UNIVERSITI PUTRA MALAYSIA

OPTIMIZATION OF RECOMBINANT VANADIUM-DEPENDENT HALOPEROXIDASE PRODUCTION FROM *GRACILARIA CHANGII*

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BACHELOR OF SCIENCE (HONS.) CELL AND MOLECULAR BIOLOGY

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SHAUNIE NG MAY QI

Thesis submitted to the Department of Cell and Molecular Biology, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, in fulfilment of the requirements for the Degree of Bachelor of Science (Hons) Cell and Molecular Biology

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ABSTRACT

Abstract of thesis presented to the Department of Cell and Molecular Biology in fulfilment of the requirement for the degree of Bachelor of Science (HONS.) Cell and Molecular Biology

OPTIMIZATION OF RECOMBINANT VANADIUM-DEPENDENT HALOPEROXIDASES PRODUCTION FROM *GRACILARIA CHANGII*

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June 2015

Chair: Associate Professor Dr. Ho Chai Ling, PhD

Faculty: Faculty of Biotechnology and Biomolecular Sciences

Vanadium-dependent haloperoxidases (VHPOs) belong to one of the three classes of haloperoxidases which can oxidize halogens including chloride (Cl^-), bromide (Br^-), and iodide (I^-). Chemical halogenation of organic compounds typically requires harsh conditions. Hence, biohalogenation has gained increasing research interest. As vanadium-dependent haloperoxidases are enzymes that catalyze halogenation of organic compounds, they are very valuable due to their potential applications in various industries as well as their stability and tolerance for different conditions. This study aims to optimize the recombinant protein expression conditions, for potentially

large scale production of vanadium-dependent bromoperoxidase 2 (GcVBPO2) that was previously isolated from *Gracilaria changii*. GcVBPO2 sequence in pET32a(+) was transformed into expression host *Escherichia coli* BL21(DE3) pLySs and induced at various temperatures. GcVBPO2 was found to be soluble when induced with 0.5mM Isopropyl β-D-1-thiogalactopyranoside (IPTG) for 16 hours in Luria-Bertani (LB) broth culture at 20°C. Purification of GcVBPO2 was performed using His-tag purification, and subsequently analysed with Western blot.

Keywords: protein expression, haloperoxidase, *Gracilaria changii*

ABSTRAK

Abstrak tesis yang dikemukakan kepada Jabatan Biologi Sel dan Molekul sebagai memenuhi keperluan untuk ijazah Bacelor Sains (Kepujian) Biologi Sel dan Molekul

PENGOPTIMUMAN PENGHASILAN PROTEIN RECOMBINAN *VANADIUM-DEPENDENT HALOPEROXIDASE* DARI *GRACILARIA CHANGII*

Oleh

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Pengerusi : Profesor Madya Dr. Ho Chai Ling
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Vanadium-dependent haloperoxidase (VHPO) tergolong dalam salah satu daripada tiga kelas *haloperoxidase* yang boleh mengoksidakan halogen termasuk klorida (Cl^-), bromida (Br^-), dan iodida (I^-). Halogenasi kimia sebatian organik biasanya memerlukan keadaan yang ketara. Oleh itu, kaedah biohalogenasi telah mendapat perhatian yang kian meningkat dalam penyelidikan. Oleh kerana *Vanadium-dependent haloperoxidase* merupakan enzim yang memangkinkan sebatian organik halogenasi, ia bernilai tinggi kerana aplikasinya dalam pelbagai industri serta kestabilan dan toleransinya dalam pelbagai keadaan. Kajian ini bertujuan untuk mengoptimumkan keadaan penghasilan protein rekombinan, bagi pengeluaran *vanadium-dependent bromoperoxidase peroxidase 2* (GcVBPO2) yang sebelum ini telah diasingkan

daripada *Gracilaria changii*, dalam skala besar. Urutan GcVBPO2 dalam pET32a (+) telah ditransformasikan ke dalam hos ungkapan *Escherichia coli* BL21 (DE3) pLySs dan dirangsangkan pada beberapa suhu. GcVBPO2 didapati terlarut apabila diinduksikan dengan 0.5mm Isopropyl β-D-1-thiogalactopyranoside (IPTG) selama 16 jam dalam kultur Luria-Bertani (LB) pada suhu 20°C. Purifikasi GcVBPO2 dilakukan dengan menggunakan penulenan *His-tag*, dan seterusnya dianalisis dengan *Western blot*.

