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PRODUCTION AND CHARACTERIZATION OF THERMOSTABLE LIPASE FROM LOCALLY ISOLATED YEAST

NURUL IZZATI ZULKIFLI

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PRODUCTION AND CHARACTERIZATION OF THERMOSTABLE LIPASE FROM LOCALLY ISOLATED YEAST

NURUL IZZATI BINTI ZULKIFLI

DEPARTMENT OF BIOCHEMISTRY

FACULTY OF BIOTECHNOLOGY AND BIOMOLECULAR SCIENCES

UNIVERSITI PUTRA MALAYSIA

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PENGESAHAN

Dengan ini adalah disahkan bahawa tesis projek yang bertajuk "Production and Characterization of Thermostable Lipase from Locally Isolated Yeast" telah disiapkan serta dikemukakan kepada Jabatan Biokimia oleh Nurul Izzati Binti Zulkifli (162815) sebagai syarat untuk kursus BCM4999 Projek.

Disahkan Oleh,

..... Tarikh: ……

(Dr. Siti Nurbaya Oslan)
Penyelia projek
Jabatan Biokimia
Fakulti Bioteknologi dan Sains Biomolekul
Universiti Putra Malaysia

..... Tarikh : .....

(Prof. Dato' Dr. Abu Bakar Salleh)
Ketua Jabatan Biokimia
Fakulti Bioteknologi dan Sains Biomolekul
Universiti Putra Malaysia

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LISTS OF ABBREVIATIONS

°C	degree Celsius
µg	microgram
µl	microliter
g	gram
g	gravity
h	hour/s
L	litre
mg	milligram
mg/ml	milligram/millilitre
min	minute/s
ml	millilitre
mm	millimeter
mM	milliMolar
OD	optical density
OD_{595}	optical density at 595 nm
OD_{600}	optical density at 600 nm
OD_{715}	optical density at 715 nm

PRODUCTION AND CHARACTERIZATION OF THERMOSTABLE LIPASE FROM LOCALLY ISOLATED YEAST

ABSTRACT

Demanding of thermostable lipase has increased for industrial processes where normally high reaction temperatures were used. *Pichia guilliermondii* strain RT (isolate RT) was previously isolated from rotten tomato at night market, Sri Serdang, Selangor. This newly isolated yeast was reported to produce an intracellular thermostable lipase. Previously, isolate RT produced low lipase activity and the characteristics of the thermostable lipase in this wild type yeast were still unknown. Therefore, finding of new lipase producers followed by optimization in order to improve and increase the efficiency of lipase production without increasing cost are very important. This study was concerned to optimize the physical factors for maximum lipase production and characterize the crude enzyme activity of the locally isolated yeast. For determining lipase activity, yeast was qualitatively screened on Rhodamine B-olive agar plate. Optimization of physical factors for maximum lipase production incorporated three different parameters: agitation rates, incubation temperature and incubation time. Then, characterization of optimum temperature and pH were evaluated to determine crude enzyme activity. This study revealed isolate RT showed maximum lipase yield at 200 rpm with incubation at 30 °C for 72 hours. The optimum temperature was at 75 °C and can retain nearly 50 % of initial activity after preincubated for 30 minutes at 75 °C. In addition, isolate RT was found to be optimum in alkaline condition at pH 9. This study indicated that favourable growth parameters and characterization of crude enzyme in wild type yeast were significant and played key roles in maximum lipase production and activity.

