UNIVERSITI PUTRA MALAYSIA

IDENTIFICATION OF THERMOSTABLE GLYCOGEN BRANCHING ENZYME FROM *GEOBACILLUS* SP. GEO5 BY GENOME MINING

NUR SYAZWANI BINTI MOHTAR

FS 2013 15

IDENTIFICATION OF THERMOSTABLE GLYCOGEN BRANCHING ENZYME FROM *GEOBACILLUS* SP. GEO5 BY GENOME MINING

NUR SYAZWANI BINTI MOHTAR

MASTER OF SCIENCE
UNIVERSITI PUTRA MALAYSIA

2013

IDENTIFICATION OF THERMOSTABLE GLYCOGEN BRANCHING ENZYME FROM *GEOBACILLUS* SP. GEO5 BY GENOME MINING

By

NUR SYAZWANI BINTI MOHTAR

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

July 2013

COPYRIGHT

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

IDENTIFICATION OF THERMOSTABLE GLYCOGEN BRANCHING ENZYME FROM *GEOBACILLUS* SP. GEO5 BY GENOME MINING

By

NUR SYAZWANI BINTI MOHTAR

July 2013

Chairman: Professor Mohd Basyarudin Bin Abdul Rahman, PhD

Faculty: Science

Glycogen branching enzyme (EC 2.4.1.18) has increasing demand from food and beverages processing industries. This enzyme, which catalyses the formation of α-1,6-glycosidic branch points in glycogen structure, is used to enhance nutritional value and quality of food and beverages. To be applicable in industries, enzymes that are stable and active at high temperature are much desired. A thermophilic bacterium, *Geobacillus* sp. Geo5, was isolated from Sungai Klah Hot Springs at 97°C and therefore it was postulated that this bacterium species would produce thermostable glycogen branching enzyme that is active at high temperature. The objectives of this research are to identify the branching enzyme gene (*glgB*) of *Geobacillus* sp. Geo5, to produce the enzyme using *Escherichia coli* and to characterise the biochemical properties of the enzyme. Using genome data mining, the nucleotide sequence of *glgB* was fished out from *Geobacillus* sp. Geo5 genome sequence provided by Malaysia Genome Institute. The size of the gene is 2013 bp and the theoretical molecular weight of the protein is 78.43 kDa. The gene sequence was then used to predict the three dimensional structure of the enzyme using an online software, I-TASSER. The percentage sequence identity of the template

(*Mycobacterium tuberculosis* H37RV; PDB ID: 3K1D) in the threading aligned region with the *Geobacillus* sp. Geo5 sequence was only 45%. Subsequently, *glgB* from *Geobacillus* sp. Geo5 was isolated using polymerase chain reaction (PCR). To study the enzyme, the gene was cloned into pET102/D-TOPO® vector by PCR cloning and overexpressed in BL21 Star ™ (DE3) *E. coli*. The expression of active enzyme was the highest when the expression was induced with 0.75 mM of IPTG, at 30°C for 8 hours. The recombinant protein was also expressed together with bacteriocin release protein to secrete the protein into *E. coli* culture medium. The study shown that induction with 5 ng/mL of mitomycin C for 8 hour was enough to secrete the recombinant protein to extracellular environment (34.1 U/mL) although not entirely since 43.0 U/mL of the activity was still in the cell. Therefore, the intracellular expression system was chosen for further studies on the enzyme. The recombinant protein from intracellular expression was then purified by affinity chromatography using HisTrap HP column with the recovery of 84%. The purified enzyme was used to study the effect of temperature and pH on enzyme activity and stability, and the inhibitory effect by metal ion on enzyme activity. This thermostable glycogen branching enzyme was found to be most active at 55°C and the half-life at 60°C and 70°C was 24 hours and 5 hours, respectively. The enzyme was stable at pH 5 to pH 9 and the optimum pH for enzyme activity was at pH 6. Metal ions, Mn^{2+}, Zn^{2+}, Cu^{2+}, Fe^{2+} and Ca^{2+} seem to inhibit the activity of this enzyme. Mg^{2+} however does not affect the enzyme activity. From this research, a thermostable glycogen branching enzyme was successfully isolated from *Geobacillus* sp. Geo5 by genome mining together with molecular biology technique. The stability of this enzyme would be very practical for industrial applications especially in carbohydrates processing such as nutraceutical, food and beverages industries.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

IDENTIFIKASI ENZIM PENCABANG GLYCOGEN TERMOSTABIL DARIPADA *GEOBACILLUS* SP. GEO5 MELALUI PERLOMBONGAN GENOM

Oleh

NUR SYAZWANI BINTI MOHTAR

Julai 2013

Pengerusi: Professor Mohd Basyarudin Bin Abdul Rahman, PhD

Fakulti: Sains

Enzim pencabang glikogen (EC 2.4.1.18) mendapat permintaan yang meningkat daripada industri pemprosesan makanan dan minuman. Enzim ini yang memangkin pembentukan titik cabangan α-1,6-glikosida dalam struktur glikogen, digunakan untuk meningkatkan nilai pemakanan dan kualiti makanan dan minuman. Untuk diguna pakai dalam industri, enzim yang stabil dan aktif pada suhu tinggi sangat diingini. Satu bakteria termofilik, *Geobacillus* sp. Geo5, telah dipencilkan dari Kolam Air Panas Sungai Klah pada 97°C dan oleh itu ia diandaikan bahawa spesis bakteria ini akan menghasilkan enzim pencabang glikogen yang termostabil and aktif pada suhu tinggi. Objektif kajian ini adalah untuk mengenal pasti gen enzim pencabang (*glgB*) daripada *Geobacillus* sp. Geo5, menghasilkan enzim tersebut menggunakan *Escherichia coli* dan mencirikan sifat-sifat biokimia enzim tersebut. Menggunakan kaedah perlombongan data genom, jujukan nukleotida gen enzim pencabang (*glgB*) dipancing keluar daripada genom *Geobacillus* sp. Geo5 yang telah dijujuk oleh Institut Genom Malaysia. Saiz gen adalah 2013 bp dan berat molekul teori protein adalah 78.43 kDa. Jujukan gen kemudiannya digunakan untuk meramal struktur tiga dimensi enzim mengunakan perisian dalam talian, I-TASSER. Peratusan identiti jujukan bagi templat (*Mycobacterium tuberculosis* H37RV; PDB ID: 3K1D)

