IN SILICO DESIGNING OF THERMOPHILIC *BACILLUS CIRCULANS* XYLANASE

BY

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ABSTRACT

Applications of Xylanase nowadays have become very important and widely used by the textile industry as the enzyme is safe and environmental friendly besides having more advantages compared to the use of chemical reagents. Previously, chlorine was widely used in the paper whitening process in pulp and paper industry. Because of the huge size of this industry, chlorine residues released to the environment became a huge environmental threat. Due to the hazardous effect of chlorine, xylanases have been proposed to decrease the usage of chlorine in pulp and paper industry. However, xylanases have to be functional at 60-70 °C which is the temperature of the incoming pulp for the bleaching operation. Generally, xylanases have an optimal activity at 55–60 °C. *Bacillus circulans* xylanase (BcX) has been proposed to be used in the pulp and paper industry because of its small size (20.4kDa), but due to the high temperature used in the process BcX will not survive. In this research, Molecular Dynamics simulation (MD) was used as an *in silico* approach to design a thermostable BcX that can survive at high temperatures. Experimentally proven thermostable *Bacillus subtilis* xylanase (BsX) is used as a reference system to identify the structural and dynamic factors responsible for the thermostability of mutant BsX. Similar structural and dynamic attributes of BsX are incorporated into BcX by suitable mutations to produce similar thermostability behavior in BcX. Molecular Dynamics simulations of BsX and BcX were performed to identify structural and dynamic factors influencing thermostability. The assumption was, if the proposed mutant has the same attributes of structural interactions and dynamic behavior as those of thermostable mutant BsX, then the proposed mutant BcX would be thermostable as well. Both BsX and BcX were examined by MD at 318 K and 338 K. Thermostability of mutant BsX was found to be contributed by the stability of the overall structure analyzed by root mean square deviation (RMSD), the existence of an important salt bridge within the active site, and an increase in the hydrophobic area. This research reported similar structural and dynamic factors between experimentally proven thermostable BsX and the proposed thermostable mutant BcX. By comparing the trends obtained in MD simulation, mutant BcX is expected to have similar or lesser thermostability compared to mutant BsX. Major factors contributing to the thermostability of BsX were also observed in mutant BcX. However, changes in the number of hydrogen bonds in mutant BcX were slightly different compared to those in BsX. Further *in silico* mutantions need to be carried out to pinpoint the thermostability factors in BcX.

خلاصة البحث

تطبيقات الأنزيم Xylanase أصبحت مهمة ومستخدمة على نطاق واسع في صناعة النسيج حيث أن الأنزيم آمن وصديق للبيئة وله فوائد أكثر بالمقارنة مع استخدام المواد الكيماوية. سابقا, كانت مادة الكلورين تستخدم في عملية تبييض الورق, ولكبر حجم صناعة الورق وما يترتب عليها من استخدام كبير لمادة الكلورين فقد أصبحت تشكل خطرا على البيئة. بسبب تأثير مادة الكلورين الخطير تم اقتراح استخدام Xylanase للتقليل من استخدام الكلورين في صناعة الورق. ولكن يجب أن يكون (Xylanase) فعالاعند درجة حرارة 60-70 درجة مئوية والتي هي درجة حرارة لب الورق عند عملية التبييض. بشكل عام, الفاعلية القصوى لأنزيمات Xylanase هي عند درجة حرارة 55-60 درجة مئوية. تم اقتراح أنزيم (Xylanase) من الميكروب *Bacillus circulans* (BcX) لاستخدامه في صناعة الورق لصغر حجمه (20.4kDa) ولكن بسبب الحرارة العالية المستخدمة في عملية التصنيع فإن الأنزيم لن ينجو. في هذا البحث تم استخدام محاكاه الديناميكا الجزيئية (MD simulation) كطريقة حسابية لتصميم BcX متحمل للحرارة حتى ينجو عند درجات حرارة عالية. تم استخدام أنزيم *Bacillus subtilis* xylanase (BsX) والذي تم التحقق مخبريا من تحمله للحرارة كنظام مرجعي لتحديد العوامل الهيكلية والديناميكية المسؤولة عن تحمل أنزيم BsX للحرارة. تم اضافة خواص هيكلية وديناميكية مستوحاه من BsX لأنزيم BcX عن طريق طفرات مناسبة للحصول على سلوك حراري مشابه. تم افتراض أنه لو كان للأنزيم المصمم الجديد نفس الخواص الهيكلية والديناميكية كتلك الموجودة في BsX فإن الأنزيم الجديد سيكون متحمل للحرارة ايضا. تم فحص كلا الأنزيمين BsX و BcX عن طريق محاكاه الديناميكا الجزيئية عند درجة حرارة 318 و 338 درجة كيلفن. وجد أن التحمل الحراري لأنزيم BsX هو نتيجة ثبات واستقرار هيكل الأنزيم الكلي والذي تم فحصه عن طريق تحليل Root Mean Square Deviation (RMSD), وجود salt bridge مهم في المنطقة الفعالة للأنزيم, بالإضافة لزيادة في المنطقة المنفرة للماء (hydrophobic area). أورد هذا البحث عوامل هيكلية وديناميكية مشابهة بين أنزيم BsX المتحمل للحرارة والأنزيم المصمم الجديد. بمقارنة التوجهات التي تم الحصول عليها عن طريق محاكاه الديناميكا الجزيئية فإن من المتوقع أن للأنزيم الجديد تحمل مماثل أو أقل للحرارة بالمقارنة مع BsX. تم رصد عوامل اساسية مساهمة في تحمل الحرارة عند BsX في أنزيم BcX الجديد. ولكن, التغيرات في عدد الروابط الهيدروجينية في BcX وجدد مختلفة قليلا بالمقارنة BsX. طفرات حسابية أخرى يجب أن تجرى للتحديد بدقة عوامل تحمل الحرارة في BcX.

