GENE AMPLIFICATION AND SEQUENCING OF HEMICELLULASE USING CULTURE DEPENDENT TECHNIQUE

NUR NAZWA BINTI SAIMON

UNIVERSITI TEKNOLOGI MALAYSIA

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NUR NAZWA BT SAIMON

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DEDICATION

To my beloved family members and friends.

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ABSTRACT

The present study aims to discover hemicellulase genes from thermophilic bacteria of *Anoxybacillus gonensis* G2^T and *Halothermobacillus malaysiensis* RA using culture dependent technique. The hemicellulase genes with the amplicon size of 2118 bp, 1143 bp, 1554 bp and 2190 bp from *A. gonensis* G2^T and *H. malaysiensis* RA were successfully obtained. Bioinformatics analyse of primary, secondary structure and tertiary structure of these proteins were performed by using bioinformatics tools. Beta-xylosidase from *A. gonensis* G2^T and alpha-glucuronidase from *H. malaysiensis* RA are intracellular proteins. While, endo-1,4-beta-xylanase and alpha-N-arabinofuranosidase from *H. malaysiensis* RA have signal peptide and secreted as extracellular protein. I-TASSER and Swiss Model server were used to model the tertiary structure of hemicellulase. Good structures were generated after energy minimization through YASARA server. Using Neighbor-Joining method, the phylogenetic analysis reveals that beta-xylosidase from *A. gonensis* G2^T (bxAg) is closely related with beta-xylosidase from *Geobacillus* sp. WSUCF1; endo-1,4-beta-xylanase from *H. malaysiensis* RA (ebxHm) is distantly related with endo-1,4-beta-xylanase from *Xanthomonas translucens* pv translucens, family GH10 *Maribacter dokdonensis* DSW-8 and *Saccharicrinis fermentans* DSM 9555JCM 21142; alpha-N-arabinofuranosidase from *H. malaysiensis* RA (anaHm) is closely related with alpha-N-arabinofuranosidase from *Gemmatimonas* sp. SG8 28; and alpha-glucuronidase from *H. malaysiensis* RA (agHm) is distantly related with alpha-glucuronidase from *Sphingomonas* sp.WG, *Caulobacter vibrioides*, *Xanthomonas oryzae* pv. oryzae and *Stenotrophomonas maltophilia*.

ABSTRAK

Kajian bertujuan untuk mencari gen hemicellulase daripada bakteria thermophilik, *Anoxybacillus gonensis* G2^{T} dan *Halothermobacillus malaysiensis* RA menggunakan pendekatan bergantung kepada pembiakan bakteria. Gen hemicellulase dengan saiz produk 2118 bp, 1143 bp, 1554 bp dan 2190 bp dari *A. gonensis* G2^{T} dan *H. malaysiensis* RA telah berjaya didapati. Analisis bioinformatik pertama, kedua dan struktur protin ketiga dibina dengan menggunakan beberapa kaedah bioinformatik. Beta-xylosidase dari *A. gonensis* G2^{T} dan alpha-glucuronidase dari *H. malaysiensis* RA adalah protin yang dirembeskan didalam sel. Manakala, endo-1,4-beta-xylanase dan alpha-N-arabinofuranosidase dari *H. malaysiensis* RA mempunyai signal peptide dan dirembeskan sebagai protin luaran. I-TASSER dan Swiss Model telah digunakan untuk membina protin hemicellulase struktur ketiga. Struktur yang baik dapat dihasilkan selepas merendahkan tenaga melalui laman YASARA. Menggunakan kaedah Neighbor-Joining, analisis phylogenetik mendedahkan beta-xylosidase dari *A. gonensis* G2^{T} (bxAg) mempunyai persamaan yang rapat dengan beta-xylosidase dari *Geobacillus* sp. WSUCF1; endo-1,4-beta-xylanase dari *H. malaysiensis* RA (ebxHm) mempunyai persamaan yang jauh dengan endo-1,4-beta-xylanase dari *Xanthomonas translucens* pv translucens, family GH10 *Maribacter dokdonensis* DSW-8 dan *Saccharicrinis fermentans* DSM 9555JCM 21142; alpha-N-arabinofuranosidase dari *H. malaysiensis* RA (anaHm) mempunyai persamaan yang dekat dengan alpha-N-arabinofuranosidase dari *Gemmatimonas* sp. SG8 28 dan alpha-glucuronidase dari *H. malaysiensis* RA (agHm) mempunyai persamaan yang jauh dengan alpha-glucuronidase dari *Sphingomonas* sp.WG, *Caulobacter vibrioides*, *Xanthomonas oryzae* pv. oryzae dan *Stenotrophomonas maltophilia*.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

3D	-	three-dimensional
A.gonensis	-	*Anoxybacillus gonensis*
agHm	-	alpha-glucurosidase from *Halothermobacillus malaysiensis* RA
anaHm	-	alpha-N-arabinofuranosidase from *Halothermobacillus malaysiensis* RA
BLAST	-	Basic Local Alignment Search Tool
bxAg	-	Beta-xylosidase from *Anoxybacillus gonensis* $G2^T$
CAZy	-	Carbohydrate-Active enZymes
dH_2O	-	distilled water
DNA	-	Deoxyribonucleic acid
dNTP	-	Deoxyribonucleotide Triphosphate
ebxHm	-	endo-1,4-beta-xylanase from *Halothermobacillus malaysiensis* RA
EC	-	Enzyme Commision
E.coli	-	*Escherichia coli*
etc	-	et cetera
ExPASy	-	Expert Protein Analysis System
F1	-	forward primer
GH	-	glycosidase hydrolase
GRAVY	-	Grand Average of Hydropathy

