Journal of Nepal Biotechnology Association, 7 (1), 2026: 75-84
DOI: https://doi.org/10.3126/jnba.v7i1.92087

Contents available at https://www.nepjol.info/index.php/jnba

Journal of Nepal Biotechnology Association

ISSN: 1728-1628

Journal Homepage: https://www.nepalbiotech.org/nba-journal

Research Article

Screening of Vip Gene in *Bacillus thuringiensis* Isolated from Different Geographical Areas of Nepal and Vip Protein Effect on Fall Armyworm

Elisha Upadhyaya, Mohammad Ataullah Siddiqui, Sunil Regmi, Ram Chandra Poudel, Deegendra Khadka, Nisha Rana, Jyoti Maharjan, Jaishree Sijapati

Molecular Biotechnology Unit, Nepal Academy of Science and Technology, Khumaltar, Lalitpur, Nepal

ARTICLE INFO

ARTICLE HISTORY

Received: 18/02/2026
Revised: 19/03/2026
Accepted: 20/03/2026

CORRESPONDENCE

Jyoti Maharjan; Jaishree Sijapati

Molecular Biotechnology Unit, Nepal Academy of Science and Technology, Khumaltar, Lalitpur, Nepal
Email: jyoti.maharjan@nast.org.np; sijajaya@gmail.com
https://orcid.org/0000-0003-3671-9853; https://orcid.org/0000-0002-1481-7316

ABSTRACT

Incautious use of chemical pesticides in the agricultural field has led to detriment of lands and decline soil fertility. It also poses human health risk and environmental degradation. *Bacillus thuringiensis*, a spore forming soil bacterium, predominantly produces insecticidal proteins, is a promising solution for sustainable pest management in agriculture. The present work aims to screen Vip genes in isolated stock samples of *B. thuringiensis* and to show the insecticidal effect of Vip 3 protein on Fall armyworm larva. Fifty *B. thuringiensis* isolates along with two commercial strains, *B. thuriengiensis* subsp. *galleriae* HD8 (BGSC 4G1) and *B. thuriengiensis* subsp. *morrisoni* HD12 (BGSC 4K1), were used for screening of vegetative insecticidal protein coding genes (Vip1, Vip2 and Vip3) by conventional PCR and sequencing. Gram staining was performed for bacterial morphology and only bipyramidal shaped protein crystals were visualized during Coomassie Brilliant Blue staining. Among 50 crystal-forming isolates, 90% strains were found to carry the Vip3 gene but none of the isolates were found to possess the Vip1 and Vip2 genes. However, Vip2 gene was detected in reference strains (BGSC 4G1 and BGSC 4K1). The pairwise comparisons and phylogenetic tree analysis of Vip3 genes from five Bt isolates showed 100% sequence similarities with worldwide distributed *B. thuringiensis* Vip3 gene. Ammonium sulfate precipitation was followed by partial purification of Vip3 protein. SDS-PAGE analysis revealed the molecular weight of the Vip3 protein around 90 KDa. From bioassay of Fall armyworm, lethal concentration (LC_{50}) of Vip3 protein of reference sample BGSC 4G1 was 16.2607 ± 5.4239 µg/ml and isolate Bt Kasara showed 19.1268 ± 8.289 µg/ml in 7 days. Protein of BGSC 4G1 showed strong effectiveness (needed 17.63% less protein) as compared to Bt Kasara Vip3 protein. Therefore, Vip3 protein showed a potential biopesticidal effect and it may be utilized as a sustainable pest management approach.

Keywords: *Bacillus thuringiensis*; Biopesticides; Pest management; Vegetative insecticidal protein

Introduction

The development of insect resistance to synthetic pesticides and their chemical hazards to the environment has led to the extensive interest in *Bacillus thuringiensis* (Bt) in pest-controlling technologies (Ibrahim et al., 2010; Watkins et al., 2012). *B. thuringiensis* (Bt) is a Gram-positive, motile, rod and spore-forming bacterium belonging to the family Bacillaceae widely known for bioinsecticides. *B. thuringiensis* was first discovered in 1901 by Shigetane Ishiwatari as a cause of sudden-collapse disease in silkworm larvae, later named by German scientist Ernst Berliner (Hannay & Fitz-James, 1955), reisolated from flour moth larvae in the state of Thuringia (Milner, 1994).

Many species of *B. thuringiensis* have shown broad-spectrum activity against different orders of insects, including Lepidoptera, Coleoptera, Diptera, and Hemiptera, as well as some nematodes and mosquitoes (El-Gaied et al., 2020; Ma et al., 2023; Şahin et al., 2018). Crystal (Cry or Cyt) protein (Bechtel & Bulla, 1976; Labaw, 1964), also known as (σ-endotoxins) and vegetative insecticidal protein (Vip) (Yu et al., 1997) are two major proteins secreted by *B. thuringiensis* during their respective stationary and vegetative growth phases. The vegetative insecticidal protein has distinct genomic sequences and binding sites with Cry protein (Aswathi et al., 2024; Yan et al., 2020). The family of vegetative insecticidal protein is classified into four subfamilies, Vip1, Vip2, Vip3 and Vip4, within which a total of more than 100 types, based on the amino acid sequence homology (Gupta et al., 2021). Subfamily Vip1 and Vip2 toxin form a heterodimer and their combination exhibits activity within Coleoptera and Hemipteran insect orders, whereas Vip3 is effective against many species of Lepidoptera (Syed et al., 2020). Among the Vip family, Vip3 proteins are the most widely studied and present in greater parts than others. Vip3 is divided into three classes (Vip3A, Vip3B and Vip3C), and their classes are subdivided again into 10 Vip3A (Vip3Aa to Vip3Aaj), three Vip3B (Vip3Ba, Vip3Bb, and Vip3Bc) and one Vip3Ca subclasses (Chakrabarty et al., 2020; Estruch et al., 1996; Gupta et al., 2021; Palma et al., 2012). Vip4, on the other hand, is the least characterized, and its pesticidal activity has been under research. The lethal effectiveness of Bt protein is based on the specificity of ligand-receptor binding (Wang & Cheung, 1994). The crystalline form of protein undergoes protease activation, following conformational changes, which drives pore formation and destruction of the larval midgut membrane (Bravo et al., 2007; Kumar et al., 1996). Normally, Vip3 protein (around 90 KDa) is in an inactive state. During activation, trypsin-like enzymes in the midgut of insect, cut the protoxin at 198 amino acid residues, releasing nearly 20 kDa N-terminal and 65 kDa C-terminal as minor and major fragments (Chakrabarty et al., 2020; Jiang et al., 2020; Núñez-Ramírez et al., 2020). The specific receptor present in the microvilli of epithelial cells can recognize the activated protein (Lee et al., 2003). The membrane pore formation process is still unclear; the fibroblast growth factor receptor (Fgfr) mediated endocytosis probably the cell lethality has been reported (Jiang et al., 2018).