APPROVAL PAGE

I certify that I have supervised and read this study and that in my opinion, it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Master of Science (Biotechnology Engineering).

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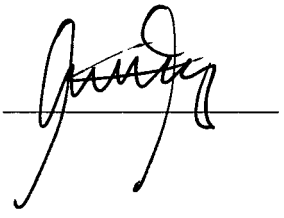
Dean, Kulliyyah of Engineering

DECLARATION

I hereby declare that this dissertation is the result of my own investigations, except where otherwise stated. I also declare that it has not been previously or concurrently submitted as a whole for any other degrees at IIUM or other institutions.

Muaz Abdul Hadi

Signature  Date 20 JULAI 2012

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LIST OF ABBREVIATIONS

MD	Molecular dynamics
BsX	*Bacillus subtilis* Xylanase
BcX	*Bacillus circulans* Xylanase
RMSD	Root Mean Square Deviation
SASA	Solvent Accessible Surface Area
PSF	Protein Structure File
PME	Particle Mesh Ewald
CHARMM	Chemistry at HARvard Molecular Mechanics
NAMD	NAnoscalable Molecular Dynamics
VMD	Visual Molecular Dynamics
PDB	Protein Data Bank
RCSB	Research Collaboratory for Structural Bioinformatics

LIST OF SYMBOLS

σ_i	solvation parameter
$\epsilon(r)$	poison-dependent dielectric
$\Psi(r)$	electrostatic potential
$\rho^f(r)$	charge density of solute
$\lambda(r)$	factor for the position-dependent accessibility of position *r*
ε_0	permittivity of free space
ε	dielectric constant
q_i	electrostatic charge of particle *i*
r_{ij}	distance between particles *i* and *j*
S_{ij}	score of amino acids *i* and *j*
p_{ij}	probability of amino acids *i* and *j* replacing each other in a homologous sequence
F_i	force on particle *i*
m_i	mass of particle *i*
a_i	acceleration of particle *i*
k_c	electrostatic constant
U	total potential energy
U_{bonded}	total bonded potential energy
U_{unbonded}	total unbonded potential energy
ζ	collision frequency
γ	friction coefficient

CHAPTER ONE

INTRODUCTION

1.1 OVERVIEW OF STUDY

Enzyme technology is an alternative to chemical technology in industrial processes and has already been developed and used widely. Due to the large scale production of a variety of products in the chemical technology field, the world is threatened by the disposal of hazardous wastes and serious problems caused by industrial effluents such as effluents from the paper industry and toxic wastes from the rubber industry. Even though the chemical technology has boosted the production capacity, however, its side effects are hard to tolerate any longer. The need for safer and environmental friendly technologies has become essential. Therefore enzyme technology alternatives over polluting chemical technologies are considered worthwhile and practical for future applications. As enzymes originated from nature, limitations of their properties such as temperature and pH constitute factors that obstruct the total substitution of chemical technology by enzymes. Compatibility of enzymes to industrial processes can be improved by protein engineering techniques; specifically the mutagenesis approach (Kazlauskas and Bornscheuer, 2009). Enzymes can be modified or altered to achieve desired enzymatic properties for specific purposes.