Kata kunci: Ungkapan protein, *haloperoxidase*, *Gracilaria changii*

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APPROVAL

This thesis was submitted to the Department of Cell and Molecular Biology, Faculty of Biotechnology and Biomolecular Sciences and has been accepted as fulfilment of the requirement for the degree of Bachelor of Science (HONS.) Cell and Molecular Biology. The member of the Supervisory Committee was as follows:

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Date:

DECLARATION

Declaration by undergraduate student

I hereby confirm that:

- this thesis is my original work;
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Shaunie Ng May Qi, 161398

Declaration by Supervisor

This is to confirm that:

- the research conducted and the writing of this thesis was under supervision

_____

Associate Professor Dr. Ho Chai Ling

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LIST OF ABBREVIATIONS

HPO	Haloperoxidase
VHPO	Vanadium-dependent haloperoxidase
VBPO	Vanadium-dependent bromoperoxidase
VCPO	Vanadium-dependent chloroperoxidase
VIPO	Vanadium-dependent iodoperoxidase
Gc	*Gracilaria changii*
kDa	Kilo Dalton
cDNA	Complementary deoxyribonucleic acid
DMSO	Dimethyl sulfoxide
LB	Luria-Bertani
PCR	Polymerase chain reaction
IPTG	Isopropyl β-D-1-thiogalactopyranoside
OD	Optical density
SDS-PAGE	Sodium dodecyl sulphate-polyacrylamide gel electrophoresis
TEMED	Tetramethylethylenediamine
APS	Ammonium persulfate
PVDF	Polyvinylidene fluoride
DAB	3,3'-Diaminobenzidine
HRP	Horseradish peroxidase
dH_2O	Distilled water
µg	Microgram
µl	Microliter

ml	Milliliter
mM	Millimole
nm	Nanometer
×g	G force

CHAPTER 1

INTRODUCTION

1. Introduction

Gracilaria changii (*G. changii*) is a red seaweed known for its high agar yield and gel strength. It is found abundantly in Malaysia (Phang et al., 1996). Seaweeds are plant-like macro marine algae that live in coastal areas, attached to rocks or other substratum. Seaweeds have many applications as food, medicine, and others (Chapman, 1970).

Similar to sponges, bacteria and various other marine organisms, seaweeds synthesize organohalogens, organic compounds containing at least one halogen. In the presence of hydrogen peroxide, haloperoxidases (HPOs) can oxidize halogens. HPOs may have a role in the defence system of seaweeds since some of the halogenated compounds such as hypobromous acid (HOBr) are bacteriacidal (Wever et al., 2001).

Vanadium-dependent haloperoxidases (VHPOs) belong to one of the three classes of HPOs. VHPOs are named according to the most electronegative halogen they can oxidize. Vanadium-dependent chloroperoxidase (VCPO) is a vanadium enzyme that can oxidize chloride (Cl^-), bromide (Br^-) and iodide (I^-); vanadium-dependent bromoperoxidase (VBPO) can oxidize bromide (Br^-) and iodide (I^-); while vanadium-dependent iodoperoxidase (VIPO) can only oxidize iodide (I^-) (Colin et al., 2003). VHPOs can halogenate organic compounds *in vivo* thus were suggested to serve as a component of an allelopathic defense system (Johnson et al., 2011). These halogenated compounds in algae have antimicrobial and bioactive properties (Butler et

al., 2004). Apart from having important defensive roles in nature, VHPOs are also potentially useful in various industries in the catalyzation of halogenated organic compounds (Baharum et al., 2013). As such, VHPOs are very valuable and of high research interest due to their potential applications in various industries as well as their stability and tolerance for different conditions (de Boer et al., 1987; Renirie et al., 2003). They are used to catalyze oxygen transfer to organic compounds in the presence of vanadium and hydrogen peroxide, producing valuable halogenated natural products (Chen et al., 2008).

VBPOs have previously been isolated from various marine algae, expressed and characterized (Baharum et al., 2003; Colin et al., 2003; de Boer et al., 1986). GcVBPO1 from *G. changii* was expressed in *Escherichia coli* (*E. coli*) BL21 (DE3) pLysS, fused with His-tag as a recombinant protein of 84kDa, and was found to be able to catalyze the oxidation of Br^- and I^- in the presence of vanadium. pH values close to that of seawater were found to be optimum for GcVBPO1 activity (Baharum et al., 2003). Since the studies on VBPOs from marine algae are still limiting (Baharum et al., 2003), this study aims to optimize the culture conditions following transformation of pET32a(+) containing the open reading frame (ORF) of GcVBPO2 from *G. changii* into *E. coli* BL21 (DE3) to maximize the protein yield of GvVBPO2. The findings would be potentially useful to determine the most efficient way to produce these proteins in large scale for industrial usage.

Foreign genes can be introduced into cells and be expressed to produce recombinant proteins. In a previous study, the cDNA encoding GcVBPO2 from *G. changii* was succesfully cloned into pET32a(+) vector with His-tag at the N-terminus. Vector

pET32a under the regulation of T7 promoter can be induced using isopropyl β-D-1-thiogalactopyranoside (IPTG) for protein expression (Peti et al., 2007). Since gene expression is affected by the culture environment (López-Maury et al., 2008), different culture conditions with different parameters will affect the yield and quality of the expressed protein. This study aims to transform GcVBPO2 into expression host *Escherichia coli* BL21 (DE3) pLySs, and to optimize the expression of recombinant GcVBPO2 under various conditions.

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