The use of Bt protein, especially, cry protein is one of the sustainable approaches to developing a safer way of agricultural reform. The worldwide use of biopesticides has already been for a century (Nester et al., 2002), Bt protein is used as a major biopesticide (Kumar et al., 2021), accounting for 90% of biopesticides from Bt protein of total biopesticides in the United States (Sanchis & Bourguet, 2008). Besides the broad action of Cry protein against insects, the development of insect resistance to Bt crystal protein (Tabashnik, 1994; Van Rie & Ferré, 2000) and against a group of commercial pesticides (Washim et al., 2024), the researcher has increasing interest in the vegetative insecticidal protein. Vip protein has a crucial role in resistance pest management since it harbors activity against a target group of insects, along with a group of insect-resistant pesticides and Bt crystal protein-resistant pests.

In Nepal, the use of pesticides in agriculture has a long history and plays an important role in the commercial production of agricultural commodities. Vegetables are the major produce with nearly 90% use out of the national average of 396 gm of a.i/ha total pesticide use (Parajuli et al., 2021). Similarly, use of pesticides is increasing about 20% per year, with insecticides sharing the second highest imported pesticides, that is 33% (Ghimire & GC, 2018).

However, due to the poor integrated pest management strategy, the haphazard use of chemical pesticides is increasing day by day. Large amounts of pesticide residues have been reported, especially in plant-based food (Nyaupane, 2022), which directly affects human health and livelihood (Karki & Dangol, 2023). So, this situation redirects to an alternative search for sustainable agriculture practices. The application of Bt protein as a biopesticide in Nepal is lagging behind despite its worldwide use. One of the reason behind this is, the research and development of the biopesticides and its industrial production is less prioritized by the government and concern bodies. However, small-scale research is conducted in different institutions and research academics. Bioassay and lethal dose determination of different insects using chemical pesticides and biopesticides have been showed in the previous studies (Limbu et al., 2020; Sharma et al., 2022). Till now, research on the bioassay of Fall armyworms using Vip protein in Nepal has not been conducted yet. The study of Vip3 protein isolated from a diverse altitude ranging from 75-5050 meters (Rana et al., 2002) is one of the works demonstrating the specific insecticidal effect of the protein. Approaching its very distinct features, here we try to figure out the screening, characterization, and sequencing of the Vip3 gene encoding a protein of *B. thuringiensis* and its bioassay. Extracted Vip3 protein may be utilized as a sustainable pest management approach, contributing to reduce environmental impact, and exploited as a safer alternative to chemical pesticides.

Materials and Methods

Bacillus thuringiensis isolates and growth conditions

A total of 50, *Bacillus thuringiensis* lyophilized stock which were previously collected and biochemically characterized samples from six geographical regions of Nepal (Figure 1 and Figure 2) along with two references *B. thuriengiensis* subsp. *galleriae* HD8 and *B. thuriengiensis* subsp. *morrisoni* HD12 (BGSC 4G1 and 4K1), kindly provided by Prof. Dr. Zeigler (*Bacillus* Genetic Stock Center, Columbus, Ohio, USA) were used for this study. All stocks were revived in T3 agar media (per liter: 3 g of tryptone, 2 g of tryptose, 1.5 g of yeast extract, 0.05 M sodium phosphate [pH 6.8], and 0.005 g of MnCl; Travers et al., 1987).

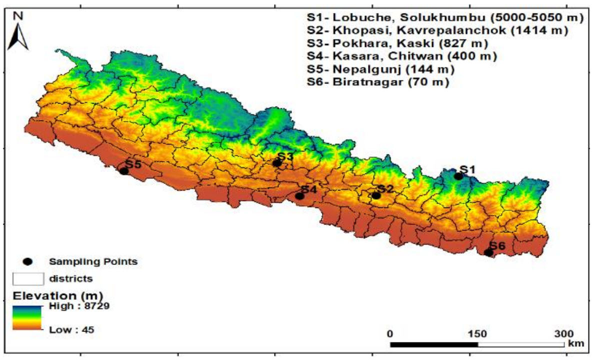

Figure 1: Map of Nepal showing six different geographical regions from where samples were collected. It covered an altitude from 70 to 5050 m asl.

Gram staining and coomassie brilliant blue (CBB) staining

Gram staining of Bt samples was performed thoroughly by smear preparation, CV-I complex formation using crystal violet and iodine, followed by decolorization with acetone and safranin as a counter stain. Bt spore crystal was observed by the Coomassie Brilliant Blue staining (CBB) technique. For this, loopful colonies of Bt isolates were inoculated in 50 ml T3 broth media and incubated at 110 rpm in a 30°C shaking incubator for 5 days. After incubation, 5 µl spore crystal mixture was transferred onto a glass slide, then it was air dried and heat-fixed. The slides were flooded with 0.133% CBB stain in 50% acetic acid CBB stain for 1 min, followed by rinsing with destaining solution (10% acetic acid, 50% methanol, and water). The type of spore crystal was visualized under the microscope.

