CHAPTER 1

Introduction

Penicilliosis marneffei, caused by the thermal dimorphic fungus *Penicillium marneffei*, recently changed to *Talaromyces marneffei* (Samson *et al*., 2011), is a systemic human mycosis geographically restricted to Southeast Asia and southern China (Supparatpinyo *et al*., 1994). It is believed that the infectious process involves the inhalation of small conidia produced by the environmental mold form of the fungus that subsequently undergo morphogenic transformation into yeast within the lungs. At present, *P. marneffei* affects mainly patients with advanced HIV infection living within the endemic area, particularly in northern Thailand (Supparatpinyo *et al*., 1992). In AIDS patients, infection with *P. marneffei* typically presents as a disseminated illness characterized by fever, cough, weight loss, skin lesions and pancytopenia (Tsui *et al*., 1992; Sirisanthana and Sirisanthana 1995), and it is fatal without antifungal treatment.

P. marneffei grows as saprophytic mycelia that produce infective conidia propagules, which are inhaled into the lungs where the conidia change to the parasitic yeast cells. The adherence of *P. marneffei* conidia to the lungs which recognized fibronectin and bind to laminin via a sialic acid-specific lectin is important for the successful establishment of infection (Hamilton *et al*., 1998, 1999). Upon host infection, one of the most essential aspects that contribute to the disease outcome is the initial interaction of the *P. marneffei*cells with the phagocytic cells such as resident macrophages and dendritic cells (Ngaosuwankul *et al*., 2008). This leads to the

induction of inflammatory cytokines from the phagocytic cells to defend against fungus.

The putative virulence properties in *P. marneffei* are believed to involve in several factors including adhesion (Hamilton *et al*., 1998, 1999), dimorphism switching (Sternberg 1994; Borneman *et al*. 2000; Boyce *et al*. 2009, production of melanin or melanin-like (Youngchim *et al*., 2005), and expression of enzyme laccase (Sapmak *et al*., 2012).

Fungal laccases are involved in fungal development, morphogenesis, detoxification process, pathogenicity and the synthesis of pigments (Thurston 1994; Nagai *et al*., 2003). Expression of laccase is induced by environmental stress such as oxidative stress, acidic condition, and nutrient deprivation (Morozova *et al*., 2007). Laccase is an important virulence factor in many pathogenic fungi. A laccase-like activity was detected in protein extracts of *Paracoccidioides brasiliensis* that was implicated in the enzymatic synthesis of melanin in yeast cells. In addition, the presence of laccase-like activity has been observed in cytoplasmatic extracts of *Histoplasma capsulatum* incubated with L-DOPA (Nosanchuk *et al*., 2002). Furthermore, in *Cryptococcus neoformans*, laccase is encoded by two laccase genes, *LAC1* and *LAC2*. *LAC1* is predominant isoform responsible for virulence because deletion *LAC2* does not affect virulence in mice (Pukkila-Worley *et al*., 2005; Salas *et al*., 1996). Laccase has been shown to directly protect *C. neoformans* against the antifungal activity of macrophage by oxidation of phagosomal iron to Fe^{3+} leading to hydroxyl radical formation of macrophage decreasing (Liu *et al*., 1999). Infection with laccase-positive (melanotic) *C. neoformans* cells elicited higher level of interleukin-4 (IL-4) and monocyte chemotactic protein-1 (MCP-1) than did infection with laccase-negative cells

(Mednick *et al*., 2005). Additionally, cryptococcal laccase deletion significantly decreased production of IL-4 and IL-10 and increased production IFN-γ (Interferon gamma), TNF-α (tumor necrosis factor-α) and IL-17A compared with the production of these cytokines in wild type-infected mice (Qiu *et al*., 2012). In *P. marneffei* displayed four putative laccase-encoding genes in whole genome sequence. All laccases including *lac1*, *lac2*, *lac3* and *arb2* genes were related to other fungal laccases. Losing of each gene did not affect growth ability of this fungus. Losing of four *lac* genes did not affect growth rate, however, the conidiation seemed to be slightly slower and lower in amount. Deletion of *arb2* gene resulted in changing the grayish green to be light brown colony at 28^{o}C. A single laccase gene deletion did not affect the resistance to stress. Losing of four laccases could not abrogate melanin production but laccase activity existed in a lower level. Deletion of four laccase-encoding genes was more sensitive to oxidative stress (H_2O_2), cell wall stress (SDS), and antifungal agents such as itraconazole, fluconazole and cotrimazole (Sapmak *et al*., 2012). However, the exact role of laccase in pathogenicity of *P. marneffei* is still unclear and needed to be investigated. In this study, the presence or absence of laccase altered innate immune response and cytokine production in macrophage has been investigated. Analysis of the role of this enzyme may lead to insights into pathogenicity and target for antifungal agent discovery.