Faculty of Science
Dept. of Microbiology

Production and Biochemical Studies on Thrombolytic Enzyme from some Indigenous *Bacilli* of Egyptian Environments.

Thesis submitted to
The Faculty of Science, Ain Shams University for the Award of the Degree of Philosophy (In Microbiology)
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ABSTRACT

This work included the isolation of 126 strains of *Bacillus* spp. from different Governorates from Egyptian environment and they were screened for their ability to produce fibrinolytic enzyme. *Bacillus* isolate No.26 achieved the highest fibrinolytic enzyme activity and it was identified by 16S rRNA sequencing and data analysis as *Bacillus subtilis* strain with 99% similarity. Central composite design was employed to optimize and investigate interactions between critical variables needed for enzyme production. In submerged fermentation the practical application of the optimal concentrations of each parameter produced 16.6 U/ml of the enzyme under study. But in solid state fermentation, the fibrinolytic enzyme activity was 141.9 U/g. Purification of fibrinolytic enzymes from *Bacillus subtilis* Egy. takes place by three-steps purification scheme, partial purifiation by salting out with ammonium sulphate (30-60% saturation). Under these conditions, specific activity reached 231.7 U/mg protein and yield recovery 35.6%. Then, after dialysis against 50mM Tris-HCl buffer pH 8.0 over night, this partially purified enzyme was applied to ion exchange column on DEAE cellulose, third purification step (Gel filtration on Sephadex G-100), the specific activity of 1270 U/mg protein and yield recovery of 5.26%. The overall purification scheme, purification steps, purification folds and percentage of yield recovery are presented. Accordingly, the fibrinolytic enzyme of *Bacillus subtilis* Egy. was purified 21.5 times with final yield recovery of 5.26%. The molecular weight of the purified enzyme is 28.5 KDa by SDS PAGE electrophorasis. Purified enzyme is heat stable upon heating to 30^{o}C it's activity increased progressively with incubation reaction temperature up to 50^{o}C at pH 8.0, by using different reaction incubation times on purified enzyme activity shows increasing with increase the reaction incubation time till 60 min. the enzyme activity responded linearly to the increase in substrate concentration up to 8 mg/mL. Thrombolytic activity of purified fibrinolytic enzyme of *Bacillus subtilis* Egy. showed significant degradation of blood clot and it is safe to use because it exhibited no mortality after 15 days of subcutaneous administration of rats.

Key words: *Bacillus subtilis*, blood clots, fibrinolytic enzyme, solid state fermentation, purification.

List of Abbreviations

ANOVA	Analysis of variance
AMI	Acute myocardial infarction
CBC	Complete blood count
CCD	Central Composite Design
CCRD	central composite rotary design
CVD	Cardiovascular diseases
DEAE	Diethylaminoethyl
Df	Degree of freedom.
EDTA	Ethylenediaminetetraacetic acid
Hx and E	Hx (Hemotoxaline) and E (Eosin)
hr.	hour
KDa	Kilo dalton
(MEGA)	Molecular Evolutionary Genetics Analysis
NBS	N Bromosuccinimide
NRC	National Research Centre.
O.D	Optical Denisty
PMSF	phenylmethanesulfonyl fluoride
RBCs	Red Blood Cells
RSM	Response Surface Methodology
SDS	Sodium dodecyl sulfate.

SE	Stander Error
SSF	Solid-State Fermentation
TAE buffer	(**T**: Tris, **A**: acetic acid and **E**: EDTA) buffer
TCA	Trichloroacetic acid
U/g, U/mg	Unit/gram, Unit/miligram
(UPGMA)	Unweighted Pair Group Method with Arithmetic

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