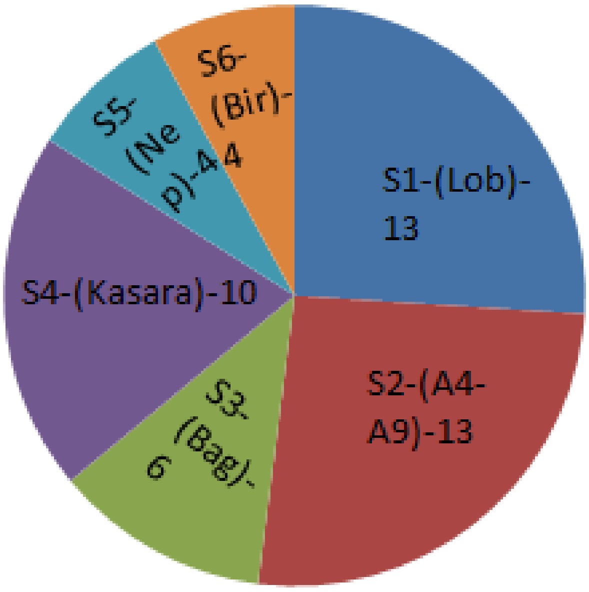

Figure 2: Pie chart showing the total number of samples and their first name of sample code from the respective region (S1 to S6) according to Figure 1.

DNA extraction and PCR amplification of Vip gene

The boiling method was used for DNA extraction of all samples (Abdelhai, 2016). Brielfly, overnight cultures of *B. thuringiensis* were prepared in Luria Bertani (LB) broth, and it was centrifuged at 13,000 rpm for 5 min. Pellets were resuspended in sterile distilled water and left to boil (100 °C) for 10 min. The suspension was again centrifuged at 10000 rpm for 2 minutes and the collected supernatant was stored at 4 °C for further use.

PCR amplification of the Vip gene from the selected *Bacillus* isolates was performed using three primer sets: Vip1, Vip2, and Vip3 consisting reference primer sequences, Vip1 forward-5' TTATTAGATAAACAACAACAAGAATATCAATCTATTMGNTGGATHGG 3', Vip1 reverse-5' GATCTATATCTCTAGCTGCTTTTTCATAATCTSARTANGGRTC 3', Vip2 forward-5' GATAAAGAAAAAGCAAAAGAATGGGRNAARRA 3', Vip2 reverse-5' CCACACCATCTATATACAGT

AATATTTTCTGGDATNGG 3' and Vip3 forward-5' TGCCACTGGTATCAARGA 3', Vip3 reverse-5' TCCTCCTGTATGATCTACATATGCATTYTTRTT RTT 3' (Hernández-Rodríguez et al., 2009). The PCR reaction mixture was prepared in a final volume of 20 µL consisting of 2X master mix, 10 pM/µl of primer concentration, and maintaining 100 ng of template. An Applied Biosystem PCR thermocycler is used to carry out PCR amplification. PCR condition set up for the *Vip* gene was initial denaturation of 95°C for 5 minutes, followed by 35 cycles of denaturation at 95°C for 1 minute, annealing at 51°C for 1 minute, extension at 72°C for 1.5 minutes, and one cycle of final extension at 72°C for 10 minutes. Expected product sizes, 585 bp, 845 bp, and 1621 bp for Vip1, Vip2 and Vip3 genes respectively were analyzed under 1.5% agarose gel electrophoresis.

Sequencing of Vip3 gene

Out of 50 samples, five strains from five geographical regions were taken for sequencing of the Vip3 genes. Sequencing was performed in a 3500xL Genetic Analyzer (Applied Biosystems) in the Molecular Biology Unit Laboratory of Nepal Academy of Science and Technology. Both obtained forward and reverse Sequences were aligned and edited using BioEdit. The sequence homology was determined by NCBI nucleotide BLAST. The phylogenetic tree was constructed using Mega 11 (Bootstrap 1000 repetition), the Neighbor-joining (NJ) method.

Extraction and purification of protein

Vegetative insecticidal protein was purified using a slightly modified method described by (Estruch et al., 1996). Selected Bt strains were grown in Terrific broth media (12% tryptone, 2.4% yeast extract, 0.04% glycerol, 0.17 M KH_2PO_4, 0.72 M K_2HPO_4) for 16 hours with 30°C shaking incubation. After the day, the protein suspension was separated at 5000 g for 20 minutes. Ammonium sulfate (80% W/V saturation) was added to the supernatant at 4°C with continuous stirring (magnetic) for protein precipitation, and the precipitate was collected again through centrifugation at 5000g for 15 min. After centrifugation, the pellets were resuspended in 1 ml of 100 mM Tris-HCl buffer (pH 7.5). Dialysis against 20 mM pH 7.5 Tris-HCl buffer was followed at 4 °C overnight to remove especially the salts and small molecules.

Analysis of Vip protein

Partially purified proteins were separated based on their molecular weight by Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE), Invitrogen Mini Gel Tank System. A separating gel (12%) and 5% stacking gel were used for Electrophoresis. Before loading, Protein samples were heated at 95 °C for 10min, and prepared in sample loading buffer containing 0.01% bromophenol blue. Electrophoresis was performed using 1X SDS-PAGE running buffer at 120 volts for 2 to 3 hours. After completion of electrophoresis, proteins were stained with 0.1% CBB, and a destaining solution was applied to remove the background stain.

