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

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Source: PROTEIN EXPRESSION AND PURIFICATION **Volume:** 94 **Pages:** 15-21 **DOI:** 10.1016/j.pep.2013.10.012 **Published:** FEB 2014

Times Cited in Web of Science Core Collection: 7

Total Times Cited: 8

Usage Count (Last 180 days): 0

Usage Count (Since 2013): 24

Cited Reference Count: 34

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. (C) 2013 Elsevier Inc. All rights reserved.

Accession Number: WOS:000329563200003

PubMed ID: 24184232

Language: English

Document Type: Article

Author Keywords: Antigen; Candida tropicalis; Pichia pastoris; Recombinant protein; Squalene synthase

KeyWords Plus: HETEROLOGOUS PROTEIN EXPRESSION; INVASIVE CANDIDIASIS; EARLY-DIAGNOSIS; ALBICANS; ANTIBODIES; INFECTIONS; GENES; YEAST; IDENTIFICATION; EPIDEMIOLOGY

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Publisher: ACADEMIC PRESS INC ELSEVIER SCIENCE

Publisher Address: 525 B ST, STE 1900, SAN DIEGO, CA 92101-4495 USA

Web of Science Categories: Biochemical Research Methods; Biochemistry & Molecular Biology; Biotechnology & Applied Microbiology

Research Areas: Biochemistry & Molecular Biology; Biotechnology & Applied Microbiology

IDS Number: 287ST

ISSN: 1046-5928

eISSN: 1096-0279

29-char Source Abbrev.: PROTEIN EXPRES PURIF

ISO Source Abbrev.: Protein Expr. Purif.

Source Item Page Count: 7

Funding:

Funding Agency	Grant Number
E-Science Fund, Malaysia	02-01-04-SF0761
Ministry of Science, Technology and Innovation (MOSTI), Malaysia	

This work was supported by the E-Science Fund, Malaysia (Project No. 02-01-04-SF0761) sponsored by Ministry of Science, Technology and Innovation (MOSTI), Malaysia.

Output Date: 2018-01-02

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