IN-SILICO STRUCTURAL ANALYSIS OF A BET-AMYLASE FROM *Clostridium thermosulfuregenes*

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DEDICATION

This thesis is dedicated to my father and mother who taught me that the best kind of knowledge to have is that which is learned for its own sake.

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ABSTRACT

β-amylase is a hydrolytic enzyme that is involved in breaking down starch and producing energy. Since the discovery of β-amylase, it has been applied in various applications especially in the food industry. In this study, a novel β-amylase from *Clostridium thermosuluregen*, a thermophilic anaerobic bacterium that ferments its extracellular emulsion to ethanol at 62 ℃ was modelled and studied using bioinformatics tools and compared with *B. cereus* β-amylases that functions at mesophilic conditions. The results showed that the overall structural conformations, secondary structures, and important residues involved in active and binding sites were identified in both proteins. The results revealed that the modelled β-amylase of *C. thermosulfuregen* is very similar with respect to the global conformation, location of active and binding sites. Both proteins showed identical structural domains with the thermophilic variant possessing a high percentage of hydrophobic amino acid residues, polar amino acid residues, and differences in secondary composition such as loops and beta sheets as the potential evolutionary thermal adaptations that make it stable enzyme that functions up to 70 ℃. The results suggest that the thermal stability are not dependent on one single unique mechanism and may use one or a combination of the mechanisms to sustain its structural conformation at a higher operating temperature. Overall, considering the common properties of this modelled protein with the β-amylase of *B. cereus*, it can be assumed that if the β-amylase of *C. thermosulfuregen* were expressed *in-vitro*, it would produce a stable protein that possesses the hydrolysis function for *C. thermosulfuregen* to break down the starch and sugar formation.

ABSTRAK

β-amilase adalah enzim hidrolitik yang terlibat dalam pemecahan kanji dan penghasilan tenaga. Sejak penemuan β-amilase, ia telah gunapakai dalam pelbagai aplikasi terutama dalam industri makanan. Dalam kajian ini, β-amylase baru dari *Clostridium thermosuluregen*, bakteria anaerobik termofilik yang berupaya menapai emulsi ekstraselularnya menjadi etanol pada 62 ℃ telah dimodelkan dan dikaji menggunakan kaedah bioinformatik dan dibandingkan dengan enzim β-amilase dari *B. cereus* yang berfungsi pada suhu mesofilik. Hasil kajian menunjukkan bahawa keseluruhan konfigurasi struktur, struktur sekunder dan residu penting yang terlibat dalam tapak aktif dan pengikat adalah sama pada kedua-dua protein. Hasil kajian menunjukkan bahawa β-amilase *C. thermosulfuregen* yang dimodelkan sangat mirip utic keselumahan konformasi global, lokasi tapak aktif dan tapak pengikatan. Kedua-dua protein *B. cereus* menunjukken domain struktur yang sama dengan varian termofilik yang mempunyai peratusan tinggi residu asid amino hidrofobik, residu asid amino polar dan perbezaan komposisi sekunder seperti “gelung” dan beta sebagai evolusi penyesuaian suhu tinggi yang mumpu berpotensis menjadikannya satu enzim stabil yang berfungsi hingga 70 ℃. Keputusan menunjukkan bahawa kestabilan terma tidak bergantung pada satu mekanisme unik dan mungkin menggunakan gabungan bebrapa mekanisme untuk mengekalkan konformasi strukturnya pada suhu operasi yang lebih tinggi. Secara keseluruhan, dengan mempertimbangkan sifat umum protein yang dimodelkan ini dengan β-amilase *B. cereus*, dapat diandaikan bahawa jika β-amilase *C. thermosulfuregen* dinyatakan secara *in-vitro*, ia akan menghasilkan protein stabil yang memiliki fungsi hidrolisis untuk pemecahan kanji dan penphasilaa gula.

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LIST OF ABBREVIATIONS

PDB	-	Protein Data Bank
3D	-	Three-Dimensional
1D	-	One-Dimensional

LIST OF SYMBOLS

β	-	Beta
α	-	Alpha
℃	-	Degree Celsius
kDa	-	Kilo Dalton
Da	-	Dalton
γ	-	Gamma
Å	-	Angstrom

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CHAPTER 1

INTRODUCTION

1.1 Background of study

The β-amylase (EC 3.2.1.2) enzyme, or known as α-1,4-glucan maltohydrolase, is an exo-type enzyme and catalyses the β-anomeric maltose from the non-reducing end of starch to produce maltose. It belongs to the family 14 of the glycoside hydrolase (GH) (Schomburg et al., 2002). β-amylase can be found in plants, fungi, and bacteria. The β-amylase enzyme has important applications in industries due to its saccharogenic activity. It is useful in the pharmaceutical industry due to its digestive activity, used for the preparation of malto-oligosaccharides, a reagent that is used for research, as nutrients in the health industry substitute for other saccharides and used in the production of the malto-oligomer mixture, an ingredient used for the preparation of chewing gum, buttercream, cakes, jellies, canned cocoa and fruit drinks (Saini et al., 2017).

