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THE USE OF A LOCALLY ISOLATED YEAST IN THE EXPRESSION OF RECOMBINANT W200R PROTEASE

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PENGESAHAN

Dengan ini adalah disahkan bahawa laporan bertajuk "The Use of a Locally Isolated Yeast in The Expression of Recombinant W200R Protease" telah disiapkan serta dikemukakan kepada Jabatan Biokimia oleh Fatin Syuhada Binti Abdul Razak sebagai syarat untuk kursus BCH 4999, projek dari Fakulti Bioteknologi dan Sains Biomolekul, Universiti Putra Malaysia.

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LIST OF ABBREVIATIONS

%	Percentage
℃	Degree celsius
g	Gram
mg	Milligram
mL	Milliliter
M	Molar
mM	Millimolar
min	Minutes
µl	Microliter
L	Liter
rpm	Rotation per minute
g	Relative centrifugal force in unit of x *g*
OD	Optical density
nm	Nanometer
h	hour
bp	base pair
Ω	ohm

ABSTRACT

Yeasts are well-received industrial hosts for the production of recombinant protein due to efficient expression. The thermostable F1 protease contained the mutation of tryptophan at residue 200, substituting arginine (W200R). The gene then was cloned into *Pichia pastoris* commercial yeast system. However, the production of W200R protease was low (38.71 U/mL) while the optimal incubation time for expression was recorded at 48h. Meanwhile, a newly isolated yeast, *Pichia* sp. strain SO, bearing 99% of similarity with *Pichia guillermondii* has been screened for protease activity and no thermostable protease was found. Hence, the recombinant mutated W200R protease gene was transformed into a new host, *Pichia* sp. SO through electroporation method with pPICZαB as the vector, similar to *Pichia pastoris* expression vector. Following this, seven colonies were screened for protease activity whereby colony R3 exhibited the highest protease secretion. R3 was selected for optimization which includes methanol induction and time course study. In the end, the cultivation of recombinant R3 in yeast peptone tryptic methanol (YPTM) medium supplemented with 2.5% (v/v) methanol demonstrated alleviated protease productivity with 100% relative activity after 18 hours of post induction. In conclusion, this study has shown that isolate SO is a promising host to express W200R protease and gain new insights for the development of a local yeast for the expression of heterologous recombinant protein.

ABSTRAK

Yis telah diterima sebagai sistem untuk penghasilan protein heterolog dalam industri. Protease F1 tahan panas mengandungi mutasi triptofan di residu 200, digantikan dengan arginin (W200R). Gen kemudiannya telah diklon ke dalam sistem yis komersial iaitu *Pichia pastoris*. Walau bagaimanapun, pengeluaran W200R protease adalah rendah (38.7 U/mL) manakala masa inkubasi optimum bagi penghasilan protease dicatatkan adalah 48 jam. Sementara itu, pencilan baru, strain *Pichia* sp. strain SO, yang mengandungi 99% persamaan dengan *Pichia guillermondii*, telah disaring untuk mengesan aktiviti protease dan hasilnya tiada protease tahan panas ditemui. Oleh itu, mutasi rekombinan protease W200R telah dimasukkan ke dalam hos baru, *Pichia* SO melalui kaedah elektroporasi, menggunakan vektor pPICZαB sama seperti *Pichia pastoris*. Berikutan itu, tujuh koloni telah disaring untuk aktiviti protease di mana koloni R3 menunjukkan penghasilan protease tertinggi. R3 telah dipilih untuk dioptimumkan, termasuk proses induksi metanol dan tempoh inkubasi. Akhirnya, rekombinan R3 dalam 2.5% (v/v) metanol ditambah yis pepton metanol tryptic (YPTM) menunjukkan produktiviti protease dengan 100% aktiviti relatif dalam masa 18 jam selepas induksi dengan 2.5% metanol. Kesimpulannya, penyelidikan ini telah menunjukkan kebolehan pencilan tempatan SO sebagai hos untuk mengekspreskan protease W200R. Oleh itu, pencilan tempatan SO mempunyai potensi untuk digunakan sebagai hos untuk mengekspreskan protein rekombinan.

CHAPTER 1: INTRODUCTION

Enzymes are important proteins that catalyzed many biochemical reactions. The uses of enzymes in industry, particularly in food, have been used long time ago. However, at that time enzymes were not in any pure or well characterized form. The enzyme industry as we see today is the result of rapid development seen primarily over the past four decades. The major type of enzymes used in industry are hydrolytic enzymes, and proteases is the dominant type of enzyme widely used in dairy and detergents industry (Kirk *et al*., 2000).

Thermostable enzymes have potential in biotechnological applications, because their inherent stability at high temperature, the presence of organic solvents and denaturing agents enable their use in process that normally restricts conventional enzymes. *Bacillus stearothermophilus* F1, a thermophile organism isolated from decomposed oil palm, was found to produce thermostable alkaline protease. This enzyme has potential to be commercialized because it is stable at high temperature (70 ^{0}C) and optimally active in alkaline (pH 9) (Rahman *et al*., 1994). An improvement for thermostability was made by construction of mutated F1 protease gene by substitution of tryptophan amino acid to arginine, created a mutant W200R protease with additional ion pairs that may increase the thermostability of the F1 protease (Rahman *et al*., 2011).

Yeasts are widely used for the expression of heterologous protein. *Saccharomyces cerevisiae* genome was fully sequenced and characterized. It has been used to express wide variety of recombinant protein (Celik, 2012). Since then methylotrophic yeasts has been used as the alternative hosts for heterologous protein expression for example *Hansenula polymorpha,* and *Pichia pastoris* (Law *et al*., 2011).

A new yeast was isolated and characterized from spoiled orange, and it was designed as isolate SO. The yeast was closely related to *Pichia guilliermondii* with 99% similarity. Zeocin was found to be a selection markers for the new isolated yeast SO (Oslan *et al*., 2012). *P. guilliermondii* and *P. pastoris* utilise methanol as a carbon source, and the AOX gene regulate heterologous protein expression in methylotrophic yeasts. Therefore it is expected that isolate SO can be used as host for expression of W200R protease by using AOX promoter.

Objectives

1. To screen for protease activity in isolate SO.
2. To express recombinant W200R protease in the new yeast system.
3. To optimize the production of W200R protease in the new yeast system.

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