PENGELUARAN DAN PENCIRIAN LIPASE TERMOSTABIL DARIPADA YIS NEGARA TEMPATAN

ABSTRAK

Permintaan lipase termostabil telah meningkat untuk proses industri yang lazimnya suhu tindak balas yang tinggi digunakan. *Pichia guilliermondii* strain RT sebelum ini telah dipencilkan daripada tomato yang reput di pasar malam Sri Serdang Selangor. Selain itu, pencilan ini telah dilaporkan menghasilkan satu lipase termostabil intraselular. Sebelum ini, pencilan RT dilaporkan mengeluarkan lipase yang rendah dan ciri lipase termostabil dalam jenis yis liar ini tidak diketahui. Oleh itu, pencarian pengeluar lipase baru diikuti dengan pengoptimuman untuk memperbaiki dan meningkatkan kecekapan pengeluaran lipase tanpa meningkatkan kos adalah sangat penting. Kajian ini adalah berkenaan untuk mengoptimumkan faktor fizikal untuk pengeluaran lipase maksimum dan mencirikan aktiviti enzim mentah daripada yis pencilan dalam negara. Untuk menentukan pengeluaran lipase, yis telah disaring secara kualitatif di Rhodamine B-minyak zaitun plat agar. Pengoptimuman faktor fizikal untuk pengeluaran lipase maksimum merangkumi tiga parameter yang berbeza: kadar agitasi, suhu inkubasi dan masa inkubasi. Kemudian, pencirian suhu optimum dan pH dinilai untuk menentukan aktiviti enzim mentah. Kajian ini mendedahkan terpencil RT menunjukkan hasil lipase maksimum pada 200 rpm dengan inkubasi pada suhu 30 °C selama 72 jam. Lipase ini adalah termostabil pada 75 °C dan boleh mengekalkan hampir 50 % daripada aktiviti awal selepas diinkubasi selama 30 minit pada 75 °C. Di samping itu, terpencil RT didapati optimum pada pH alkali 9. Kajian ini menunjukkan bahawa parameter pertumbuhan yang menggalakkan dan pencirian enzim mentah dalam jenis yis liar adalah penting dan memainkan peranan utama dalam pengeluaran dan aktiviti lipase yang maksimum.

CHAPTER 1

INTRODUCTION

1.1 Background study

Lipases (triacylglycerol acylhydrolases, EC 3.1.1.3) is an ubiquitous enzyme and belong to the class of hydrolase which catalyse the hydrolysis of triacylglycerides in oil-water interface to glycerol and long-chain fatty acids (Saxena et al., 1999). Lipases are categorized as the third largest group of enzymes used in industrial processes after proteases and carbohydrates (Hasan et al., 2006). Thus, lipases are found to hold major commercial in biotechnology industry such as detergents formulations, oleochemical, food, dairy, paper manufacture, cosmetics and pharmaceuticals industry. In addition, lipase has speciality that capable to catalyse several reactions under controlled conditions such as ester synthesis, interesterification, transesterification, acidolysis, aminolysis and alcoholysis (Joseph et al., 2008). These reactions can only occurred in the presence of organic solvents. Besides, lipases also possess other interesting characteristic. Firstly, they generally display chemoselectivity, regioselectivity and stereoselectivity. Secondly, this hydrolytic enzyme is readily available in large quantities because can produce abundantly from lipase producing microorganism such as bacteria and fungi. Next, many lipase crystal structure have been solved. Moreover, lipases usually do not require cofactor when they catalyse side reactions. These characteristics make lipase most commonly used group of biocatalyst in organic chemistry. Lipases are widespread occurrence in nature such as from animals, plants and microorganism. Normally, lipases from microbial origin give greater significant effect in biotechnology industry because they can be produced in abundant, easy to be

manipulated genetically as compared to animals and plant. Other than that, they have rapid growth on the inexpensive media (Hasan et al., 2009). Nowadays, demanding of thermostable lipase is increasing because it is compatible at higher temperature and can possess higher activity at elevated temperature. On the other hand thermostable lipase resists to chemical denaturation, thus makes these hydrolytic enzyme valuable to industrial application where normally high reaction temperature and organic solvents are used.

Therefore, it is essential to produce the thermostable lipase in a large amount of quantity for the industry. Studies of the enzyme production and characterization are important to provide new lipase with the best quality and wider range of applications. Thermostable lipase can be isolated in wide array in nature for instance soil, hot spring, compost, effluent of palm oil mill (Rahman et al., 2007) and rotten fruit. Yeast other than bacteria is known to be the potential producer of intracellular and extracellular lipase. Moreover, yeasts are also easy to be grow, and handled than bacteria (Demain et al., 2009). From the previous study, a new yeast species has been discovered as lipase producing strain. It was isolated from rotten tomato and identified as *Pichia* sp. strain RT (Oslan et al., 2012).

1.2 Significance of study

Studies of production and characterization of thermostable lipase in locally isolated yeast could provide contribution for the large scale industrial application. This effort hope can fulfil the industry demand and strengthen country economy because many valuable foreign exchanges are spent on the import of industrial enzymes.

1.3 Objectives

This thesis is concerned with the following objectives:

1. To optimize the physical factors for optimum lipase production in locally isolated yeast.

2. To characterize the crude enzyme activity of the lipase produced in local yeast.

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