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PURIFICATION AND CHARACTERIZATION OF RECOMBINANT THERMOSTABLE T1 LIPASE EXPRESSED FROM Pichia pastoris

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PURIFICATION AND CHARACTERIZATION OF RECOMBINANT THERMOSTABLE T1 LIPASE EXPRESSED FROM *Pichia pastoris*

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PENGESAHAN

Dengan ini adalah disahkan bahawa laporan bertajuk "Purification and Characterization of Recombinant Thermostable T1 Lipase Expressed from *Pichia pastoris*" telah disiapkan serta dikemukakan kepada Jabatan Biokimia oleh Nalini a/p Periyasamy (161821) sebagai syarat untuk kursus BCH 4999, projek dari Fakulti Bioteknologi dan Sains Biomolekul, Universiti Putra Malaysia.

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LIST OF ABBREVIATIONS

%	Percentage
°C	Degree Celsius
g	Gram
mg	Milligram
ml	Milliliter
M	Molar
mM	Milli Molar
min	Minutes
µl	Microliter
L	Liter
Rpm	Rotation per minute
g	Relative centrifugal force in unit of x *g*
kDa	Kilo Dalton
OD	Optical density
PAGE	Poly-acrylamide gel electrophoresis
Nm	Nanometer

ABSTRACT

Thermostable lipases have high demand in various industries owing to its interesting features. Thermostable T1 lipase gene of *Geobacillus zalihae* was successfully cloned in *Pichia pastoris*. However, the characterization of purified lipase is not performed yet. Therefore, this study was conducted to determine the characteristic of the purified T1 lipase expressed in *P. pastoris*. The pH of the supernatant was adjusted to pH 7.4 using 1.5 M Tris-HCl and subjected to purification using Nickel-Sepharose resin. The recombinant T1 lipase was purified with 68.6 % recovery and 8.47 fold. The molecular weight of recombinant T1 lipase obtained was approximately 44 kDa. This enzyme exhibited maximum activity at temperature 70 °C and was found to be stable for two hours at 60 °C and 65 °C with relative activity of 80 % and 50 %, respectively. The optimum pH of recombinant T1 lipase was at pH 9 in 0.05 M Tris-HCl. The enzyme was found to be stable at pH 8 and pH 9 with 0.05 M Tris-HCl as its buffer. Finally, the enzyme was found to have broad substrate specificity for natural oils especially with coconut oil with 172 % relative activity. In conclusion, the recombinant T1 lipase expressed in *P. pastoris* was purified with the highest purification fold and its important characteristics were fully characterized.

ABSTRAK

Lipase yang tahan panas mendapat permintaan yang menggalakkan dalam pelbagai industri disebabkan ciri-cirinya yang menarik. Gen lipase T1 terma stabil yang diperoleh dari *Geobacillus zalihae* telah berjaya diklonkan ke dalam hos *Pichia pastoris*. Namun, ciri-ciri lipase yang tulen ini masih belum dicirikan. Oleh demikian, kajian telah dijalankan bagi mengenalpasti ciri-ciri lipase T1 yang tulen di dalam *P. pastoris*. pH supernatan telah dilaraskan pada pH 7.4 dengan menggunakan 1.5 M Tris-HCl dan seterusnya ditulenkan menggunakan resin Nickel-Sepharose. Recombinan lipase T1 ini telah ditulenkan dengan peratusan pengembalian enzim sebanyak 68.6 % dan penulenannya adalah sebanyak 8.47 lipat. Saiz molekul recombinan lipase T1 yang diperolehi adalah kira-kira 44 kDa. Enzim ini menunjukkan aktiviti yang maksimum pada suhu 70 °C dan didapati stabil selama dua jam pada suhu 60 °C dan 65 °C dengan aktiviti relatif masing-masing sebanyak 80 % dan 50 %. pH optimum recombinant lipase T1 adalah pada pH 9 dalam larutan 0.05 M Tris-HCl. Enzim ini didapati stabil pada pH 8 dan pH 9 apabila 0.05 M Tris-HCl digunakan sebagai penampan. Akhirnya, enzim ini didapati boleh bertindak balas terhadap pelbagai minyak semula jadi terutamanya minyak kelapa dengan relatif aktiviti 172 %. Kesimpulannya, recombinan lipase T1 dari hos *P. pastoris* telah ditulenkan dengan gandaaan penulenan yang paling tinggi dan ciri-ciri penting telah dikaji.