di kawasan yang sejajar dengan jujukan *Geobacillus* sp. Geo5 adalah hanya 45%. Seterusnya, *glgB* daripada *Geobacillus* sp. Geo5 telah dipencilkan menggunakan tindak balas berantai polimerase (PCR). Untuk mengkaji enzim tersebut, gen diklon ke dalam vektor pET102/D-TOPO® melalui pengklonan PCR dan diekspres di dalam *E. coli* BL21 Star TM (DE3). Penghasilan enzim yang aktif adalah paling banyak apabila ekspresi diaruh dengan 0.75 mM IPTG, pada 30°C selama 8 jam. Protein rekombinan juga diekspres bersama-sama dengan protein pelepasan bakteriosin untuk merembeskan protein tersebut ke dalam media kultur *E. coli*. Kajian ini menunjukkan bahawa induksi dengan 5 ng/mL mitomycin C cukup untuk merembeskan protein rekombinan ke persekitaran ekstrasel (34.1 U/mL) walaupun tidak keseluruhannya memandangkan aktiviti sebanyak 43.0 U/mL masih di dalam sel. Disebabkan itu, sistem pengekspresan intrasel dipilih untuk kajian lanjut ke atas enzim. Protein rekombinan daripada pengekspresas intrasel seterusnya ditulenkan melalui kromatografi afiniti menggunakan turus HisTrap HP dengan pemulihan sebanyak 84%. Enzim yang telah ditulenkan digunakan untuk mengkaji kesan suhu dan pH ke atas aktiviti dan kestabilan enzim, dan kesan rencatan oleh ion logam pada aktiviti enzim. Enzim pencabang glikogen didapati paling aktif pada 55°C dan separuh hayat pada 60°C dan 70°C masing-masing adalah 24 jam dan 5 jam. Enzim stabil pada pH 5 hingga pH 9 dan pH optimum bagi aktiviti enzim adalah pada pH 6. Ion-ion logam, Mn^{2+}, Zn^{2+}, Cu^{2+}, Fe^{2+} dan Ca^{2+} didapati telah merencat aktiviti enzim ini. Mg^{2+} bagaimanapun tidak menjejaskan aktiviti enzim. Daripada kajian ini, enzim pencabang glikogen yang termostabil telah berjaya dipencil daripada *Geobacillus* sp. Geo5 melalui kaedah perlombongan genom bersama-sama dengan teknik biologi molekul. Kestabilan enzim ini akan menjadi sangat praktikal untuk aplikasi perindustrian terutamanya dalam pemprosesan karbohidrat seperti industri nutraseutikal, makanan dan minuman.

ACKNOWLEDGEMENTS

Alhamdulillah, all praises and thanks to Allah, The Most Gracious and The Most Merciful. I would like to express my gratitude to MGI and their personnel who were generous enough provide this research with the *Geobacillus* sp. Geo5 isolate and its genome sequence. I would also like to express my utmost appreciation to my supervisors Professor Dr. Mohd Basyaruddin Abdul Rahman, Professor Dr. Raja Noor Zaliha Raja Abdul Rahman and Professor Dato' Dr. Abu Bakar Salleh, for their dedication, guidance and encouragement through the course of this project. Dr Adam Leow Thean Chor and Dr. Mohd. Syukuri, I really appreciate their generous ideas and guidance.

I would like to give special thanks to my friends from Enzyme Technology Lab, IBS and 140 Lab, Faculty of Biotechnology and Biomolecular Sciences who have been together with me throughout the course. To all who has helped me and gave me the possibility to complete this thesis, thank you very much. I also thank National Science Fellowship for sponsoring my Master degree.

Most importantly, I am forever grateful to family for their endless love and support, they always believe in me and stay by my side. May we always be in His blessings.

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

Mohd Basyaruddin Bin Abdul Rahman, PhD
Professor
Faculty of Science
Universiti Putra Malaysia
(Chairman)

Raja Noor Zaliha Binti Raja Abdul Rahman, PhD
Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Member)

_____

BUJANG BIN KIM HUAT, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

DECLARATION

I declare that the thesis is my original work except for quotations and citations, which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institutions.

_____

NUR SYAZWANI BINTI MOHTAR

Date: 12 July 2013

TABLE OF CONTENTS

		Page
ABSTRACT		ii
ABSTRAK		iv
ACKNOWLEDGEMENTS		vi
APPROVAL		vii
DECLARATION		ix
LIST OF TABLES		xiii
LIST OF FIGURES		xiv
LIST OF ABBREVIATIONS		xvi
CHAPTER		
1	**INTRODUCTION**	1
2	**LITERATURE REVIEW**	4
2.1	Glycogen	4
2.2	Glycogen branching enzyme	6
2.2.1	Mechanism of action of glycogen branching enzyme	10
2.2.2	Thermostable glycogen branching enzyme	12
2.2.3	Applications of glycogen branching enzyme	14
2.3	Genome mining	17
2.4	Structural studies of glycogen branching enzyme	19
2.4.1	Crystal structures of branching enzyme	20
2.4.2	Protein structure prediction	24
2.5	Lab-scale production of GBE in *Escherichia coli* expression system	29
2.5.1	Cloning of PCR product	31
2.5.2	Transformation of *Escherichia coli* with plasmid	31
2.5.3	Expression of recombinant protein	32
2.5.4	Secretory expression of recombinant protein	33
2.6	Purification of recombinant protein	34
3	**MATERIALS AND METHODS**	36
3.1	Materials	36
3.2	Genome mining	36
3.3	Enzyme structure prediction	37
3.4	Culturing *Geobacillus* sp. Geo5	37
3.4.1	Thermus broth preparation	38
3.4.2	Thermus agar preparation	38
3.5	Genomic DNA extraction of *Geobacillus* sp. Geo5	38
3.6	Polymerase chain reaction of *glgB*	39

3.7 Polymerase chain assembly 40
3.8 PCR purification by gel extraction 42
3.9 Cloning of PCR product 42
3.10 Recombinant plasmid purification 43
3.11 Analysing transformed recombinant plasmids 43
3.11.1 PCR of recombinant plasmid DNA 44
3.11.2 *Pst*I and *Bgl*II digestion 44
3.11.3 DNA sequencing of recombinant plasmid DNA 45
3.12 Intracellular expression of G5GBE 45
3.13 Determination of G5GBE expression level 46
3.13.1 Iodine stain assay 46
3.13.2 Branching linkage assay 47
3.13.3 SDS-PAGE 48
3.14 Secretory expression of G5GBE 48
3.14.1 Co-transformation of pSW1 vector 49
3.14.2 Analysis of positive transformants 49
3.14.3 Quasi-lysis test 49
3.14.4 Effect of mitomycin C concentration on secretory expression 50
3.15 Purification of G5GBE 50
3.15.1 MALDI TOF/TOF analysis 51
3.16 Characterisation of purified GBE 52
3.16.1 Effect of temperature on enzyme activity 52
3.16.2 Effect of temperature on enzyme stability 52
3.16.3 Effect of pH on enzyme activity 53
3.16.4 Effect of pH on enzyme stability 53
3.16.5 Effect of metal ions on enzyme activity 53