Preparation of protein standard and detection of extracted protein concentration

The concentration of protein was determined by the Biuret method. A total of six stocks of concentration of BSA from 0.5 mg/ml to 10 mg/ml were taken for standard preparation. For the Biuret test, 25 µl of protein (partially purified protein for the test sample and BSA for standard) and 125 µl of Biuret reagent were mixed, and it was incubated for 30 min at room temperature in the dark conditions. Tris buffer with biuret reagent was taken for the blank. After incubation, absorbance was read at 540 nm. The concentration of test samples was calculated using a standard equation.

Bioassay of insects

Fall armyworm (FAW) eggs were collected from a laboratory of the Entomology Division, Nepal Agriculture Research Council (NARC), Khumaltar, Lalitpur. Eggs were reared for up to 3 days until the larvae reached their third instar phase by feeding on fresh leaves of maize. Third-instar larvae were used for the assay.

The *in vitro* bioassay against FAW was performed by taking the reference from different sources (Estruch et al., 1996; Maheesha et al., 2022; Sharma et al., 2022). Different concentrations of protein were prepared: 10 µg/ml, 25 µg/ml, 50 µg/ml, 75 µg/ml, and 100 µg/ml for activity against larvae.

Fresh Maize leaves were dipped into 100 ml of a particular concentration of protein solution for 30 seconds. Treated leaves were air-dried for a few minutes and placed into bioassay containers containing tissue at the bottom. Larvae were allowed to feed on protein-treated maize leaves. For control larvae, protein untreated fresh leaves were feeded accordingly. This process was repeated up to 6-7 days at 24-hour intervals. Old leaves were removed daily, and freshly treated leaves were provided for feeding. Containers were kept well-labeled, provided with proper ventilation, and maintained at 30 °C room temperature. Mortality was recorded after 6 days.

Results and Discussion

Staining of vegetative cells and crystal protein

Gram staining of all 50 samples showed Gram-positive bacteria. Rod-shaped purple-coloured bacteria were observed under a 100X (immersion oil) microscope. Figure 3 shows the Gram staining and crystal staining of the *Bacillus thuringiensis* bacterium. All 50 isolates of *B. thuringiensis* form crystal protein during sporulation. Under microscopic observation, released crystals could be distinguished from spores since they stained purple and displayed a unique diamond shape, while spores remained white and elliptical in appearance (Figure 3).

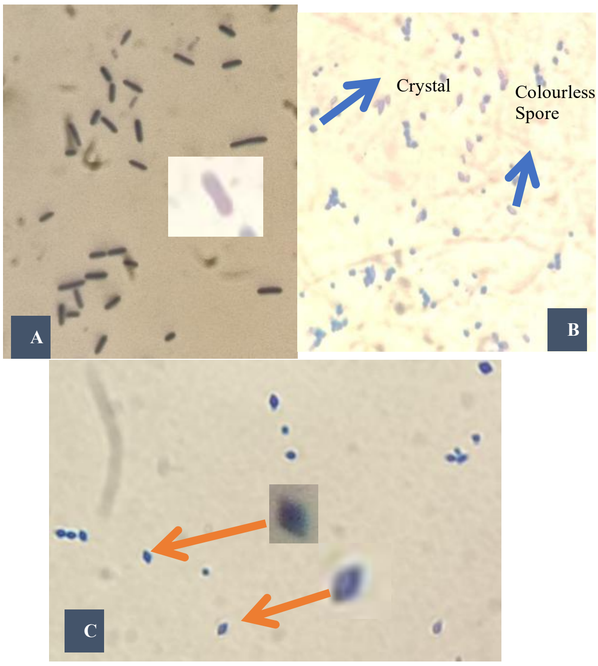

Figure 3: Gram staining and spore staining of *Bacillus thuringiensis* isolates. Rod shaped bacterium (A), Spore protein mixture (B) and Bypiramidal shaped crystal structure of Bt protein (C).

PCR amplification of the vegetative insecticidal protein (VIP) gene

A total of 45 samples, along with two reference strains 4G1 and 4K1, showed the Vip3 gene positive among all 52 (50 isolates and 2 references) samples tested, with the amplicon around 1621 bp (Figure 4). The Vip1 gene was amplified only in two commercial strains of BGSC 4G1 and 4K1 around the product size of 585bp, but none of the Bt strains were found to carry the Vip2 gene. The occurrence of the Vip3 gene has been found in around 90% of total samples.

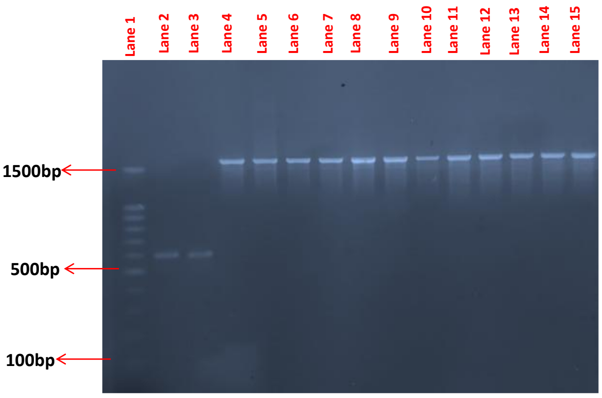

Figure 4: Screening of Vip genes. Lane 1; 100 bp Promega DNA ladder, Lane 2 and 3; 585 bp amplicon of vip1 gene, Lane 4 to lane 15 amplified PCR product of Vip3 gene size around 1621 bp.

Construction of a phylogenetic tree of the Vip 3 protein gene

The Vip3 gene from five Bt strains (Bt Nep LA, Bt Lob 10 LA, Bt A9 b LA, Bt Kasara, Bt Bir) showed a 100% match with Bt strains reported across various regions worldwide, including MZ191101.1 *B. thuringiensis* Vip3 gene India, CP001910.1 *B. thuringiensis* Vip3 gene China, KY780302.1 *B. thuringiensis* Vip3 gene Pakistan, MH318013.2 *B. thuringiensis* Vip3 gene Turkey, DQ426899.1 *B. thuringiensis* Vip3 gene Thailand, as shown in Figure 5.