CHAPTER 1

INTRODUCTION

1.1 General

Lipases also known as acylglycerol hydrolases are ubiquitous in nature and can be produced by most plants, animals, and microorganisms (Arpigny et al., 1999). Even though there are various types of lipases available, microbial lipases are shown more interest due to their ability to catalyze a wide variety of reactions in aqueous and non aqueous state, possess greater stability, have low production costs and are more easily available than lipases from plants and animals (Kumar et al., 2005). Fernández et al. (2006) deduced that the ability of lipases to carry out enzymatic reactions under mild conditions and their high selectivity make them to be a good candidate to perform some transformations which are difficult to achieve by chemical processes.

Lipases derived from mesophilic organisms are not preferable as biocatalyst because they suffer denaturation at high temperature. Since majority of the industrial processes are carried out at elevated temperature, lipases from thermophilic microorganisms have high commercial value for this purpose. They do not only catalyze biochemical transformation, but also maintain their adaptability efficiency and reproducibility. Biocatalysis at high temperature are significant because they offer some benefits such as increasing solubility of compounds in organic solvents, reducing viscosity, increasing product yield and lowering contamination probability (Sharma et al., 2013).

Although thermophiles are often recognized as best candidate in producing thermostable lipases, it is impractical due to low expression of enzymes and high

temperature fermentations are required to grow the thermophiles. As a solution, molecular approaches through genetic engineering become a best substitute to produce high level expression of enzyme (Leow et al., 2014). Many studies have been reported of using heterologous host to express thermostable lipases intracellularly and also extracellularly. For example, Shariff et al. (2011) integrated thermostable L2 lipase gene from *Bacillus* sp. into *Escherichia coli* for intracellular expression while Quyen et al. (2003) integrated thermostable lipase gene from *Bacillus thermocatenulatus* in *P. pastoris* for extracellular expression.

Purification is often done prior studying about the personality and properties of enzyme to ensure the activity observed is from the individual enzyme. According to Deutscher (1990), purification is a process in which the protein of interest was isolated from the pool of proteins. The enzymes studied had most if not all been purified prior to subsequent tests. The conversion of a substance to its product as a whole is considered pointless if the enzymes are not pure.

To understand more about how an enzyme works, characterization is needed. Characterizations of a purified enzyme enable us to identify how the enzyme reacts to diverse external environment or stress and identify the optimum condition of the enzyme prefer to work best. This property of an enzyme might differ if the expression host is altered. For example, the characterization of recombinant L2 lipase expressed from *E. coli* showed the optimum temperature and pH are 70 °C and pH 9 respectively (Shariff et al., 2011). On the other hand, Sabri et al. (2009) found out that the same enzyme, L2 lipase expressed from *P. pastoris* exhibited optimum temperature and pH at 65 °C to 75 °C and pH 8, respectively.

This study reports the characterizations of purified recombinant thermostable T1 lipase expressed from *P. pastoris* expression system.

1.2 Problem Statement

Rahman et al. (2007) isolated a thermophile from palm oil mill effluent and identified it as *Geobacillus zalihae* strain T1. The T1 lipase gene was successfully cloned into pPICZαB vector and expressed in *Pichia pastoris* under regulation of alcohol oxidase promoter (Oslan et al., 2014). However the T1 lipase expressed using secretory *P. pastoris* expression system has not been purified and characterized yet.

1.3 Objectives

This study was carried out to characterize the purified recombinant T1 lipase expressed from *P. pastoris*. Therefore, the objectives of the study are listed below;

1) To purify the recombinant T1 lipase (his-tag) expressed from *P. pastoris* using affinity chromatography.

2) To characterize the purified T1 lipase for optimum temperature, temperature stability, optimum pH, pH stability and substrate specificity.

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