4 RESULTS AND DISCUSSIONS 54
4.1 Genome mining 54
4.2 Enzyme structure prediction 59
4.3 Culturing *Geobacillus* sp. Geo5 66
4.4 Genomic DNA extraction of *Geobacillus* sp. Geo5 66
4.5 Polymerase chain reaction of *glgB* 67
4.5.1 DNA sequencing of PCR product 68
4.6 Polymerase chain assembly 69
4.7 Cloning of PCR product 70
4.8 Analysing transformed recombinant plasmids 71
4.9 Expression of G5GBE in *Escherichia coli* 73
4.9.1 Intracellular expression of G5GBE 74
4.9.1.1 Effect of IPTG concentration 74
4.9.1.2 Effect of induction temperature 77
4.9.1.3 Effect of induction time 79

4.9.2 Secretory expression of G5GBE 81
4.9.2.1 Analysis of positive transformants 81
4.9.2.2 Quasi-lysis test 81
4.9.2.3 Effect of mitomycin C concentration on secretory expression 82
4.10 Purification of G5GBE 87
4.10.1 MALDI TOF/TOF analysis 89
4.11 Biochemical characterisation of purified G5GBE 91
4.11.1 Effect of temperature on enzyme activity and stability 91
4.11.2 Effect of pH on enzyme activity and stability 94
4.11.3 Effect of metal ions on enzyme activity 97

5 CONCLUSION AND RECOMMENDATIONS 99
5.1 Conclusion 99
5.2 Recommendations 100

REFERENCES 101
APPENDICES 113

LIST OF TABLES

Table		Page
1	Four conserved regions of amino acid sequence of α-amylase family enzymes	7
2	Glycoside hydrolase family 13 enzymes	9
3	Conserved regions in glycogen branching enzyme from *Geobacillus* spp., *Escherichia coli* and *Mycobacterium tuberculosis*	57
4	Percentage of aromatic amino acids, glutamine and asparagine in relation to the temperature optimum for activity and stability of three bacterial and two maize branching enzymes	59
5	C-score of the predicted models by I-TASSER	60
6	Summary of the PROCHECK analysis	64
7	Purification of GBE from *Geobacillus* sp. Geo5 using affinity chromatography	89

LIST OF FIGURES

Figure		Page
1	Structural formula of glycogen	5
2	Reaction mechanism of glycogen branching enzyme	11
3	X-ray crystal structure of truncated *Escherichia coli* branching enzyme	21
4	X-ray crystal structure of *Mycobacterium tuberculosis* branching enzyme	23
5	X-ray crystal structure of *Thermus thermophilus* branching enzyme	25
6	Accuracy and applications of protein models	27
7	PCR and PCA	41
8	Nucleotide and amino acid sequence of *glgB* from *Geobacillus* sp. Geo5	56
9	Five predicted structures of glycogen branching enzyme by I-TASSER	61
10	Ramachandran plots	63
11	The catalytic site of glycogen branching enzyme from *Geobacillus* sp. Geo5	65
12	Gel electrophoresis of PCR products	68
13	Gel electrophoresis of PCR and PCA products	71
14	Gel electrophoresis of PCR product from pET/GBE	72
15	Gel electrophoresis of restriction enzyme digestion of pET/GBE	73
16	Expression of G5GBE by *Escherichia coli* BL21 Star™ (DE3) when induced with different concentrations of IPTG	76
17	SDS-PAGE of expressed G5GBE when induced with different concentrations of IPTG	76
18	Expression of G5GBE by *Escherichia coli* BL21 Star™ (DE3) when induced at different temperatures	78
19	SDS-PAGE of expressed G5GBE when induced at different	78

	temperatures	
20	Expression of G5GBE by *Escherichia coli* BL21 Star™ (DE3) when induced up to 28 hours and the growth curve of the *Escherichia coli*	80
21	Gel electrophoresis of restriction enzyme digestion of pSW1 and pET/GBE	82
22	Quasi-lysis test done on *Escherichia coli* BL21 (DE3) Star™ harbouring pSW1 and pET/GBE	83
23	Effect of mitomycin C concentration on secretory expression of G5GBE	84
24	Secretory expression of G5GBE	85
25	Chromatogram of affinity chromatography performed using Äkta Explorer	88
26	SDS-PAGE of purified enzyme	88
27	Hits from the MALDI TOF/TOF analysis	90
28	Effect of temperature on enzyme activity	92
29	Effect of temperature on enzyme stability	93
30	Effect of pH on enzyme activity	95
31	Effect of pH on enzyme stability	96
32	Effect of metal ion on enzyme activity	98

LIST OF ABBREVIATIONS

3D	Three-dimensional
A_{260}	Absorbance at 260 nm
$A_{260/280}$	Ratio of absorbance at 260 nm and 280 nm
A_{600}	Absorbance at 600 nm
ADP	Adenosine diphosphate
bp	Base pair
BRP	Bacteriocin release protein
CASP	Critical Assessment of Structure Prediction
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
EC	Enzyme classification
G5GBE	Recombinant GBE from *Geobacillus* sp. Geo5
GBE	Glycogen branching enzyme
GH	Glycoside hydrolase
HCl	Hydrochloric acid
I_2	Iodine
IPTG	Isopropyl-β-D-thiogalactopyranoside
kb	Kilo base pair
kDa	Kilo Dalton
KI	Potassium iodide
LB	Luria-Bertani
M	Molar
MALDI TOF/TOF	Matrix-assisted laser desorption ionization tandem time-of-flight
mg	Milligram
MGI	Malaysia Genome Institute

mL	Millilitre
mL/min	Millilitre per minute
mm	Millimetre
mM	Millimolar
NaCl	Sodium chloride
NaOH	Sodium hydroxide
ng	Nanogram
ng/mL	Nanogram per millilitre
ORF	Open reading frame
PCA	Polymerase chain assembly
PCR	Polymerase chain reaction
PDB	Protein Data Bank
pET/GBE	Recombinant plasmid; *glgB* of *Geobacillus* sp. Geo5 in pET102
rpm	Rotations per minute
SDS	Sodium dodecyl sulphate
SDS-PAGE	Sodium dodecyl sulphate polyacrylamide gel electrophoresis
× g	Gravitational force
U	Unit
U/mg	Unit per milligram
U/mL	Unit per millilitre
UV	Ultraviolet
v/v	Volume per volume
w/v	Weight per volume
µg/mL	Microgram per millilitre
µL	Microlitre
µM	Micromolar
µmol	Micromole

