Screening, Characterization and Biochemical Studies for Novel Polyhydroxybutyrate (PHB) Producing and/or degrading bacteria.

Thesis submitted for Partial Fulfillment of Master degree of Science (Biochemistry)

Submitted by

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Development of gastric vaccines
Holding campany of bioproduct and Vaccines

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APPROVAL SHEET FOR SUBMISSION

Title of M.Sc. the Thesis: **Screening, Characterization and Biochemical Studies for Novel Polyhydroxybutyrate (PHB) Producing and/or degrading bacteria.**

Name of Candidate: **Eman Mohamed Mostafa Fath El-Din**

This thesis has been approved for submission by the supervisors:

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2. **Dr. Moataz fouad Abd El-Ahany**
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ABSTRACT

Name : Eman Mohamed Mostafa Fath El-din

Title of M.Sc. Thesis:

Screening, Characterization and Biochemical Studies for Novel Polyhydroxybutyrate (PHB) Producing and/or degrading bacteria.

Degree : Master degree in Science

In this study five Polyhydroxybutyrate (PHB)-degrading bacterial strains were isolated from soil samples collected from different agricultural sites in Egypt. These strains designated as MEM 1, MEM 2, MEM 3, MEM 4 and MEM 5. They were able to use Polyhydroxybutyrate [1 g/l] as a sole carbon source in the growth media. MEM1 shows the highest activity of degradation of the PHB. It was therefore chosen for further studies. MEM1 Isolated strain was preliminarily identified by API ® 20 NE as *Alcaligenes faecalis*. PCR amplification and partial sequence analysis of 16S rRNA genes identified MEM1 isolate as *Alcaligenes faecalis* strain ST1. The degradation potential of strains was examined and detected using Optical density, measured by a spectrophotometer. The level of succinic acid (one of PHB degradation products) in the culture broth was utilized as indirect indicator for the ability of growing bacteria to assimilate polymer. Attempts were made to amplify the gene involved in PHB degradation (depolymerase gene) by PCR amplifications. PCR product with the right size was formed. The DNA sequencing and alignment analysis indicated that the fragment very unlikely to contain the depolymerase gene, Further study is being done to clone the new depolymerase from the isolated strain.

Key Words:

Polyhydroxybutyrate(PHB), degradation, *Alcaligenes faecalis* and depolymerase.

signature:

Supervisors: **1- Dr. Mervat Elsayed Mohammed**

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List of Abbreviations

˚ C	: Degree centigrade
Abs	: Absorbance
bp	: Base pair
Da	: Daltons
DE	: depolmerase
DNA	: Deoxyribonucleic acid
EBI	: European bioinformatics institute
EDTA	: Ethylene Diamin Tetra Acetic Acid
F	: Forward
Fig.	: Figure
g/gm	: Gram
IUPAC	: International Union of Pure and Applied Chemistry
J.	: Journal
Kb	: Kilobase
Kg	: Kilogram
Kp	: Kilo base pair
L	: Liter
MPa	: millipascal
mcl-PHA	: medium-side-chain PHA
min	: minute
Mix	: mixture
ml	: milliliter
MSM	: minimal salt media
MT	: Metric Ton
NCBI	: national center for biotechnology information
nm	: nanometer
no	: Number
O.D.	: Optical density
PBS	: poly(butylene succinate)
PCL	: Polycaprolactone

PES	: poly(ethylene succinate)
PCR	: Polymerase Chain Reaction
PHA	: Polyhydroxyalkanoate
PHAs	: Polyhydroxyalkanoates
PHB	: polyhydroxybutyrate
P(HB-HV)	: poly(hydroxybutyrate/hydroxyvalerate)
PHV	: hydroxyvalerate
L-PLA	: poly(L-lactide)
PP	: Polypropylene
ppm	: Part per million
R, Rev.	: Reverse
Rpm	: revolutions per minute
rRNA	: Ribosomal ribonucleic acid
Rs	: reduce, reuse and recycle
scl-PHA	: short-side-chain PHA
SDS	: Sodium Dodecylsulphate
SDS-PAGE	: Sodium Dodecylsulphate-Polyacrylamide Gel
Sec	: Second
sp.	: Species
TBE	: Tris Borate buffer
Tg	: glass transition temperature
Tm	: Melting temperature
Tris	: Trishydroxymethylaminomethane
TSA	: Trypticase soy agar media
UPVC	: Unplasticized polyvinyl chloride
UV	: ultra violet
w/v	: Weight to volume
wt	: weight
µl	: micro liter

Chapter 1

INTRODUCTION
and Aim of the work