GENETIC IMPROVEMENT OF BACTERIAL XYLANASE PRODUCTION

By

MAHA TAIMOUR HASSAN EMAM

B.Sc. Agric. Sc. (Biotechnology), Cairo University, 2003
M.Sc. Agric. Sc.(Genetics), Ain Shamus University, 2010

A Thesis Submitted in Partial Fulfillment

Of

The Requirement for the Degree of

DOCTOR OF PHILOSOPHY
in

Agricultural Sciences
(Genetics)

Department of Genetics
Faculty of Agriculture
Ain Shams University

2017

Approval Sheet

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ABSTRACT

Maha Taimour Hassan Emam: Genetic Improvement of Bacterial Xylanase production. Unpublished PhD Thesis, Department of Genetics, Faculty of Agriculture, Ain Shams University, 2017.

Evaluation of the collected bacterial strains revealed that, the maximum xylanase activity of *Bacillus pumilus* GH and *Geobacillus. sterothermophilus* 2027 was 80 and 40 U/ml, respectively at 60^{o}C and pH7 with negligible amounts of cellulase activity. Xylanase activity from both strains showed tolerance to high temperature and alkaline conditions. Both strains were subjected to UV and EMS mutagenesis. The results proved that EMS is more effective as a mutagenic agent than UV irradiation for induction of high xylanase production. The best mutants of *B. pumilus* GH and *G. sterothermophilus* 2027 were selected for construction of intraspecific and intergeneric protoplast fusion. The highest xylanase activity (294.3 U/ml) was obtained from intraspecific protoplast fusion between *B. pumilus* mutants which increased by about two folds and 3.67 folds in comparison to parental strains and *B. pumilus* GH wild type, respectively. The thermostable endo-1,4-beta-xylanase gene of *B. pumilus* GH strain was isolated from chromosomal DNA using specific primers, then cloned into pET29a (+) vector and transformed into *E. coli* DH5α. The positive clone was selected, sequenced and submitted to gene bank with the accession number KT757524.1. The open reading frame of the xylanase gene was 687 bp encoding a protein of 228 amino acids with a molecular mass of 23 kDa. The recombinant plasmid containing xylanase gene was transformed to expression host *E.coli* BL21 (DE3) and the xylanase gene was successfully expressed but xylanase activity is lower than *B. pumilus* GH wild type strain.

Key Words: *Bacillus, E. coli*; xylanase; UV mutation; EMS; protoplast fusion; sequence analysis; gene cloning and expression.

ACKNOWLEDGMENT

First and foremost praises and thanks to **Allah**, the Lord, the most Gracious, and the most Merciful. **Allah** given me the power to believe in my passion and pursue my dreams. I would never have been able to finish my dissertation without the guidance of my committee members, help from friends, and support from my family.

I would like to express my deep appreciation and gratitude to **Prof. Dr. Samir Abd El-Aziz Ibrahim** for his guidance, encouragement, valuable discussions, suggestion and creative ideas which helped me during the progress of this work.

I would like to express my special gratitude and thanks to **Prof. Dr. Karima Abd El-Hafez Mohamed** for her kindness, patience, valuable advices and motivation during the laboratory work and for her great effort during writing the thesis. She always was being like my mother.

I would like to thank **Prof. Dr. Fatma Mohammad Ibrahim Badawy** for her kindness, guidance and facilities offered during this work.

I would like express my great thanks and gratitude to all the **staff members** of the Department of Genetics, Faculty of Agriculture, Ain Shams University for their help.

Gratefulness and thanks to my colleagues at **Applied Microbial genetics lab**, for their cooperation and assistance during this work and many thanks to all my friends at National Research Center for their help and moral support

Finally I would like to thank **my family** specially **my mother** for their tremendous support, encouragement and constant love in all my life.

DEDICATION

I dedicate this work to all my family members**; My Father**, **My Mother**, My brother (**Tamer**), My dear sister (**Sara**) and My lovely nephews (**Sondos and Hamza**)

Maha Taimour

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LIST OF ABBREVIATION

ABBREVIATION	MEAN
Arg	Arginine
bp	Base pair
BSA	Bovine serum albumin
CMC	Carboxy methyl cellulose
DNA	Deoxy ribonucleic acid
DNS	Dinitrosalicylic acid
EDTA	Ethylenediaminetetraacetic acid
EMS	Ethyl methane sulfonate
GH	Glycoside hydrolase
His	Histidine
IPTG	Isopropyl β-D-1-thiogalactopyranoside
kDa	kilo dalton
LB	Luria-Bertani
M	Marker
MeGA	4-O-Methyl-a-D-glucuronic acid
MM	Minimal medium

MW	Molecular weight
NA	Nutrient agar
O.D	Optical density
ORF	Open reading frame
PAGE	Poly acrylamide gel electrophoresis
PCR	Polymerase chain reaction
PEG	Poly ethylene glycol
rpm	Rotation per minutes
rRNA	Ribosomal ribonucleic acid
SDS	Sodium dodecyl sulphate
UV	Ultraviolet
Val	Valine
XLM	Xylan liquid medium
XOs	Xylooligosaccharides

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