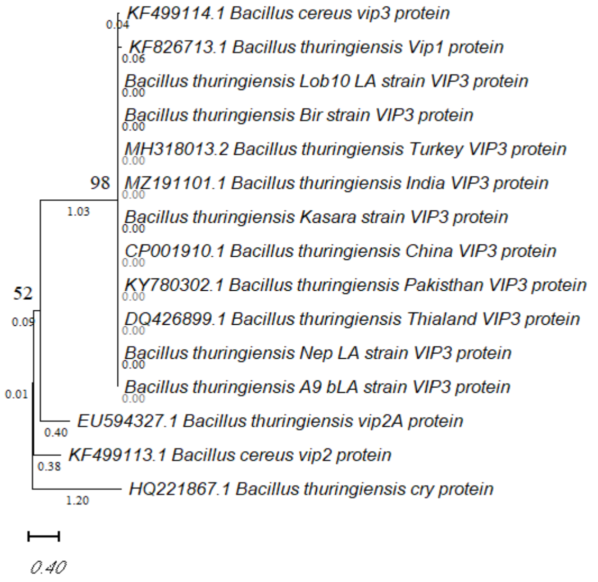

Figure 5: Phylogenetic tree analysis based on Vip3 gene of five Bt strains (Bt Nep LA, Bt Lob 10 LA, Bt A9 b LA, Bt Kasara, Bt Bir) constructed using neighbor joining method of Mega 11 software with 1000 bootstrap values.

Concentration and molecular weight determination of extracted protein

The concentration of different proteins was calculated using a BSA standard curve from the Biuret test. The concentration of six Bt isolates (Bt Nep LA, Bt Bag LA, Bt Lob 10 LA, Bt A9 b LA, Bt Kasara, Bt Bir) along with reference strain 4G1 (Table 1) were calculated using BSA as a standard ($y = 0.0636x + 0.1116$, with the correlation coefficient ($R^2 = 0.9903$). Among the six Bt isolates from six geographical regions, the highest concentration of Vip3 protein was found in the isolate of Bt Kasara, 9.566 mg/ml, with the lowest concentration 2.71 mg/ml from isolates Bt Lob 10 LA (Table 1). Isolate Bt Kasara was selected for the bioassay to compare activity with reference strain 4G1 of 10.242 mg/ml protein concentration (Table 1).

Table 1: Concentration of protein from different isolates and positive control.

Sample	Concentration (mg/ml)	Bacterial type	Geographical region
Bt Nep LA	7.33	Isolate	Nepalgunj
Bt Bag LA	9.25	Isolate	Pokhara, Kaski
Bt Lob 10 LA	2.71	Isolate	Lobuche, Solukhumbhu
Bt A9 b LA	9.17	Isolate	Khopasi, Kavrepanchok
Bt Kasara	9.566	Isolate	Kasara, Chitwan
Bt Bir	3.11	Isolate	Biratnagar
4G1	10.242	Reference strain	
4K1	9.581	Reference strain	

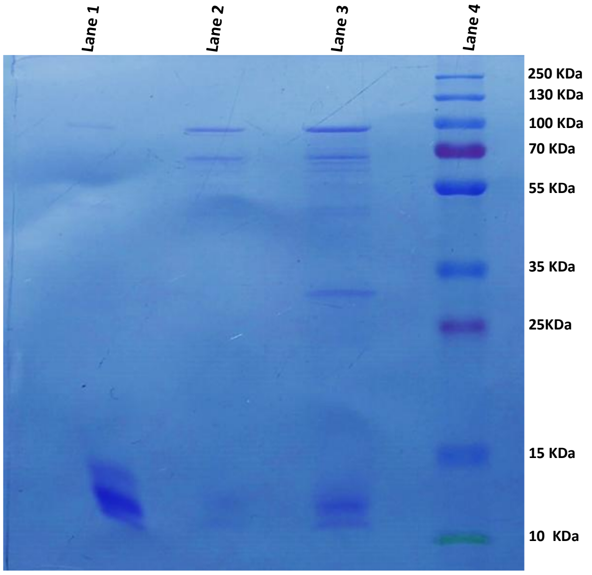

Figure 6: SDS PAGE of BT Vip3 protein. Lane 1 to 3 : Vip 3 protein of BT Bir, BT Kasara, and BT reference strain 4G1 respectiveyly. Lane 4; protein marker (Thermofisher Scientific prestained PAGE Ruler).

In our result, the molecular weight of the Vip3 protein is found around 90 kDa, which is shown in Figure 6. Lanes 1 and 2 are the Vip3 protein band of isolates Bt Bir and Bt Kasara, and lane 3 is the band from the protein extract of the 4G1 reference strain. The size was compared with the protein marker (Thermofisher Scientific prestained PAGE Ruler. This preliminary protein could be useful for further characterization and bioassay of the Vip protein against different lepidopteran pests.

Bioassay of Vip protein

The lethal concentration (LC$_{50}$) is the concentration of a compound that causes the death of *50%* population of test animals. From probit analysis, the Lethal Concentration of Bt Kasara Vip3 protein was determined as 19.1268 µg/ml and 16.2607 µg/ml for BGSC 4G1 at 7 days (Table 2). Bt Kasara, with an average mortality 60% and 64% respectively (data not shown). The detailed study to determine the LC50 value of Vip3 protein from all isolates is underway.

Table 2: Toxicity of Vip3 protein and LC$_{50}$ determination.

