

**GENETIC SIMILARITIES AMONG *BACILLUS*
THURINGIENSIS STRAINS FROM DIFFERENT CLIMATIC
ZONES OF NEPAL**

A

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of the Degree of Master of Science in Microbiology**

By

Nirajan Bhattarai

Central Department of Microbiology

Tribhuvan University

Kirtipur, Kathmandu

Nepal

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RECOMMENDATION

This is to certify that **Mr. Nirajan Bhattarai** has completed this dissertation work entitled “**GENETIC SIMILARITIES AMONG *BACILLUS THURINGIENSIS* STRAINS FROM DIFFERENT CLIMATIC ZONES OF NEPAL**” as a partial fulfillment of M. Sc. degree in Microbiology under our supervision. To our knowledge, this thesis work has not been submitted for any other degree.

Prof. Dr. Vishwanath Prasad Agrawal

Academician
Nepal Academy of Science and
Technology (NAST)
Kathmandu, Nepal

Director
Universal Science College
Kathmandu, Nepal

Executive Director
Research Laboratory for Agricultural
Biotechnology and Biochemistry
(RLABB)
Kathmandu, Nepal

Dr. Prakash Ghimire, PhD

Associate Professor
Central Department of Microbiology
Tribhuvan University
Kirtipur, Kathmandu
Nepal

Dr. Shreekant Adhikari, PhD

Associate Professor
Central Department of Microbiology
Tribhuvan University
Kirtipur, Kathmandu
Nepal

CERTIFICATE OF APPROVAL

On the recommendation of **Prof. Dr. Vishwanath Prasad Agrawal, Dr. Prakash Ghimire, and Dr. Shreekant Adhikari**, this dissertation work by **Mr. Nirajan Bhattarai**, entitled “**GENETIC SIMILARITIES AMONG *BACILLUS THURINGIENSIS* STRAINS FROM DIFFERENT CLIMATIC ZONES OF NEPAL**” has been approved for the examination and is submitted to the Tribhuvan University in partial fulfillment of the requirement for M. Sc. degree in Microbiology.

Dr. Anjana Singh, PhD

Head of Department

Central Department of Microbiology

Tribhuvan University

Kirtipur, Kathmandu

Nepal.

Date:-

BOARD OF EXAMINERS

Recommended by:

Prof. Dr. Vishwanath Prasad Agrawal
Supervisor

Dr. Prakash Ghimire, PhD
Supervisor

Dr. Shreekant Adhikari, PhD
Supervisor

Approved by:

Dr. Anjana Singh, PhD
Head of Department

Examined by:

Bishnu Raj Tiwari
External Examiner

Dr. Dwij Raj Bhatta, PhD
Internal Examiner

Date:

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Date:.....

Nirajan Bhattarai

ABSTRACT

Bacillus thuringiensis is a spore forming bacterium showing the unusual ability to produce endogenous crystals during sporulation that are toxic for some pest insects. This work was performed to study the composition, ecological distribution of biochemical types and genetic similarities among the isolates of this entomopathogenic bacterium from high altitude region (Tangboche, Khumbu region), and Terai region (Biratnagar). Using acetate selection methods to screen soil samples, 115 presumptive *B. thuringiensis* were isolated from 43 soil samples. Of these strains, 64% (74/115) were classified as *B. thuringiensis* on the basis of their crystal proteins visualized by microscopy. The percentages of samples with *B. thuringiensis* were 78.9 and 83.4 for the Khumbu region and Biratnagar respectively. The respective *B. thuringiensis* indexes were 0.7 and 0.61 for the regions. Of the total 64 *B. thuringiensis* isolates, 13.51% were novel, while rest of the isolates (86.49%) were identified upto subspecies level as evident by the defined biochemical typing. *B. thuringiensis* subspecies *thuringiensis*, *indiana* and type 16 were recovered from both high altitude and Terai region. Randomly amplified polymorphic DNA (RAPD)-Polymerase Chain reaction (PCR) was used to study the genetic similarities. Based on the RAPD patterns obtained with random primer, *B. thuringiensis* subspecies *indiana* isolates from Khumbu region showed more than 65% genetic similarities, whereas those from Biratnagar showed more diverse among the strains.

Key Words: *Bacillus thuringiensis* subspecies, RAPD-PCR, genetic similarity, Khumbu, Biratnagar.

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LIST OF ABBREVIATIONS

APN	Aminopeptidase N
AP-PCR	Arbitrary primed PCR
bp	Base Pairs
BBMV	Brush border membrane vesicles
<i>cry</i> gene	Crystal protein producing gene
<i>cyt</i> gene	Cytolytic toxin producing gene
DAF	DNA Amplification Fingerprinting
D value	Discriminatory value
DDT	Dichlorodiphenyl trichloroethane
DMSO	Dimethoxy sulphoxide
D/W	Distilled Water
EDTA	Ethylenediaminetetraacetic acid
FAO	Food and Agricultural Organization
ICP	Insecticidal Crystal Protein
kDa	kilo Dalton
LB	Lauria Broth
MPCA	Microbial pest control agents
MR test	Methyl red test
NA	Nutrient Agar
PCR	Polymerase Chain reaction
RAPD	Randomly Amplified Polymorphic DNA
rRNA	Ribosomal RNA
Tn element	Transposable element
VP test	Voges-Proskauer test

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