CHAPTER 1

INTRODUCTION

Enzymes are amazing catalysts that are naturally occurring in all living organisms, efficiently catalyse from simple to complex reactions by accepting selectively a wide range of molecules as substrates. As an alternative to conventional chemical catalysts, enzymatic reactions produce fewer by-products that are mostly harmless, hence making enzymes as an environmentally friendly choice (Schmid *et al.*, 2001). Efficient selective catalysis is essential and therefore has enormous potential especially in the industrial processing and synthesis of fine chemicals, food and pharmaceuticals (Burton *et al.*, 2002). BCC Research reported that in 2010, the value of global market for industrial enzymes was US$3.6 billion and the largest segment of the industrial enzymes industry comes from food and beverage enzymes with revenues of nearly US$1.2 billion (BCC Research, 2012). These enzymes are used either to increase the nutritional value or to make the food and drinks more appealing. Therefore enzymes like amylase, lipase and protease have gained a lot of interest in the research field. Other than aforementioned enzymes, one of the enzymes that have a rising demand in food and beverages industries is branching enzyme (van der Maarel, 2009).

Branching enzyme (EC 2.4.1.18) is a type of transferase that is found naturally in plants, animals and microorganisms. This enzyme carries out the transglycosylation reaction of starch and glycogen. The transglycosylation reaction transfers

an α-1,4-glycosidic linkage to α-1,6 making the starch and glycogen structure branched out. These branches in starch and glycogen structures help the digestion of the polysaccharides as they are more soluble and easier to be absorbed by the cells (Abad *et al.*, 2002). As glycogen branching enzyme produces more branches than starch branching enzymes, many studies are focusing on commercialisation of glycogen branching enzymes for applications in beverage, food processing and nutraceutical industries. In order to be practical in industries, enzymes that can withstand high temperature would be very sought after. One of the major concerns is to find enzymes that are naturally active and stable in high temperature. For that reason, researchers have been isolating thermostable glycogen branching enzymes from thermophilic microorganisms. However, the production of this enzyme in its thermophilic host is very low. Therefore recombinant DNA technology, such as *Escherichia coli* cloning and expression systems, were often utilised in order to maximise enzyme production to supply for industries and studies on the enzyme. *E. coli* system often preferred as this system is easy to manipulate, capable to produce enzyme rapidly and reasonably cheap.

Studies on branching enzymes have made the gene sequence of the enzyme accessible (van der Maarel *et al.*, 2002). On top of that, the venture in genome sequencing is contributing vast information on new genetic sequences and therefore abundance of novel enzymes and proteins remain to be explored. When put together, that knowledge can be exploited in the search for novel biocatalysts by a technique called genome mining. The term 'genome mining' brings the meaning of a post genome analysis where the desired gene is fished out of a genome sequence using bioinformatics tools and databases. However, genetic sequence alone cannot be used

to predict the exact catalytic function of putative enzymes unless their structural information is analysed and then verified through molecular biology experimentation. Nevertheless, this genetic codes can be use to generate 3D structures of the proteins through computational prediction by protein threading and homology modelling methods (Zhang and Skolnick, 2005; Bowie *et al.*, 1991). From the structural information, the enzyme characteristics such as enzyme stability, substrate preference and mechanism of the enzyme can be predicted. From there, further studies can be done to improve the enzyme. Although about 90% of protein structures deposited in Protein Databank (PDB) were elucidated by X-ray crystallography, this technique takes time and requires a big budget. Therefore researchers often use protein structure prediction as a foundation to study the characteristic and structure of the desired protein.

In this research, genome mining was used to isolate glycogen branching enzyme gene (*glgB*) from *Geobacillus* sp. Geo5 genome, which was sequenced by Malaysia Genome Institute (MGI) under their Whole Genome Sequencing Project. This *Geobacillus* sp. Geo5 was isolated from a 97°C hot spring in Sungai Klah, Perak and therefore the enzyme isolated is likely to be thermostable and active at high temperature. Thus, this research is conducted with the following objectives:

1. To identify thermostable glycogen branching enzyme gene from *Geobacillus* sp. Geo5 genome sequence.
2. To produce thermostable recombinant glycogen branching enzyme in *Escherichia coli* expression system.
3. To characterise the biochemical properties of the purified recombinant glycogen branching enzyme.

REFERENCES

Abad, M., Binderup, K., Rios-Steiner, J., Arni, R. and Preiss, J. (2002). The X-ray crystallographic structure of *Escherichia coli* branching enzyme. *The Journal of Biological Chemistry*. 277 (44): 42164–42170.

Amersham Pharmacia Biotech (1999). *Protein Purification Handbook*. Upsala: Amersham Pharmacia Biotech AB.

Arnau, J., Lauritzen, C., Petersen, G. and Pedersen, J. (2006). Current strategies for the use of affinity tags and tag removal for the purification of recombinant proteins. *Protein Expression and Purification.* 48:1-13.

Arner, E. and Holmgren, A. (2000). Physiological functions of thioredoxin and thioredoxin reductase. *European Journal of Biochemistry*. 267: 6102-6109.

Baker, D. (2000). A surprising simplicity to protein folding. *Nature*. 405: 39-42.

Baker, D. and Sali, A. (2001). Protein structure prediction and structural genomics. *Science.* 294: 93-96.

Baneyx, F. (1999). Recombinant protein expression in *Escherichia coli*. *Current Opinion in Biotechnology.* 10(5): 411-421.

Baneyx, F. and Mujacic, M. (2004). Recombinant protein folding and misfolding. *Nature Biotechnology*. 22(11): 1139-1408.

Banskota, A., McAlpine, J., Sørensen, D., Ibrahim, A., Aouidate, M., Piraee, M., Alarco, A., Farnet, C. and Zazopoulos, E. (2006). Genomic Analyses Lead to Novel Secondary Metabolites. *Journal of Antibiotics*. 59(9): 533–542.

Bartlett, J. and Sterling, D. (2003). A short history of the polymerase chain reaction. *Methods in Molecular Biology*. 226: 3-6.

BCC Research. (2012). *Global Markets for Enzymes in Industrial Applications* (Publication no. BIO030G). Wellesley, MA: BCC Research. Retrieved from: http://www.bccresearch.com/report/enzymes-industrial-applications-markets-bio030g.html.

Berg, J. (1987). Metal ions in proteins: Structural and functional roles. *Cold Spring Harbour Symposia on Quantitative Biology*. 52: 579-585.

Berger, D. (2004). Gene-mining the *Arabidopsis thaliana* genome: Applications for biotechnology in Africa. *South African Journal of Botany.* 70(1): 173-180.