H. malay-siensis RA	-	*Halothermobacillus malaysiensis* RA
I-TASSER	-	Iterative Threading ASSEmbly Refinement
MEGA 7	-	Molecular Evolutionary Genetics Analysis
NCBI	-	National Centre of Biotechnology Information
NJ	-	Neighbor-joining
OH^-	-	Hydroxide ion
OligoCalc	-	Oligonucleotide Properties Calculator
PCR	-	Polymerase Chain Reaction
PDB	-	Protein DataBank
R1	-	reverse primer
RAST	-	Rapid Annotation using Subsystem Technology
RNase	-	ribonuclease
rRNA	-	ribosomal RNA
sp.	-	species
TAE	-	Tris-Acetate-EDTA
TIM	-	Triosephosphate isomerase
Tris	-	Tris(hydroxymethyl)methylamine
UniProt	-	Universal Protein resource
YASARA	-	Yet Another Scientific Artificial Reality Application

LIST OF SYMBOLS

α	-	alpha
β	-	beta
>	-	greater than
<	-	less than
%	-	percentage
°C	-	degree celcius
$(\beta/\alpha)_8$	-	TIM barrel
®	-	registered mark
™	-	unregistered mark
∞	-	infinity
bp	-	base pair
g	-	gram
kPa	-	kilopascal
ml	-	mililiter
ng/µl	-	nanogram per microliter
nm	-	nanometer
pH	-	potential of Hydrogen
pI	-	Isoelectric point
rpm	-	revolutions per minute
sec	-	seconds
T	-	temperature

µg/Ml	-	microgram per mililiter
µl	-	microliter
µm	-	micrometer
V	-	voltage
(v/v)	-	volume per volume
(w/v)	-	weight per volume

LIST OF APPENDICES

CHAPTER 1

INTRODUCTION

1.1 Background of study

Hemicellulase is one of the most versatile and viable enzymes; for the degradation of hemicellulose complex in plant polysaccharides. There are four main hemicellulase enzymes (i) endo-1,4-beta-xylanases (EC 3.2.1.8) and (ii) beta-xylosidases (EC 3.2.1.37) which are required for the degradation of xylan backbone; (iii) alpha-N-arabinofuranosidase (EC 3.2.1.55), and (iv) alpha-glucuronidase (EC 3.2.1.139) for completing degradation of xylan (Amore *et al.*, 2013).

Microbial origin, in particularly thermophiles produce thermostable enzymes that suit industrial applications. Thousands of thermostable enzymes that have been identified and used commercially as these enzymes exhibit unique characteristics (Chirumamilla *et al.*, 2001). Enzymes that are stable and can withstand high temperature is a major characteristics in determining its use in various biotechnology applications (Markov *et al.*, 2006). Biotechnology industries or white biotechnology commonly used high temperature, thus heat labile enzymes or biocatalysts that originated from mesophilic environment are less suitable. Changing the selection of microorganism; from mesophile microorganism to those of thermophile microorganism could lead to increased product yield, reduce viscosity, and decrease contamination during bioprocessing as enzymes stay active at high temperature and in organic solvent.

Most current researches on hemicellulases focused on fungi origins. Thermostable hemicellulase from thermophilic prokaryotes is increasingly becoming of interest to industry, thus, encouraging further study on enzymes from thermophilic microorganisms with the optimal activity of >55°C. Culture dependent technique to mine thermostable enzymes have provided a powerful tool in determining diversity of enzymes.

Discovery of hemicellulase gene from thermophilic bacteria *Anoxybacillus gonensis* G2^{T} and from novel genus, *Halothermobacillus malaysiensis* RA have not been studied. Hence, in this study, hemicellulase gene that present in pure culture of *A. gonensis* G2^{T} and *H. malaysiensis* RA was performed. Beta-xylosidase from *A. gonensis* G2^{T}, endo-1,4-beta-xylanase from *H. malaysiensis* RA, alpha-N-arabinofuranosidase *H. malaysiensis* RA and alpha-glucuronidase from *H. malaysiensis* RA were successfully amplified and sequenced, and the protein sequences undergone further bioinformatics analysis. The analyses involved primary structure analysis, secondary structure prediction, tertiary structure prediction, validation and estimation of homology model of protein and phylogenetic tree.

1.2 Objectives

The objectives of this study were to:

i. Amplify hemicellulase gene from pure culture of *A. gonensis* G2^{T} and *H. malaysiensis* RA.

ii. Analyse primary, secondary prediction structure and tertiary prediction structures of selected hemicellulases by using bioinformatics tools.

1.3 Scope of Study

i. Genome from *A. gonensis* $G2^T$ and novel genus *H. malaysiensis* RA were extracted and their hemicellulase gene were amplified by PCR.

ii. Amplicon of hemicellulase genes were visualized by gel electrophoresis.

iii. Hemicellulase gene sequence was confirmed through gene sequencing and were analyzed using bioinformatics tools including primary analysis, secondary prediction structure, tertiary prediction structure and validation of structure.

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