CLONING, EXPRESSION AND CHARACTERIZATION OF TREHALOSE SYNTHASE FROM *PYROBACULUM CALIDIFONTIS*

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CLONING, EXPRESSION AND CHARACTERIZATION OF TREHALOSE SYNTHASE FROM *PYROBACULUM CALIDIFONTIS*

Submitted to University of Management and Technology Lahore

In partial fulfillment of the requirements

For the award of degree of

MS

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By

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LAHORE, PAKISTAN

DECLARATION

I, Rabbia Muzaffar student of MS biotechnology ID: 15001254001 aware of and understand the university's policy on plagiarism and I certify that this thesis titled **"Cloning, Expression and Characterization of Trehalose Synthase from *Pyrobaculum calidifontis*"** is my own work, except where indicated by referencing, and the work presented in it has not been submitted in support of another degree or qualification from this or any other university or institute of learning. All experimental work belong to me; the collaborative contributions have been indicated clearly and acknowledged. Due references have been provided on all supporting literature and resources.

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RESEARCH COMPLETION CERTIFICATE

This is to certify that the thesis entitled **"Cloning, Expression and Characterization of Trehalose Synthase from *Pyrobaculum calidifontis*"**submitted by Rabbia Muzaffar ID: 15001254001has been accepted towards the partial fulfillment of the requirement for MS degree in Biotechnology**.** The quantum and quality of the work contained in this thesis is adequate for the award of the degree.

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DEDICATION

This humble effort is dedicated to my
Parents
Who gave me the greatest gift anyone could give another person, they believed in me.

Abstract

Trehalose is a multifunctional disaccharide sugar which is produced in different organisms like plants, animals, fungi and other microbes. It is known for its ability to preserve the important proteins and biomolecules of organisms inside the body under stress conditions i.e. temperature fluctuations and desiccation. Due to this potent nature, it can be used in pharmaceutical, food and cosmetic industries as stabilizer. It is difficult to attain this commercially valuable product from those organisms in high amount. Therefore, enzymes can be used for in vitro synthesis of trehalose at large scale. The most efficient enzyme for the production of trehalose is the Trehalose synthase, a glycosyltransferase. It involves a one-step pathway that convert maltose into trehalose. The enzyme of trehalose synthase was successfully cloned and expressed from *Pyrobaculum calidifontis.* The ORF of the gene was *Pcal_1359.* Thegene was expressed in *E. coli* expression system through *pET-21a (+)* vector. The protein was produced as insoluble inclusion bodies. That was refolded by various strategies. The considerable activity was observed by refolding with Urea. The 3D structure was not available, therefore it was made by homology modelling technique. Furthermore, the structure was refined by Molecular dynamics (MD) simulation studies that have indicated that the enzyme can survive at high temperature range from 300K to 400K. The docking studies was also performed by using maltose as substrate and acrabose, cathomycin and ValidamycinA as inhibitors. The active site residues of Pcal_1359 was Asp_{119}, His_{139}, Arg_{222}, and Glu_{308}. The binding affinity was highest with cathomycin that indicated it as a potential inhibitor for the enzyme.

Acknowledgement

With pen in hand, I pause to think that what words do justice to express my thanks to **ALMIGHTY ALLAH** for HIS unlimited kindness. The Omnipotent, the Merciful and the Beneficent who is the entire source of all knowledge and wisdom to mankind. I offer my humblest thanks from the deepest core of my heart to the greatest man of the world, the Holy **PROPHET MUHAMMAD (PEACE BE UPON HIM)**, a lofty personality and eternal source of guidance, knowledge and blessings for the mankind as a whole.

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Lastly, I feel weak and deficient in vocabulary to find suitable words to express my feelings with tearful eyes for my loving **Parents** who taught me to take the first step, to speak the first word and inspired me throughout of my life, whose hands are always raised for prayers which made me successful in every field of my life. They supported me to achieve sources in my academic endeavors and other spheres of life. Their day and night prayers enable me to join higher ideas of life, solving all of problems and boosted my moral to accomplish my goals.

Rabbia Muzaffar

List of Abbreviations

APS	Ammonium per sulfate
$CaCl_2$	calcium chloride
DNA	deoxyribonucleic acid
dNTP	deoxyribonucleotide triphosphate
IPTG	isopropyl-beta-D-1-thiogalactopyranoside
LB	Lauria Bertani
mg	milligram
mL	mili liter
mM	mili molar
$MgCl_2$	magnesium chloride
mM	millimolar
NaCl	sodium chloride
NaOH	sodium hydroxide
ng	nanogram
nt	nucleotide
OD	optical density
ORF	open reading frame
PCR	polymerase chain reaction
pH	paviour of hydrogen
RNA	ribonucleic acid
rpm	revolutions per minute
SDS	Sodium dodecyl sulfate
TAE	tris-acetate EDTA
Taq	*Thermus aquaticus*
UV	ultraviolet
X-Gal	5-Bromo-4-chloro-3-indolyl-b-D-galactopyranoside
µl	microliter
µg	microgram

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CHAPTER1

INTRODUCTION

1.1 TREHALOSE

1.1.1 History

Trehalose ($C_{12}H_{22}O_{11}$) is a non-reducing sugar, composed of two D-glucose monomers linked by α,α- 1,1 glycosidic linkage. The discovery of this sugar was credited to H.A. Wiggers in 1832 during his research on ergot of rye solutins. Afterwards, Mitscherlich have isolated it from mushrooms in 1858 and named as “mycose”. In the same year, Berthelot extracted it from cocoon samples which he had collected from the Middle East and named this sugar as “trehalique glucose” or “trehalose”. He noted that its properties were similar with the mycose that was isolated from mushrooms. In early times, it was considered as rare sugar. Later, it was found in various plants, fungi, insects, and some animals in abundance. Due to tenuous purification from different organisms, it is difficult to obtain this extraordinary sugar at large scale (Harding, 1923).