Berrow, N., Bussow, K., Coutard, B., Diprose, J., Ekberg, M., Folkers, G., Levy, N., Lieu, V., Owens, R., Peleg, Y., Pinaglia, C., Quevillon-Cheruel, S., Salim, L., Scheich, C., Vincentellic R. and Bussoh, D. (2006). Recombinant protein expression and solubility screening in *Escherichia coli*: A comparative study. *Acta Crystallographica Section D*. D62:1218-1226.

Binderup, K., Mikkelsen, R., and Preiss, J. (2002). Truncation of the amino terminus of branching enzyme changes its chain transfer pattern. *Archives of Biochemistry and Biophysics.* 397 (2): 279-285.

Bischof, J. and He, X. (2005). Thermal Stability of Proteins. *Annals of the New York Academy of Sciences.* 1066: 12–33.

Bowie, J., Luthy, R. and Eisenberg, D. (1991). A method to identify protein sequences that fold into a known three-dimensional structure. *Science.* 253 (5016): 164-170.

Bruno, C., Servidei, S., Shanske, S., Karpati, G., Carpenter, S., McKee, D., Barohn, R., Hirano., Rtfai, Z. and DiMauro, S. (1993). Glycogen branching enzyme deficiency in adult polyglucosan body disease. *Annals of Neurology*. 33(1): 88-93.

Burley, S. and Petsko, G. (1985). Aromatic-aromatic interaction: A mechanism of protein structure stabilization. *Science*. 229: 23-28.

Burton, S., Cowen, D. and Woodley, J. (2002). The search for the ideal biocatalyst. *Nature Biotechnology.* 20: 37-45.

Challis, G. (2008). Genome mining for novel natural product discovery. *Journal of Medicinal Chemistry.* 51: 2618–2628.

Cheng, C., and Shuman, S. (2000). Recombinogenic flap ligation pathway for intrinsic repair of topoisomerase IB-induced double-strand breaks. *Molecular and Cellular Biolology.* 20: 8059-8068.

Corre, C. and Challis, G. (2007). Heavy tools for genome mining. *Chemistry & Biology.* 14: 7-9.

Demirjian, D. C., Morís-Varas, F., and Cassidy, C. S. (2001). Enzymes from extremophiles. *Current Opinion in Chemical Biology.* 5(2): 144-151.

Devillers, C., Piper, M., Ballicora, M., and Preiss, J. (2003). Characterization of the branching patterns of glycogen branching enzyme truncated on the N-terminus. *Archives of Biochemistry and Biophysics.* 418: 34–38.

Ferrer, M., Martínez-Arbaca, F. and Golyshin, P. (2005). Mining genomes and 'metagenomes' for novel catalysts. *Current Opinion in Biotechnology*. 16:588–593.

Fraaije, M., Wu, J., Heuts, D., van Hellemond, E., Spelberg, J. and Janssen, D. (2005). Discovery of a thermostable Baeyer–Villiger monooxygenase by genome mining. *Applied Microbiology and Biotechnology*. 66: 393–400.

Fujiwara, S. (2002). Extremophiles: Developments of their special functions and potential resources. *Journal of Bioscience and Bioengineering.* 94(6): 518-525.

Garg, S., Alam, S., Kishan, R. and Agrawal, P. (2007). Expression and characterization of α-(1,4)-glucan branching enzyme Rv1326c of *Mycobacterium tuberculosis* H37Rv. *Protein Expression and Purication.* 51: 198–208.

Gogichaena, N., Williams, T. and Alterman, M. (2007). MALDI TOF/TOF tandem mass spectrometry as a new tool for amino acid analysis. *Journal of the American Society for Mass Spectrometry.* 18: 279-284.

González-Montalbán, N., García-Fruitós, E. and Villaverde, A. (2007). Recombinant protein solubility- does more mean better? *Nature Biotechnology*. 25 (7): 718-720.

Guan, H., Li, P., Imparl-Radosevich, J., Preiss, J. and Keeling, P. (1997). Comparing the properties of *Escherichia coli* branching enzyme and maize branching enzyme. *Archives of Biochemistry and Biophysics*. 342 (1): 92–98.

Guenther, B., Onrust, R., Sali, A., O'Donnell, M. and Kuriyan, J. (1997). Crystal structure of the δ' subunit of the clamp-loader complex of *E. coli* DNA polymerase III. *Cell*. 91: 335–345.

Hanahan, D. (1983). Studies on transformation of *Escherichia coli* with plasmids. *Journal of Molecular Biology.* 166: 557-580.

Hanahan, D., Jesse, J and Bloom, F. (1991). Plasmid transformation of *Escherichia coli* and other bacteria. *Methods in Enzymology.* 204: 63-113.

Henrissat, B. (1991). A classification of glycosyl hydrolases based on amino acid sequence similarities. *Biochemical Journal.* 280: 309-316.

Hilden, I., Leggio, L., Larsen, S., and Poulsen, P. (2000). Characterization and crystallization of an active N-terminally truncated form of the *Escherichia coli* glycogen branching enzyme. *European Journal of Biochemistry*. 267: 2510-2155.

Jespersen, H., MacGregor, A., Henrissat, B., Sierks, M. and Svensson, B. (1993). Starch- and glycogen-debranching and branching enzymes: Prediction of structural features of the catalytic $(\beta/\alpha)_8$-barrel domain and evolutionary relationship to other amylolytic enzymes. *Journal of Protein Chemistry*. 12(6): 791-805.

Jonasson, P., Liljeqvist, S., Nygren, P.A. and Stahl, S. (2002). Genetic design for facilitated production and recovery of recombinant proteins in *Escherichia coli. Biotechnology and Applied Biochemistry*. 35: 91-105.

Kawabata, Y., Toeda, k., Takahashi, T., Shibamoto, M. and Kobayashi, M. (2002). Preparation of highly branch starch by glycogen branching enzyme from *Neurospora crassa* N2-44 and its characterization. *Journal of Applied Glycoscience.* 49 (3): 273-279.

Kiel, J., Boels, J., Beldman, G. and Venema, G. (1990). Nucleotide sequence of the *Synechococcus* sp. PCC7942 branching enzyme gene (*glgB*): expression in *Bacillus subtilis*. *Gene.* 89: 77-84.

Kim, W. S., Kim, J., Krishnan, H. and Nahm, B. H. (2005). Expression of *Escherichia coli* branching enzyme in caryopses of transgenic rice results in amylopectin with an increased degree of branching. *Planta.* 220: 689–695.