Protein engineering involves high costs if it is to be carried out experimentally. Mutational processes, for instance, can be preliminarily performed computationally (*in silico*) to study their effect and to see whether mutations have positive or negative effect on the structure of the protein. By doing this, the scope of mutations can be

narrowed down to one that is predicted to contain promising points of mutation (Noorbatcha et al., 2009). For example, to do a mutation on a structure with 300 residues, every single amino acid residue has the possibility to be substituted with nineteen other amino acids. Therefore, it is a tedious task to perform random mutations experimentally. However, computer simulations help reduce the cost and time of trial and error of this process by providing insights to narrow down the number of possible promising mutations.

This study describes the *in silico* designing of a thermostable *Bacillus circulans* xylanase (BcX). Molecular Dynamics (MD) simulation is used to investigate the dynamic behavior of experimentally proven thermostable *Bacillus subtilis* xylanase (BsX) by comparing the structures of the wild type with mutant enzyme at different temperatures to study the effect temperature has on the enzyme structure and its stability. The information learned from MD simulations of BsX was then taken to be a reference to design a new thermostable BcX.

1.2 PROBLEM STATEMENT AND ITS SIGNIFICANCE

Xylanases have been proposed for applications in the pulp and paper industry. They serve as an effective bio-reagent used for bio-bleaching, which would replace the poisonous chlorine compounds commonly used to achieve pulp brightness. In addition, xylanases are used in the manufacture of high-quality paper products. Meanwhile, xylanases are required to function at temperatures ranging from 60-70 °C, which is the temperature of pulps at the time of bleaching process, under high alkaline conditions (Srinivasan and Rele, 1999). However, most xylanases display optimal activity at 55-60 °C and pH 5.0-7.0 (Subramaniyan and Prema, 2002)

Family 11 xylanases have several advantages over other xylanases when used in the pulp industry. Most of family 11 xylanases are smaller in size compared to xylanses from other families. Having an enzyme with small size helps penetrate the pulp fibers to release xylan from the pulp and improve its bleaching. Furthermore, unlike xylanases from other families, xylanases from family 11 only hydrolyze xylan but do not hydrolyze cellulose at the same time. The hydrolysis of cellulose damages the pulp and is unacceptable in commercial mills.

Bacillus circulans xylanase, from family 11, makes a very good candidate for industrial use as it has a small size and an optimum pH of 7.0-8.0 which meets the requirements of the bleaching process (Torronen and Rouvinen, 1995). Nevertheless, the enzyme has an optimum temperature of 45 °C which is a limitation to its potential use. As a result, it is crucial to design a new *Bacillus circulans* xylanase enzyme that (in addition to its ideal size and pH profile) has an optimum temperature of 60-70 °C. Employing computational methods to improve the thermostability of xylanase can save a lot of time and cost often invested in experimental methods.

1.3 RESEARCH OBJECTIVES

The ultimate objective of this research is to design, using computational methods, a mutant *Bacillus circulans* xylanase that is functional at temperatures of 60-70 °C to be used in the bio-bleaching industry. The specific objectives of this study are as follows:

a) To conduct *in silico* mutations on *Bacillus circulans* xylanase (BcX) to improve its thermostability.

b) To conduct Molecular Dynamics (MD) simulations on both BsX and BcX at different temperatures to study the dynamic behavior of the enzymes.

c) To analyze the interactions and structural properties of the experimentally proven thermostable *Bacillus subtilis* xylanase (BsX) and to identify the factors responsible for thermostability.

1.4 RESEARCH METHODOLOGY

This study employs computational methods to achieve its objectives. Various methods and tools will be used to study the structure and different static and dynamic properties of BsX and BcX. Mathematical algorithms and force field calculations (such as CHARMM force field) employed in Molecular Dynamics (MD) simulation (Figure 1.1) will be used to study the changes in behavior of enzymes under different temperatures. Improvements in enzyme stability would be reflected in its dynamic behavior represented by the Root Mean Square Deviation (RMSD) of the atoms and residues of the protein, which will be generated using MD simulation. RMSD values will be calculated for backbone, beta-sheet, alpha-helix, turn, and coil atoms. In addition, radius of gyration of the molecule will be calculated to analyze the compactness of the structure.