S.N.	Bt Strain	LC$_{50}$ (µg/ml)
1.	Bt Kasara	19.1268±8.289
2.	BGSC 4G1	16.2607±5.4239

Discussion

In the present study, the majority of Bt strains were dominated (in all isolates we used in our study) by bipyramidal-shaped crystal protein. But the crystal protein shape may vary between the initially and most studied bipyramidal (Hannay & Fitz-James, 1955) to spherical, cuboidal, and rectangular shapes (Handayani et al., 2025). Among the three primers tested for screening of Vip genes, the presence of the Vip3 gene was found more prevalent than the Vip1 and Vip2 genes. This denotes that the Vip3 gene has a high abundance among other genes. Although we have samples from different geographic regions, there is a huge difference (nil to 90%) in the distribution of Vip1/Vip2, and Vip3 genes. This frequency may vary due to the difference in environmental conditions of collected sources, like altitude elevation, texture and nutrition of the soil, aeration, temperature, etc (X. Yu et al., 2011). Some research already showed that the frequency of the Vip3 gene is much higher, about 49.8% than Vip1 and Vip2 which was 10% (Hernández-Rodríguez et al., 2009; R. Shingote et al., 2013). Also, the newly designed primer helps to provide the robustness of the primers to amplify and differentiate among different subclasses of the Vip family. Based on the sequences of the Vip3 region, we can assume a broad geographic distribution of Bt strains found in Nepal. Variation in Vip gene sequences leads to variation in amino acid sequences, which influences the level of toxicity in the Vip protein (Li et al., 2007).

Although the research on *B. thuriengiensis* has been reported in Nepal (Limbu et al., 2020; Parajuli et al., 2015), the extensive and systematic analysis in distribution and diversity of Vip gene is not highly studied. So it might be the potential work to determine the sequence similarity in Vip genes present in Bt strains of Nepal.

In this study, we use partially purified Vip3 protein for characterization and bioassay. The fully purified protein has a high concentration and higher binding affinity, so even at low concentration, it can be more effective against the target. The variation in protein profiling may depend on the diversity of the soil sample collection. As our results showed that the highest protein concentration was 9.566 mg/ml, which was the Bt strain from an altitude of 400 m asl. A similar range of proteins was extracted from the remaining isolates from 144 m asl to 1414 m asl height (Table 1, Figure 1). Similarly, the lower concentrations ranged from 2.71 mg/ml to 3.11 mg/ml, which were geographic elevations from 70 and 5050 m asl (Table 1, Figure 1).

Protein profiling of various *Bt* isolates helps in studying the diversity of insecticidal proteins and their functionality. The vegetative insecticidal protein of Bt is secreted during the vegetative growth phase (Jiang et al., 2023). The Vip3 gene encodes a protein of around 90 kDa (Chakrabarty et al., 2020) having 787 to 789 amino acids (Syed et al., 2020). SDS-PAGE confirmed the presence of Vip3 protein in the *Bt* isolates, showing visible bands at ~ 90 kDa similar to the reference strain 4G1 Figure 5. The Vip3 proteins can be cleaved into N-terminal and C-terminal fragments of about 20 kDa and 66 kDa, similar to the Vip3Aa type protein responsible for specificity and toxicity. The deletion of amino acids from the N-terminal enhances the toxicity of Vip3 protein, and this can be achieved by the trypsinization of the protein (Jiang et al., 2023). This divergence in protein profile might be because of Bt strains from different geographic soil samples. As many researchers showed that protein profile is also dependent on the Bt strains of diverse geography (Baranek et al., 2023; Maheesha et al., 2022; Nair et al., 2018).

Bioassay results revealed that Vip protein from isolate Bt Kasara showed a lethal concentration (LC_{50}) of 19.1268±8.289 μg/ml at 7 days, which was 17.63% greater concentration needed to affect fall armyworm larvae than that of the control strain 4G1. The lethal concentration of insecticidal protein depends on the percentage of mortality rate, the type of protein and purification methods we use, the stages of larval stage, the group of insect species, and the type of diet provided for the insect (Lone et al., 2016). A previous study demonstrated a large range of (0 to 100%) mortality rate on *Plutella xylostella* larvae at 25 μg/ml using a spore crystal mixture of different Bt isolates (Navya et al., 2021). *B. thuringiensis* Bt294 Vip3A toxin's LC_{50} values of 187.1 ng/cm^2 at 7 days against third instar larvae of *Spodoptera exigua* when they use artificial diet as a supplement (Nutaratat et al., 2023). Similarly, 100% mortality was found using *E. coli* AB88 (pCIB7104) cloned Vip3A protein treated in *Agrotis ipsilon* larvae, and while using neonates of fall armyworm (*S. frugiperda*) as a host, they also showed 100% mortality at 140 ng per cm^2, pCIB 7105 Vip3A clone protein concentration (Estruch et al., 1996). A very low (4 ng/cm^2) Vip3A protein concentration needed for Fall armyworm gut paralysis and above 40 ng/cm^2 for larval death (Yu et al., 1997). These results suggest that if we use purified cloned Vip3 protein, they have high activity against the target at low concentration since it has a strong affinity to bind at specific sites. Although our isolates showed less activity compared to the reference, if we go through further characterization and purification processes, then we can figure out the lowest concentration of Vip3 protein needed for different lepidopteran insects. However, it is necessary for extensive research on biopesticides to protect against environmental detriment and the development of sustainable agricultural practices.

Conclusion

From this study, we concluded that Vip3 gene has abundantly present in the *Bacillus thuriengiensis* strains native to different geographical soil of Nepal. The partially purified Vip 3 protein is effective against one of the insects of lepideptera order. Our preliminary result above supports the development of reproducible and validated Vip protein which can be used as a sustainable biopesticides.

Acknowledgements

Authors thankful to Nepal Academy of Science and Technology, Lalitpur for providing laboratory facilities. The contribution of Ms Prabina Rana in this research is highly acknowledged. We also acknowledge the laboratory of Entomology Division, Nepal Agriculture Research Council, Lalitpur for providing insect larva.