The characterization of β-amylase and determination of its 3D structure has been documented for a range of species; from bacteria *Bacillus cereus* (Hirata et al., 2004), *Glycine max* (soybean) (Mikami et al., 1999), *Ipomoea batatas* (sweet potato) (Cheong et al., 1995), *Hordeum vulgare* (barley) (Rejzek et al., 2011), and *Triticum aestivum* (wheat) (Hofer et al., 2019). All of these β-amylase structures share common characteristics that suggest highly conserved regions especially at the active centre in the region of $(\alpha/\beta)_8$ barrel. Only the tertiary structure barrel configuration differs between that of plant and bacterial β-amylase (Hirata et al., 2004). Although plant-based β-amylases are well documented, the breadth of the sources is limited to five plants and one bacterial origin. Therefore, it is of interest to expand the source of the enzyme to include a thermostable β-amylase that is active at higher temperatures, increasing its potential applicability in the industry.

The β-amylase from *Clostridium thermosulfurogenes* has been shown to possess an optimum operating temperature at 75 ℃ and would be stable up to 80 ℃ (Hyun & Zeikus, 1985). Barnaud *et al.* (1997) cloned and sequenced the gene encoding the thermophilic β-amylase of *Clostridium thermosulfurogenes* in *Bacillus subtilis* and showed that the mature β-amylase has 519 amino acids and molecular weight (MW) of 57167 kDa. The β-amylase sequence showed 32% homology with β-amylase of soybean and barley. However, there is no 3D structure of this thermophilic enzyme available for study.

This study is conducted to model the structure of the thermophilic β-amylase from *Clostridium thermosulfurogenes* making it the first modelled thermophilic structure and the first from species other than *Bacillus cereus* and of plant origin. The model will be used to examine if there are differences that can be attributed to structural adaptations that allow it to function at higher temperatures. The knowledge of these differences would be contributing to the understanding of thermophilic adaptation in β-amylase and may be used in protein modification for future used.

1.2 Problem Statement

The enzyme β-amylase is one of the important enzymes used in the food production industry. Although widely used, several issue required for further study.

Firstly, existing 3D structures are limited to only a few species; bacteria *Bacillus cereus*, *Paenibacillus polymyxa* and plants; *Glycine max* (soybean) (Mikami et al., 1994), *Ipomoea batatas* (sweet potato) (Cheong et al., 1995) and *Hordeum vulgare* (barley) (Mikami et al., 1999). The availability of the enzymes comes mostly from plant sources and within the mesophilic operating range. This limited number of the elucidated structure suggests that other species have yet to be discovered and studied, especially from extremophiles that can be a novel source of potentially new β-amylase for future industrial use.

Secondly, the existing structure of β-amylase in the databases has been shown to possess an upper limit of the operational temperature of 70 °C (Li et al., 1991). However, research has reported that the β-amylase from *Clostridium thermosulfurogenes* possess a higher operating temperature at 75 ℃ and would be stable up to 80 ℃ (Hyun & Zeikus, 1985). The enzyme has a greater operating pH range of between pH 3.5 to 6.5, unlike other β-amylase that possess the optimal activity and stable only at neutral pHs. Results have also shown that the enzyme possesses an optimum activity at pH 5.5 to 6.0, making it an interesting candidate for studying the sequence and structural differences that may contribute to its ability to operate at a different temperature and pH range.

1.3 Objectives of the Study

(a) To model the 3D structure of a novel thermophilic β-amylase from *Clostridium thermosulfuregenes*.

(b) To compare between the predicted model of enzyme thermophilic β-amylase from *Clostridium thermosulfuregenes* with *Bacillus cereus* β-amylase.

1.4 Significance of the Study

Enzyme β-amylase has significant applications in industries due to its saccharogenic activity. It is also used in the pharmaceutical industry because of its digestive activity as well as in the preparation of maltooligosaccharides and the production of the maltooligomer mixtures (Saini et al., 2017).

The reported activity of the thermostable β-amylase of *Clostridium thermosulfurogenes* at higher temperatures and the optimum activity at pH 5.5 to 6.0 and the stability at pH 3.5 to 6.5 makes it an interesting candidate to study. Furthermore, anaerobic bacteria like *Clostridium* SP can convert starch directly into ethanol (Ueki et al., 1991), making any attempt to study and improve the enzyme

properties to assist in the production of ethanol under elevated heat conditions and pH makes elucidating this protein's structural adaptation mechanism useful in future protein engineering strategies.

1.5 Scope of the Study

The research scope of this study is exclusively bioinformatics and computational analysis of laboratory data derived from the primary databases such as UniProt (https://www.uniprot.org/), protein data bank (PDB) (www.rcsb.orgn), BRENDA (https://www.brenda-enzymes.org/), and others. The extent of this study only covers the computational aspects of the research as further research with laboratory proof of concept will be pursued for future doctoral work.

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Appendix A The percentage of secondary structure of *B. cereus* and *C. thermosulfuregen* protein

Protein source (organism)	Helix	Sheet	Coil or loop	Turn
B.cereus	71.60%	45.60%	47.44%	11.70%
C.thermosulfuregen	51.20%	72.10%	56.99%	12.20%

LIST OF PUBLICATIONS