Kortstee, A., Vermeesch, A., de Vries, B., Jacobsen, E., and Visser, R. (1996). Expression of *Escherichia coli* branching enzyme in tubers of amylose-free transgenic potato leads to an increased branching degree of the amylopectin . *The Plant Journal.* 10(1): 83-90 .

Kulakov,L., Poelarends,G., Janssen, D. and Larkin, M. (1999). Characterization of IS2112, a new insertion sequence from *Rhodococcus,* and its relationship with mobile elements belonging to the IS110 family. *Microbiology*. 145: 561-568.

Kuriki, T. and Imanaka, T. (1999). The concept of the α-amylase family: Structural similarity and common catalytic mechanism. *Journal of Bioscience and Bioengineering.* 87(5): 557-565.

Kuriki, T., Guan, H., Sivak, M. and Preiss, J. (1996). Analysis of the active center of branching enzyme II from maize endosperm. *Journal of Protein Chemistry.* 15(3): 305-313.

Laemmli, U. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature.* 227: 680-685.

Laskowski, R., MacArthur, M., Moss, D. and Thornton, J. (1993). PROCHECK: A program to check the stereochemical quality of protein structures. *Journal of Applied Crystallography*. 26: 283-291.

Laurent, T., Moore, E. and Reichard, P. (1964). Enzymatic synthesis of deoxyribonucleotides. *The Journal of Biological Chemistry*. 239(10): 3436-34444.

LaVallie, E., DiBlasio, E., Kovacic, S., Grant, K., Schendel, P. and McCoy, J. (1993). A thioredoxin gene fusion expression system that circumvents inclusion body formation in the *E. coli* cytoplasm. *Biotechnology*. 11(2):187-93.

Lee, C., Le, Q., Kim, Y., Shim, J., Lee, S., Park, J., Lee, K., Song, S., Auh, J., Lee, S. and Park, K. (2008). Enzymatic synthesis and properties of highly branched rice starch amylose and amylopectin cluster. *Journal of Agriculture and Food Chemistry.* 56: 126-131.

Lichty, J., Malecki, J., Agnew, H. Michelson-Horowitz, D. and Tan, S. (2005). Comparison of affnity tags for protein purification. *Protein Expression and Purification.* 41:98-105.

Lloubes, R., Vitab, N., Bernadaca, A., Shireb, D., Leplatoisb, P., G1ia, V., Frenette, M, Knibiehler, M., Lazdunski, C. and Baty, D. (1993). Colicin A lysis protein promotes extracellular release of active human growth hormone accumulated in *Escherichia coli* cytoplasm. *Biochimie.* 75(6): 451-8.

Lodish, H., Berk, A., Matsudaira, P., Kaiser, C., Krieger, M., Scott, M., Zippursky, S. and Darnel, J. (2004). *Molecular cell biology: Fifth edition*. New York: W. H. Freeman and Company.

Loper, J., and Gross, H. (2007). Genomic analysis of antifungal metabolite production by *Pseudomonas fluorescens* Pf-5. *European Journal of Plant Pathology*. 119:265–278.

Lu, Z., DiBlasio-Smith, E., Grant, K., Warne, N., LaVallie, E., Collins-Racie, L., Follettie, M., Williamson, M. and McCoy, J. (1996). Histidine patch thioredoxins. *Journal of Biological Chemistry*. 271,5059-5065

Luthy, R., James, U. and David, E. (1992). Assessment of protein models with three dimesional profiles. *Nature.* 356: 83–85.

Madigan, M. T. and Marrs, B. L. (1997). Extremophiles. *Scientific American.* 276(4): 82-87.

Makrides, S. (1996). Strategies for achieving high-level expression of genes in *Escherichia coli*. *Microbiological Reviews.* 60(3): 512-538.

Marchand, J. and Peccoud, J. (2012). Building block synthesis using the polymerase chain assembly method. *Methods in Molecular Biology.* 852: 3-10.

Marchant, M., Franzetti, A., Pavlostathis, S., Tas, D., Erdbrűgger, I., Űnyayar, A., Mazmanci, M. and Banat, I. (2008). Thermophilic bacteria in cool temperate soils: Are they metabolically active or continually added by global atmospheric transport? *Applied Microbiology and Biotechnology*. 78: 841-852.

Marchant, R. and Banat, I. (2010). The genus *Geobacillus* and hydrocarbon utilization. *Handbook of Hydrocarbon and Lipid Microbiology*. 1888-1896.

Marti-Renom, M.A., Stuart, A.C., Fiser, A., Sánchez, R., Melo, F. and Šali, A. (2000). Comparative protein structure modeling of genes and genomes. *Annual Review of Biophysics and Biomolecular Structure.* 29: 291-325.

McAlpine, J., Bachmann, B., Piraee, M., Tremblay, S., Alarco, A., Zazopoulos, E. and Farnet, C. (2005). Microbial genomics as a guide to drug discovery and structural elucidation: ECO-02301, a novel antifungal agent, as an example. *Journal of Natural Products.* 68, 493-496.

McCoy, J. and LaVallie, E. (2001). Expression and purification of thioredoxin fusion proteins. *Current Protocols in Molecular Biology.*

Murakami, T., Kanai, T., Takata, H., Kuriki, T. and Imanaka, T. (2006). A novel branching enzyme of the GH-57 Family in the hyperthermophilic archaeon *Thermococcus kodakaraensis* KOD1. *Journal of Bacteriology.* 188(16): 5915–5924.

Nazina, T., Tourova, T., Poltaraus, A., Novikova, E., Grigoryan, A., Ivanova, A., Lysenko, A., Petrunyaka, V., Osipov, G., Belyaev, S. and Ivanov, M. V. (2001). Taxonomic study of aerobic thermophilic bacilli: Descriptions of *Geobacillus subterraneus* gen. nov., sp. nov. and *Geobacillus uzenensis* sp. nov. from petroleum reservoirs and transfer of *Bacillus stearothermophilus, Bacillus thermocatenulatus, Bacillus thermoleovorans, Bacillus kaustophilus, Bacillus thermodenitrificans* to *Geobacillus* as the new combinations *G. stearothermophilus, G. thermocatenulatus, G. thermoleovorans, G. kaustophilus, G. Thermoglucosidasius* and *G. thermodenitrificans. International Journal of Systematic and Evolutionary Microbiology.* 51(2): 433-446.

Nelson, D. and Cox, M. (2005). *Lehninger Principles of Biochemistry: Fourth edition*. New York: W. H. Freeman and Company.

Ni, Y. and Chen, R. (2009). Extracellular recombinant protein production from *Escherichia coli*. *Biotechnology Letters.* 31: 1661–1670

Nielsen, J., Borchert, T. and Vriend, G. (2001). The determinants of α-amylase pH-activity profiles. *Protein Engineering*. 14(7): 505-512.

