STUDY OF DELTA ENDOTOXIN IMMUNOCROSSREACTIVITY OF *BACILLUS THURINGIENSIS* ISOLATES FROM KHUMBU BASE CAMP OF THE EVEREST REGION

A Dissertation

Submitted to the Central Department of Microbiology

Tribhuvan University

In Partial Fulfillment of the Requirements for the Award of the Degree of

Master of Science in Microbiology (Environmental Microbiology)

By
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RECOMMENDATION

This is to certify that **Mr. Upendra Thapa Shrestha** has completed this dissertation work entitled "**STUDY OF DELTA ENDOTOXIN IMMUNO-CROSSREACTIVITY OF *BACILLUS THURINGIENSIS* ISOLATES FROM KHUMBU BASE CAMP OF THE EVEREST REGION**" as a partial fulfillment of M. Sc. Degree in Microbiology under our supervision. To our knowledge this thesis work has not been for any other degree.

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ABSTRACT

Bacillus thuringiensis strains were isolated from soil samples collected from Khumbu Base Camp of the Everest region and subsequently identified by standard microbiological techniques including colonial characteristics, morphological characteristics and biochemical characteristics. The stationary phase culture broth was tested for insect bioassay and delta-endotoxins (crystal protein) were extracted from the crystal endotoxin producing strains (46 from Phereche and 40 from Sagarmatha National Park). The crystal proteins were partially purified in alkaline solution and further purified by Native-PAGE. Among the ten randomly selected isolates, *B. thuringiensis* S_6 culture and its purified crystal protein showed the highest insecticidal activity. The endotoxin was found to contain five subunits with molecular weight 107KD, 83KD, 71KD, 58KD and 40KD as revealed by SDS-PAGE. A pair of New Zealand white rabbits were used to raise polyclonal antisera against purified S_6 endotoxin. The presence of polyclonal antibody was confirmed by Ouchterlony double diffusion method against S_1, S_{2a}, S_4, S_5, S_{10}, P_2, P_6 and P_{10} antigens. Indirect ELISA was optimized using 6-8µg of the endotoxin coated microtitre plate per well. The optimal dilution of the polyclonal antibody was found to be 1000 folds corresponding to OD_{450} = 0.045 for colour observation. Of the total 86 endotoxin producing isolates, 31 (36.05%) corresponding endotoxins were 25-30% crossreactive with the polyclonal antisera. Similarly, 6 (6.97%) were 75-80% and 85-90% crossreactive each, and 4 (4.65%) were 80-85% crossreactive. Only 3 (3.49%) were more than 90% crossreactive. The Discriminatory Index (D) of the Indirect ELISA was found to be 0.92. The antibody raised against the crystal protein was finally confirmed by Western Blot.

TABLE OF CONTENTS

LISTS OF TABLES

LIST OF FIGURES

LIST OF PHOTOGRAPHS

LIST OF ABBREVIATIONS

APS	Ammonium per sulphate
BHI	Brian Heart Infusion Agar media
CBB R-250	Coomasie Brilliant Blue R-250
CFA	Complete Freund's adjuvant
cry gene	Crystal protein producing gene
cyt gene	Cytolytic toxin producing gene
D value	Discriminatory value
DDT	Dichlorodiphenyl trichloroethane
DMSO	Dimethoxy sulphdoxide
D/W	Distilled Water
EDTA	Ethylenediaminetetraacetic acid
FAO	Food and Agricultural Organization
ICP	Insecticidal Crystal Protein
ID	Immunodiffusion
IFA	Incomplete Freund's adjuvant
Ind-ELISA	Indirect Enzyme Linked Immunosorbent Assay
LB	Lauria Broth
MR test	Methyl red test
NA	Nutrient Agar
PBS	Phosphate Buffer Saline
PBS/T_w	Phosphate Buffer Saline Tween-20
PVDF	Polyvinylidene Fluoride membrane
SDS-PAGE	Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis
SIM	Sulphide-Indole motility agar
Tn element	Transposable elements
TEMED	N, N, N, N'-tetramethylenediamine
TMB	Tetramethylbenzidine
VP test	Voges-Proskauer test
WHO	World Health Organization