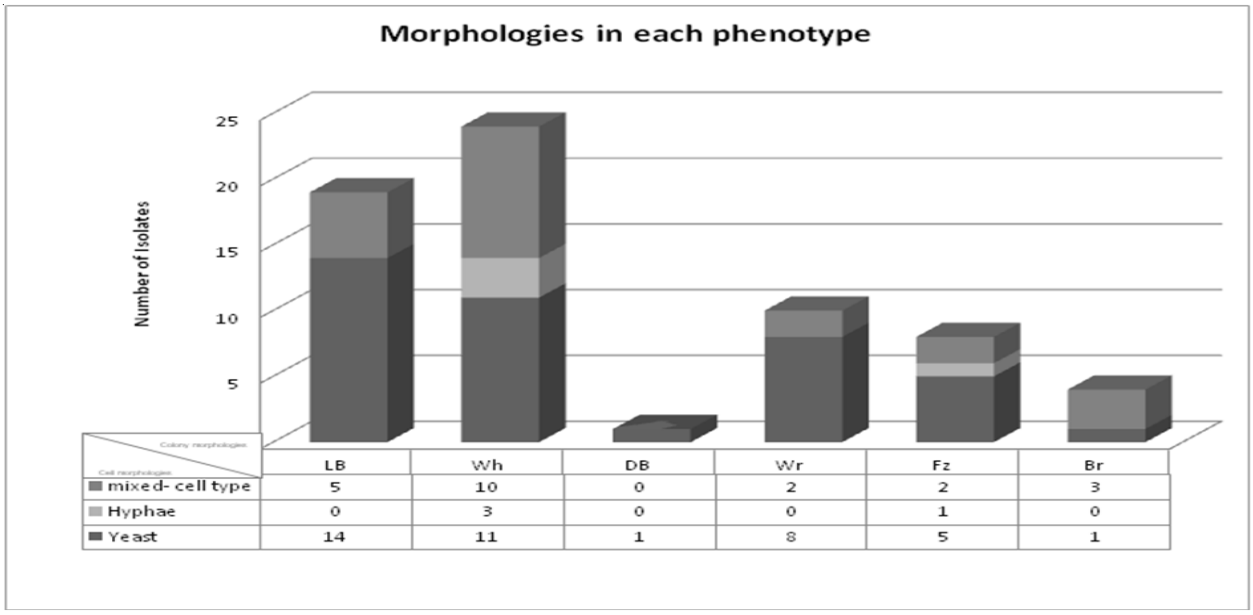

Figure 2 The cell morphology found in each switched phenotype; light brown (LB), white (Wh), dark brown (DB), wrinkle (Wr), fuzzy light brown (Fz) and brain-like appearance (Br), from 36 isolates of *Candida dubliniensis*.

Result

Phenotypic switching was also found in *Candida dubliniensis*. The switching system included white (Wh), light brown (LB), dark brown (DB), wrinkle (Wr), fuzzy light brown (Fz) and brain-like appearance (Br) phenotypes (Fig. 1). When cell morphologies in each switched phenotype were observed under the microscope, it was found that almost half of white phenotype showed mixed cells type such as yeast cell and hyphae (Fig. 1,2). Wrinkle and brain-like appearance phenotypes showed mainly elongate yeast cell. Light brown and dark brown phenotypes showed round budding yeast form, 14/19 isolates and 1/1 isolate, respectively (Fig. 1,2).

Conclusion

The ability to switch between the yeast form and the filamentous form is thought to be important for the virulence in *Candida albicans.* The microscopic morphology study, white phenotype showed much hyphae production, while other types showed budding yeast form. Filamentous cells are more invasive and better at tissue penetration, while yeast cells are easy to be delivered and disseminated in the bloodstream[3-5]. It seems that the ability of switching back and front between the two forms is important for different stage of pathogenesis[6]. This study has suggested according to Sudbery PE [6] that if filamentous form is more virulence than budding yeast form, white phenotype is possible to be a pathogenic form of *Candida dubliniensis*. It may switch from original yeast to hyphae when exposed to some stimuli or environmental cues for tissue invasion. However, for more understanding of the pathogenesis, the stimulant, the properties, and the virulence in each phenotype other than morphogenesis have been further studying.

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