References

Aswathi, N., Balakrishnan, N., Srinivasan, T., Kokiladevi, E., & Raghu, R. (2024). Diversity of Bt toxins and their utility in pest management. *Egyptian Journal of Biological Pest Control*, *34*(1), 40.

Baranek, J., Pluskota, M., Rusin, M., Konecka, E., Kaznowski, A., & Wiland-Szymańska, J. (2023). Insecticidal activity of Bacillus thuringiensis strains isolated from tropical greenhouses towards Cydia pomonella and Spodoptera exigua larvae. *BioControl*, *68*(1), 39–48.

Bechtel, D. B., & Bulla, L. A. (1976). Electron Microscope Study of Sporulation and Parasporal Crystal Formation in *Bacillus thuringiensis*. *Journal of Bacteriology*, *127*(3), 1472–1481.

Bravo, A., Gill, S. S., & Soberón, M. (2007). Mode of action of Bacillus thuringiensis Cry and Cyt toxins and their potential for insect control. *Toxicon*, *49*(4), 423–435.

Chakrabarty, S., Jin, M., Wu, C., Chakraborty, P., & Xiao, Y. (2020). Bacillus thuringiensis vegetative insecticidal protein family Vip3A and mode of action against pest Lepidoptera. *Pest Management Science*, *76*(5), 1612–1617.

El-Gaied, L., Mahmoud, A., Salem, R., Elmenofy, W., Saleh, I., Abulreesh, H. H., Arif, I. A., & Osman, G. (2020). Characterization, cloning, expression and bioassay of vip3 gene isolated from an Egyptian Bacillus thuringiensis against whiteflies. *Saudi Journal of Biological Sciences*, *27*(5), 1363–1367.

Estruch, J. J., Warren, G. W., Mullins, M. A., Nye, G. J., Craig, J. A., & Koziel, M. G. (1996). Vip3A, a novel Bacillus thuringiensis vegetative insecticidal protein with a wide spectrum of activities against lepidopteran insects. *Proceedings of the National Academy of Sciences*, *93*(11), 5389–5394.

Ghimire, K., & GC, A. (2018). Trend of Pesticide Use in Nepal. *Journal of the Plant Protection Society*, *5*, 32–42.

Gupta, M., Kumar, H., & Kaur, S. (2021). Vegetative Insecticidal Protein (Vip): A Potential Contender From Bacillus thuringiensis for Efficient Management of Various Detrimental Agricultural Pests. *Frontiers in Microbiology*, *12*, 659736.

Abdelhai, M. H. (2016). Comparative Study of Rapid DNA Extraction Methods of Pathogenic Bacteria. *American Journal of Bioscience and Bioengineering*, *4*(1), 1

Handayani, K., Indra, R. N., Agustrina, R., & Sumardi. (2025). Study of Morphology and Protein Crystal Character of Bacillus thuringiensis Isolates. In S. Hadi, F. R. Lumbanraja, & E. L. Widiastuti (Eds.), *Proceedings of the 5th International Conference on Applied Sciences, Mathematics, and Informatics (ICASMI 2024)* (Vol. 13, pp. 280–293). Atlantis Press International BV.

Hannay, C. L., & Fitz-James, P. (1955). The Protein Crystals of Bacillus Thuringiensis Berliner. *Canadian Journal of Microbiology*, *1*(8), 694–710.

Hernández-Rodríguez, C. S., Boets, A., Van Rie, J., & Ferré, J. (2009). Screening and identification of *vip* genes in *Bacillus thuringiensis* strains. *Journal of Applied Microbiology*, *107*(1), 219–225.

Ibrahim, M. A., Griko, N., Junker, M., & Bulla, L. A. (2010). Bacillus thuringiensis: A genomics and proteomics perspective. *Bioengineered Bugs*, *1*(1), 31–50.

Jiang, K., Chen, Z., Zang, Y., Shi, Y., Shang, C., Jiao, X., Cai, J., & Gao, X. (2023). Functional characterization of Vip3Aa from Bacillus thuringiensis reveals the contributions of specific domains to its insecticidal activity. *Journal of Biological Chemistry*, *299*(3), 103000.

Jiang, K., Hou, X., Han, L., Tan, T., Cao, Z., & Cai, J. (2018). Fibroblast Growth Factor Receptor, a Novel Receptor for Vegetative Insecticidal Protein Vip3Aa. *Toxins*, *10*(12), 546.

Jiang, K., Zhang, Y., Chen, Z., Wu, D., Cai, J., & Gao, X. (2020). Structural and Functional Insights into the C-terminal Fragment of Insecticidal Vip3A Toxin of Bacillus thuringiensis. *Toxins*, *12*(7), 438.

Karki, R., & Dangol, K. (2023). Pesticide use and health effects among Nepalese farmers: A cross-sectional study in Tokha municipality. *Indian Journal of Occupational and Environmental Medicine*, *27*(3), 255.

Kumar, P. A., Sharma, R. P., & Malik, V. S. (1996). The Insecticidal Proteins of Bacillus thuringiensis. In *Advances in Applied Microbiology* (Vol. 42, pp. 1–43). Elsevier.

Kumar, P., Kamle, M., Borah, R., Mahato, D. K., & Sharma, B. (2021). Bacillus thuringiensis as microbial biopesticide: Uses and application for sustainable agriculture. *Egyptian Journal of Biological Pest Control*, *31*(1), 95.

Labaw W., L. (1964). *The Structure of Bacillus Thuringiensis Berliner Crystals*. *10*, 66–75.

Lee, M. K., Walters, F. S., Hart, H., Palekar, N., & Chen, J.-S. (2003). The Mode of Action of the *Bacillus thuringiensis* Vegetative Insecticidal Protein Vip3A

Differs from That of Cry1Ab δ-Endotoxin. *Applied and Environmental Microbiology*, *69*(8), 4648–4657.