Pal, K., Kumar, S., Sharma, S., Garg, S. , Alam, M. , Xu, H., Agrawal P. and Swaminathan, K. (2010). Crystal structure of full-length *Mycobacterium tuberculosis* H37Rv glycogen branching enzyme: insights of N-terminal beta-sandwich in substrate specificity and enzymatic activity. *The Journal of Biological Chemistry.* 285 (27): 20897-20903.

Palomo, M., Pijning, T. Booiman, T., Dobruchowska, J., van der Vlist, J., Kralj, S., Planas, A., Loos, K., Kamerling, J., Dijkstra, B., van der Maarel, M., Dijkhuizen, L. and Leemhuis, H.(2011). *Thermus thermophilus* glycoside hydrolase family 57 branching enzyme: Crystal structure, mechanism of action, and products formed. *The Journal of Biological Chemistry.* 286(5): 3520-30

Preiss, J. (1984). Bacterial glycogen synthesis and its regulation. *Annual Review of Microbiology*. *38*: 419-458.

Reddy, N., Nimmagada, A. and Rao, K. (2003). An overview of the microbial α-amylase family. *African Journal of Biotechnology*. 2(12): 645-648.

Ring, C., Sunt, E., Mckerrow, J., Leet, G., Rosenthalt, P., Kuntz, I. and Cohen, F. (1993). Structure-based inhibitor design by using protein models for the development of antiparasitic agents. *Proc Natl Acad Science*. 90: 3583-3587.

Romeo, T., Kumar, A., and Preiss, J. (1988). Analysis of the *Escherichia coli* glycogen gene cluster suggests that catabolic enzymes are encoded among the biosynthetic genes. *Gene.* 70: 363-376.

Roux, k. (1995). Optimization and troubleshooting in PCR. *Genome Research.* 4: S185-S194.

Roy, A., Kucukural, A and Zhang, Y. (2010). I-TASSER: A unified platform for automated protein structure and function prediction. *Nature Protocol.* 5(4): 725-738.

Santos, C., Tonoli, C., Trindade, D., Betzel, C., Takata, H., Kuriki, T., Kanai, T., Imanaka, T., Arni, R. and Murakami, M. (2011). Structural basis for branching-enzyme activity of glycoside hydrolase family 57: Structure and stability studies of a novel branching enzyme from the hyperthermophilic archaeon *Thermococcus Kodakaraensis* KOD1. *Proteins*; 79: 547–557.

Sasangka, P., Matsuno, A., Tanaka, A., Akasaka, Y., Suyama, S., Kano, S., Miyazaki, M., Akao, T., Kato, M., Kobayashi, T. and Tsukagoshi, N. (2002). Structural features of the glycogen branching enzyme encoding genes from aspergilli. *Microbiological Research.* 157: 337–344.

Saxena, R., Sheoran, A., Giri, B. and Davidson, W. (2003). Purification strategies for microbial lipases. *Journal of Microbiological Methods.* 52: 1-18.

Schattner, P. (2009). Genomics made easier: An introductory tutorial to genome datamining. *Genomics*. 93: 187–195.

Scherlach, K. and Hertweck, C. (2006). Discovery of aspoquinolones A–D, prenylated quinoline-2-one alkaloids from *Aspergillus nidulans,* motivated by genome mining. Organic & Biomolecular Chemistry. 4, 3517–3520.

Schiraldi, C. and De Rosa, M. (2002). The production of biocatalysts and biomolecules from extremophiles. *Trends in Biotechnology.* 20(12): 515-521.

Schmid, A., Dordick, J., Hauer, B., Wubbolts, M. and Witholt, B. (2001). Industrial biocatalysis today and tomorrow. *Nature*, *409*, 258-268.

Serrano, L., Bycroft, M. and Fersht, A. (1991). Aromatic-aromatic interactions and protein stability investigation by double-mutant cycles. *Journal of Molecular Biology.* 218: 465-475.

Sezonov,G., Joseleau-Petit, D. and D'Ari, R. (2007). *Escherichia coli* physiology in luria-bertani broth. *Journal of Bacteriology*. 189 (23): 8746–8749.

Shinohara, M., Ihara, M., Abo, M., Hashida, M., Takagi, S. and Beck, T. (2001). A novel thermostable branching enzyme from an extremely thermophilic bacterial species, *Rhodothermus obamensis*. *Applied Microbiology and Biotechnology*. 57. 653–659.

Sippl, M. (1993). Recognition of errors in three-dimensional structures of proteins. *Proteins.* 17: 355-362.

Skolnick, J. and Fetrow, S. (2000). From genes to protein structure and function: novel applications of computational approaches in the genomic era. *Trends in Biotechology*. 18: 34-39.

Studier, F. (2005). Protein production by auto-induction in high-density shaking cultures. *Protein Expression and Purification.* 41(1):207-234.

Studier, F. and Moffatt, B. (1986). Use of bacteriophage T7 RNA polymerase to direct selective high-level expression of cloned genes. *Journal of Molecular Biology*. 189:113-130.

Takata, H., Akiyama, T., Kajiura, H., Kakutani, R., Furuyashiki, T., Tomioka, E., Kojima, I. and Kuriki, T. (2010). Application of branching enzyme in starch processing. *Biocatalysis and Biotransformation.* 28 (1): 60–63.

Takata, H., Kato, T., Takagi, M. and Imanaka, T. (2005). Cyclization reaction catalyzed by *Bacillus cereus* branching enzyme, and the structure of cyclic glucan produced by the enzyme from amylose. *Journal of Applied Glycoscience*. 52: 359-365.

Takata, H., Takaha, T., Kuriki, T., Okada, S., Takagi, M. and Imanaka, T. (1994). Properties and active center of the thermostable branching enzyme from *Bacillus stearothermophilus*. *Applied and Environmental Microbiology.* 60 (9): 3096-3104.

Takata, H., Takaha, T., Okada, S., Takagi, M., and Imanaka, T. (1996). Cyclization reaction catalyzed by branching enzyme. *Journal of Bacteriology.* 178 (6): 1600-1606.

Takeda, Y., Guan, H. and Preiss, J. (1993). Branching of amylose by the branching isoenzymes of maize endosperm. *Carbohydrate Research*. 240: 253-263.

Terada, Y., Yanase, M., Takata, H., Takaha, T., and Okada, S. (1997). Cyclodextrins are not the major cyclic α-1,4-glucans produced by the initial action of cyclodextrin glucanotransferase on amylose. *The Journal of Biological Chemistry.* 272 (25): 15729-15733.

