

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

### **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.

**Keyword:** Antigen; *Candida tropicalis*; *Pichia pastoris*; Recombinant protein; Squalene synthase