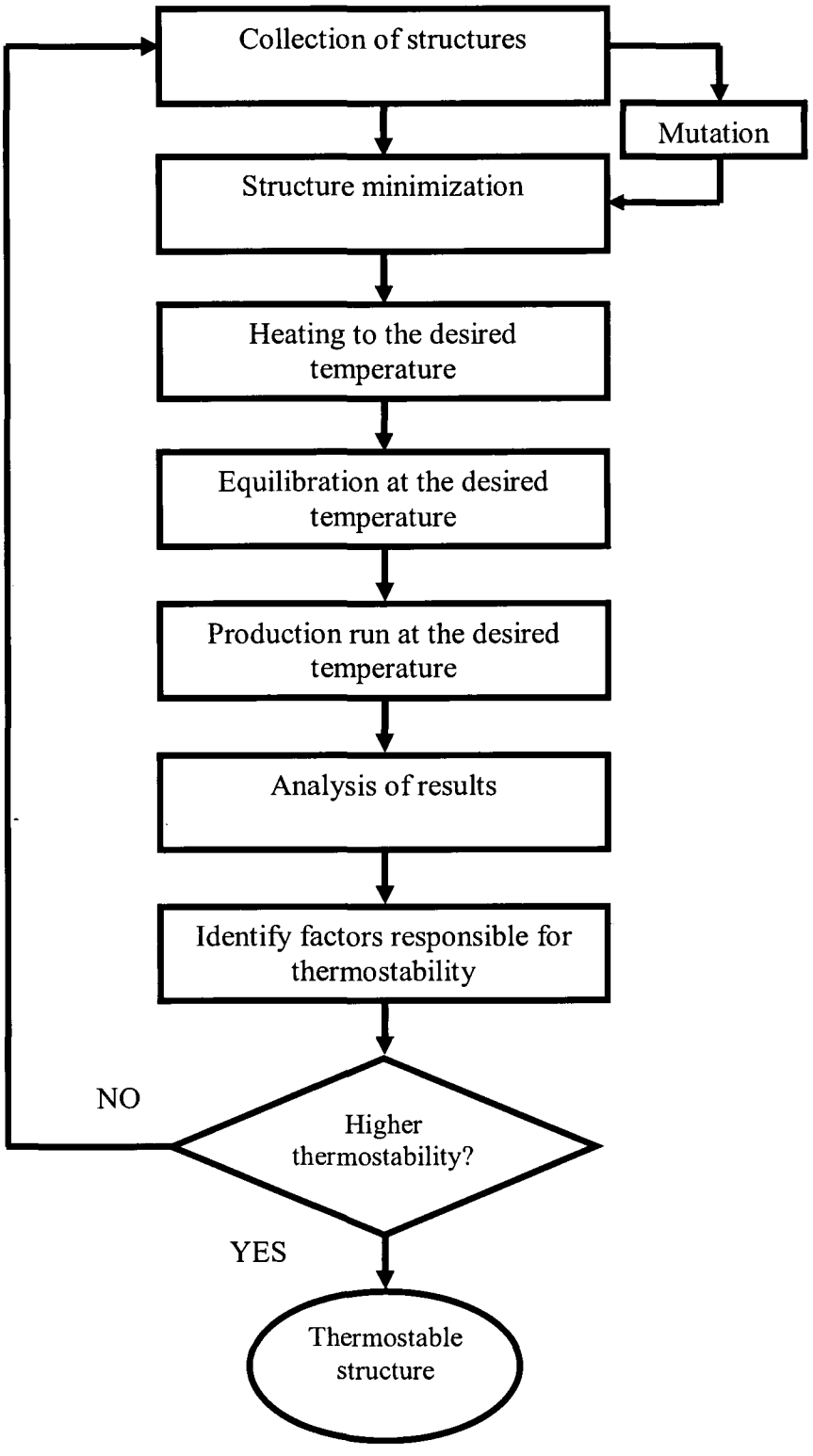

Figure 1.1: Flow chart of research methodology for overall process of the study