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Cloning, expression and purification of squalene synthase from *Candida tropicalis* in *Pichia pastoris*

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ABSTRACT

Squalene synthase (SS) is the key precursor and first committed enzyme of the sterol biosynthesis pathway. In a previous work, SS has been identified as one of the immunogenic proteins that could be a potential diagnostic candidate for the pathogenic fungus *Candida tropicalis*. In this study, SS from *C. tropicalis* was cloned and expressed as recombinant protein in *Pichia pastoris* to investigate its reactivity with serum antibodies. *ERG9* gene that encodes for SS was amplified by PCR and cloned in-frame into pPICZB expression vector. The recombinant construct was then transformed into *P. pastoris* GS115 host strain. Expression of the recombinant protein was confirmed by SDS–PAGE and Western blot analysis using anti-His tag probe. Optimal protein production was achieved by cultivating the culture with 1.0% methanol for 72 h. The recombinant protein was purified to approximately 97% pure in a single step immobilized metal affinity chromatography with a yield of 70.3%. Besides, the purified protein exhibited specific reactivity with immune sera on Western blot. This is the first report on heterologous expression of antigenic SS from *C. tropicalis* in *P. pastoris* which can be exploited for large-scale production and further research. The results also suggested that the protein might be of great value as antigen candidate for serodiagnosis of *Candida* infection.

Introduction

Candida species is a commensal fungus and also an opportunistic pathogen that can cause candidiasis, one of the most important nosocomial infections affecting people with compromised immune system. *Candida* spp. is the major human fungal pathogen causing a wide range of clinical illnesses, ranging from superficial candidiasis to disseminated invasive candidiasis. Worldwide, there has been an increase in the incidence of candidiasis and *Candida* spp. was ranked as the fourth most common cause of nosocomial bloodstream infections in United States [1]. *Candida albicans* is the predominant species isolated but a change in the epidemiology towards other *Candida* spp. has been observed [2–4]. *Candida tropicalis* is one of the most commonly isolated *Candida* spp. after *C. albicans* and its prevalence has increased significantly in recent years [5,6]. In Malaysia, notably *C. tropicalis* has emerged as a major *Candida* spp. isolated from clinical specimens after *C. albicans* [7,8].

Current diagnostic methods for candidiasis depend on traditional microbiological culture, which is relatively insensitive and time-consuming [9]. Moreover, this method often results in late diagnosis during advanced stage of infection. This subsequently causes delay in the antifungal treatment and significantly reduces survival of the patients. The aforementioned problems of the microbiological culture method highlight the need to develop more reliable, rapid, sensitive and specific diagnostic tests. Some non-culture approaches have been developed and evaluated to overcome the limitations of the microbiological culture method. This involves either detection of the fungal nucleic acids [10], metabolites [11], antigens and/or antibodies [12,13] from infected patients.

Despite numerous effort and progress so far, exploration for alternative diagnostic method is still ongoing. Serological diagnosis is one of the focuses of current research for the development of diagnostic assay for invasive candidiasis. This diagnostic strategy employs specific interaction between the target antigen and antibody to detect the presence of infection. In this context, protein

Abbreviations: AOX1, alcohol oxidase 1; bp, base pair; BSA, bovine serum albumin; BMGY, buffered glycerol-complex medium; BMMY, buffered methanol-complex medium; DTT, dithiothreitol; EDTA, ethylenediaminetetraacetic acid; LiCl, lithium chloride; PBS, phosphate-buffered saline; TBST, Tris-buffered saline with 0.05% Tween 20; PMSF, phenylmethylsulfonyl fluoride; PVDF, polyvinylidene difluoride; SS, squalene synthase; YPD, yeast extract-peptone-dextrose.

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