TerMaat, J., Pienaar, E., Whitney, S., Mamedov, T., and Subramanian, A. (2009). Gene synthesis by integrated polymerase chain assembly and PCR amplification using a high-speed thermocycler. *Journal of Microbiological Methods.* 79: 295-300.

Terpe, K. (2003). Overview of tag protein fusions: from molecular and biochemical fundamentals to commercial systems. *Applied Microbiology and Biotechnology*. 60: 523-533.

Thiemann, V., Saake, B., Vollstedt, A., Schafer, T., Puls, J., Bertoldo, C., Freudl, R and Antranikian, G. (2006). Heterologous expression and characterization of a novel branching enzyme from the thermoalkaliphilic anaerobic bacterium *Anaerobranca gottschalkii*. *Applied Microbiology and Biotechnology*. 72: 60–71.

Turner, P., Mamo, G., and Karlsson, E. (2007). Potential and utilization of thermophiles and thermostable enzymes in biorefining. *Microbial Cell Factories. 6*(1): 9.

U.S. Food and Drug Administration. (2010). Guidance for Industry: Enzyme Preparations: Recommendations for Submission of Chemical and Technological Data for Food Additive Petitions and GRAS Notices. Silver Spring, MD: U.S. Department of Health and Human Services. Retrieved from: http://www.fda.gov/Food/GuidanceComplianceRegulatoryInformation/GuidanceDocuments/FoodIngredientsandPackaging/ucm083345.htm

Utsumi, Y., Yoshida, M., Francisco, Jr., P., Sawada, T., Kitamura, S. and Nakamura, Y. (2009). Quantitative assay method for starch branching enzyme with bicinchoninic acid by measuring the reducing terminals of glucans. *Journal of Applied Glicoscience*. 56: 215-222.

van den Burg, B. (2003). Extremophiles as a source for novel enzymes. *Current Opinion in Microbiology*. 6(3): 213-218.

van der Maarel, M. (2009). Starch-Processing Enzymes. *Enzymes in Food Technology*, 320-331.

van der Maarel, M., van der Veen, B., Uitdehaag, J., Leemhuis, H., and Dijkhuizen, L. (2002). Properties and applications of starch-converting enzymes of the alpha-amylase family. *Journal of Biotechnology*. 94: 137–55.

van der Maarel, M., Vos, A., Sanders, P., and Dijkhuizen, L. (2003). Properties of the glucan branching enzyme of the hyperthermophilic bacterium *Aquifex aeolicus*. *Biocatalysis and Biotransformation.* 21 (4): 199- 207.

van der Wal, F. J., Koningstein, G., ten Hagen, C. M., Oudega, B., and Luirink, J. (1998). Optimization of bacteriocin release protein (BRP)-mediated protein release by *Escherichia coli:* Random mutagenesis of the pCloDF13-derived BRP gene to uncouple lethality and quasi-lysis from protein release. *Applied and Environmental Microbiology.* 64(2): 392-398.

van der Wal, F. J., ten Hagen-Jongman, C. M., Oudega, B., and Luirink, J. (1995). Optimization of bacteriocin-release-protein-induced protein release by

Escherichia coli: Extracellular production of the periplasmic molecular chaperone FaeE. *Applied Microbiology and Biotechnology.* 44: 459-465.

Vasina, J. and Baneyx, F. (1997). Expression of aggregation-prone recombinant proteins at low temperatures: A comparative study of the *Escherichia coli cspA* and *tac* promoter systems. *Protein Expression and Purification*. 9: 211-218.

Vieille, C., Epting, K., Kelly, R. and Zeikus, J. (2001). Bivalent cations and amino-acid composition contribute to the thermostability of *Bacillus licheniformis* xylose isomerase. *European Journal of Biochemistry*. 268: 6291-6301.

Villaverde, A., and Carrio, M. (2003). Protein aggregation in recombinant bacteria: Biological role of inclusion bodies. *Biotechnology letters.* 25(17): 1385-1395.

Voet, D. and Voet, J. (2011). *Biochemistry: Fourth edition*. Hoboken, NJ: John Wiley & Sons, Inc.

Wang, X., Li, X. and Li, Y. (2007). A modified Coomassie Brilliant Blue staining method at nanogram sensitivity compatible with proteomic analysis. *Biotechnology Letters.* 29:1599–1603.

Wilfinger, W. , Mackey, K. and Chomczynski, P. (1997). Effect of pH and ionic strength on the spectrophotometric assessment of nucleic acid purity. *BioTechniques*. 22: 474-481.

Wu, G., Fiser, A., ter Kuile, B., Sali, A. and Müller M. (1999). Convergent evolution of *Trichomonas vaginalis* lactate dehydrogenase from malate dehydrogenase. *Proceedings of the National Academy of Sciences of the United States of America.* 96: 6285-6290.

Wu, G., Wolf, J., Ibraheem, A., Vadasz, S., Gunasinghe, M. and Freeland, S. (2006). Simplified gene synthesis: A one-step approach to PCR-based gene construction. *Journal of Biotechnology*. 124(3): 496503.

Wu, S., Skolnick, J. and Zhang, Y. (2007). *Ab initio* modeling of small proteins by iterative TASSER simulations. *BMC Biology*. 5(17).

Yoon, S., Ryu, S., Lee, S. and Moon, T. (2008). Purification and characterization of branching specificity of a novel extracellular amylolytic enzyme from marine hyperthermophilic *Rhodothermus marinus*. *Journal of Microbiology and Biotechnology*. 18(3): 457-464.

Yurieva, O., Skangalis, M., Kuriyan, J. and O'Donnel, M. (1997). *Thermus thermophilis dnaX* homolog encoding γ- and τ-like proteins of the chromosomal replicase. *The Journal of Biological Chemistry.* 272: 27131-27139.

Zeigler, D (2001). The Genus *Geobacillus*. *Bacillus Genetic Stock Center 3*: 7-11.

Zhang, Y. (2008). I-TASSER server for protein 3D structure prediction. *BMC Bioinformatics.* 9(40).

Zhang, Y. (2009). -TASSER: Fully automated protein structure prediction in CASP8. *Proteins.* 77(9): 100-113.

Zhang, Y. and Skolnick, J. (2004). Scoring function for automated assessment of protein structure template quality. *Proteins*. 57 (4): 702–710.

Zhang, Y. and Skolnick, J. (2005). The protein structure prediction problem could be solved using the current PDB library. *Proceedings of the National Academy of Sciences of the USA.* 102(4): 1029-1034.

Zhu, D., Mukherjee, C., Biehl, E. and Hua, L. (2007). Discovery of a mandelonitrile hydrolase from *Bradyrhizobium japonicum* USDA110 by rational genome mining. *Journal of Biotechnology*. 129: 645–650.