بسم الله الرحمن الرحيم

شبكة المعلومـــــات الجامعية

التوثيق الالكتروني والميكروفيلم

شبكة المعلومات الجامعية

جامعة عين شمس

التوثيق الالكتروني والميكروفيلم

﴿ قـــسـم ﴾

نقسم بالله العظيم أن المادة التي تم توثيقها وتسجيلها
علي هذه الأفلام قد اعدت دون آية تغيرات

يجب أن

تحفظ هذه الأفلام بعيداً عن الغبار

في درجة حرارة من 15 – 20 مئوية ورطوبة نسبية من 20-40 %

To be kept away from dust in dry cool place of
15 – 25c and relative humidity 20-40 %

بعض الوثائق الأصلية تالفة

بالرسالة صفحات

لم ترد بالأصـــل

GROWTH PHYSIOLOGY AND PRODUCTION OF MOSQUITOCIDAL TOXINS FROM Bacillus sphaericus

Thesis

Submitted to Department of Microbiology
For the Degree of Doctor of Philosophy(Microbiology)

By
Magda Abd El-Ghaffar El-Bendary
M. Sc. Microbiology
1994
Faculty of Science
Ain Shams University

Supervised By

Prof. Dr. Mohamed R. Abu Shady
Head of Microbiology Department
Faculty of Science
Ain Shams University

Prof. Dr. Mohamed S. Foda
Prof. of Microbial Chemistry
Genetic Engineering and
Biotechnology Division
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Prof. Dr. Belal A. Soliman
Head of Biological Sciences Dept.
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List of abbreviations

°C	degree Celsius
β-ME	β-mercaptoethanol
X-Gal	5-bromo-4-chloro-3-indolyl-beta-D-galactopyranoside
A_{260}	absorbance at 260 nm
A_{280}	absorbance at 280 nm
bp	base pair.
Btx	binary toxin
btx	binary toxin gene
BSA	bovine serum albumin.
cm	centimeter
CFU	colony forming unit.
dUTP	deoxy uracil triphosphate
dATP	deoxy-adenosine triphosphate
dCTP	deoxy-cytidine triphosphate
dGTP	deoxy-guanosine triphosphate
dTTP	deoxy-thymidine triphosphate
dNTP	deoxynucleotide triphosphate
DNA	deoxyribonucleic acid
DIG	digoxigenin
EDTA	ethyldiaminetetraacetic acid
FWC	final whole culture.
g	gram
h	hour
pH	hydrogen-ion exponent
IPTG	isopropyle beta-D-thiogalactopyranoside
kDa	kilo Dalton
kb	kilobase.
kV	kilovolt
l	litre
LB	Luria-Bertani medium
µF	microfarad
µg	microgram

µl	microliter
µM	micromolar
mA	milliampere
mg	milligram
mjoule	millijoule
ml	millilitre
mM	millimolar
min	minute
M	molar
Mtx	mosquitocidal toxin
mtx	mosquitocidal toxin gene
TEMED	N, N, N`, N`-tetramethyl ethylene-diamine.
ng	nanogram
nm	nanometer
OD	optical density.
pmol	picomol
PCR	polymerase chain reaction
RNA	ribonucleic acid
r.p.m	rotations per minute
SDS	sodium dodecyl sulphate
SDS-PAGE	sodium dodecyl sulphate polyacrylamide gel electrophoresis.
LC_{50}	The concentration of sample that will theoritically kill 50% of the mosquito larvae in a certain time
TTC	tri-phenyl-tetrazolium chloride
Tris	Tris (hydroxymethyl) aminomethane
V	volt
v/v	volume/volume
w/v	weight/volume

ABSTRACT

The present work was devoted to the isolation of entomopathogenic strains of *Bacillus sphaericus* from Egyptian environments, their characterization, identification and the elucidation of the physiological factors affecting their growth, and toxin production. Special attention was paid to the application of modern genetic engineering approaches for illustrating their taxonomic position in reference to present classifications as well as the study of possible relationships between sporulation and toxin formation. An investigation on possible production of the mosquitocidal toxin from indigenous strains using locally available agro-industrial by-products in comparison to an international strain, was carried out.

The obtained results have shown that some of the local isolates were highly toxic to mosquitoe larvae and possessed the typical ribotype pattern of known pathogenic *B. sphaericus* strains (homology group IIA). While some low toxic Egyptian isolates possessed a unique ribotype pattern that was different from the typical pattern of pathogenic strains. Furthermore, some isolates with low toxicity and have Mtx toxin, when serologically classified, were found to belong to serotypes 27, 5a5b, 9a9b and 26a26b. Serotypes 27, 5a5b and 9a9b were not known before to include low pathogenic strains while serotype 26a26b was known to have strains that lack both toxin genes.

The obtained results have also shown that sporulation process was essential for crystal toxin formation. Expression of sigma factor σ^F is responsible for toxin production in stage II and continued until stage

III of sporulation.

Physiological studies have revealed that some factors influenced the production of the binary toxin of *Bacillus sphaericus*. Such factors included phosphate concentrations, level of trace elements in the growth medium as well as aeration extents and incubation temperatures.

Comparative fermentation studies using an indigenous Egyptian strain as well as a standard international strain grown on media made of leguminous seeds and locally available agro-industrial by-products gave excellent growth and high yields of mosquitocidal toxins. Best results of the fermentation studies were obtained upon the use of the Egyptian strain when grown on soy flour, cottonseed flour, offals meal and backing yeast as complete media for toxin production. The obtained results were discussed in the light of their application feasibility regarding the possible local production of the mosquitocidal toxins from the highly potent indigenous strain grown on local agroindustrial byproducts and leguminous seeds in Egypt.