Li, C., Xu, N., Huang, X., Wang, W., Cheng, J., Wu, K., & Shen, Z. (2007). *Bacillus thuringiensis* Vip3 mutant proteins: Insecticidal activity and trypsin sensitivity. *Biocontrol Science and Technology*, *17*(7), 699–708.

Limbu, J., Shrestha, B. K., Shakya, J., Khatri, S. B., & Khanal, H. (2020). Isolation of Bacillus thuringiensis and its Insecticidal Effect against Galleria mellonella. *Himalayan Journal of Science and Technology*, *4*, 96–102.

Lone, S. A., Yadav, R., Malik, A., & Padaria, J. C. (2016). Molecular and insecticidal characterization of Vip3A protein producing *Bacillus thuringiensis* strains toxic against *Helicoverpa armigera* (Lepidoptera: Noctuidae). *Canadian Journal of Microbiology*, *62*(2), 179–190.

Ma, X., Hu, J., Ding, C., Portieles, R., Xu, H., Gao, J., Du, L., Gao, X., Yue, Q., Zhao, L., & Borrás-Hidalgo, O. (2023). New native Bacillus thuringiensis strains induce high insecticidal action against Culex pipiens pallens larvae and adults. *BMC Microbiology*, *23*(1), 100.

Maheesha, M., Balasubramani, V., Murugan, M., Raveendran, M., Rajadurai, G., Tamilnayagan, T., Kokiladevi, E., & Sathiah, N. (2022). Characterisation of native *Bacillus thuringiensis* isolates toxicity to fall armyworm, *Spodoptera frugiperda* (J.E. Smith). *Journal of Biological Control*, 171–180.

Milner, R. J. (1994). History of *Bacillus thuringiensis*. *Agriculture, Ecosystems & Environment*, *49*(1), 9–13.

Nair, K., Al-Thani, R., Al-Thani, D., Al-Yafei, F., Ahmed, T., & Jaoua, S. (2018). Diversity of Bacillus thuringiensis Strains From Qatar as Shown by Crystal Morphology, δ-Endotoxins and Cry Gene Content. *Frontiers in Microbiology*, *9*, 708.

Navya, R. N. S., Balasubramani, V., Raveendran, M., Murugan, M., & Lakshmanan, A. (2021). Diversity of indigenous Bacillus thuringiensis isolates toxic to the diamondback moth, Plutella xylostella (L.) (Plutellidae: Lepidoptera). *Egyptian Journal of Biological Pest Control*, *31*(1), 151.

Nester, E. W., Thomashow, L. S., Metz, M., & Gordon, M. (2002). *100 Years of Bacillis thuringiensis, a Paradigm for Producing Transgenic Organisms: A Critical Scientific Assessment*. American Academy of Microbiology.

Núñez-Ramírez, R., Huesa, J., Bel, Y., Ferré, J., Casino, P., & Arias-Palomo, E. (2020). Molecular architecture and activation of the insecticidal protein Vip3Aa from Bacillus thuringiensis. *Nature Communications*, *11*(1), 3974.

Nutaratat, P., Werapan, B., Phosrithong, N., Trakulnaleamsai, C., Rungrod, A., Utamatho, M., Soonsanga, S., Promdonkoy, B., Malairuang, K., & Prathumpai, W. (2023). Vegetative insecticidal protein (Vip3A) production by Bacillus thuringiensis Bt294 and its efficacy against Lepidopteran pests (Spodoptera exigua). *Biotechnology Reports*, *40*, e00812.

Nyaupane, S. (2022). Use of Insecticides in Nepal, Its Impact and Alternatives of Insecticides for Nepalese Farmers. In R. Eduardo Rebolledo Ranz (Ed.), *Insecticides—Impact and Benefits of Its Use for Humanity*. IntechOpen.

Palma, L., Hernï¿½ndez-Rodrï¿½guez, C. S., Maeztu, M., Hernï¿½ndez-Martï¿½nez, P., Ruiz De Escudero, I., Escriche, B., Muï¿½oz, D., Van Rie, J., Ferrï¿½, J., & Caballero, P. (2012). Vip3C, a Novel Class of Vegetative Insecticidal Proteins from Bacillus thuringiensis. *Applied and Environmental Microbiology*, *78*(19), 7163–7165.

Parajuli, P., Rana, N., Shrestha, S., Maharjan, J., Rana, P., & Sijapati, J. (2015). Isolation and Characterization of Bacillus Thuringiensis Strains Containing Cryi Genes from Soil Samples of Nepal. *Asian Journal of Microbiology, Biotechnology and Environmental Sciences*, *17*(3), 99–104.

Parajuli, S., Mahatara, B., Budhathoki, S., Paudel, M., Parajuli, K., & Adhikari, A. (2021). Scenario of Pesticide Import, Formulation, Consumption and the Residue Status among Agricultural Crops in Nepal. *Big Data In Agriculture*, *3*(2), 94–99.

R. Shingote, P., P. Moharil, M., R. Dhumale, D., V. Jadhav, P., S. Satpute, N., & S. Dudhare, M. (2013). Screening of vip1/vip2 binary toxin gene and its isolation and cloning from local Bacillus thuringiensis isolates. *ScienceAsia*, *39*(6), 620.

Rana, P., Sijapat, J., Rana, N., & Regmi, C. (2002). Distribution of Bacillus thuringiensis in the Soils of Nepal and Their Bioassay. *Nepal Journal of Science and Technology*, *4*(1), 27-31.

Şahin, B., Gomis-Cebolla, J., Güneş, H., & Ferré, J. (2018). Characterization of Bacillus thuringiensis isolates by their insecticidal activity and their production of Cry and Vip3 proteins. *PLOS ONE*, *13*(11), e0206813.

Sanchis, V., & Bourguet, D. (2008). Bacillus thuringiensis: Applications in agriculture and insect resistance management. A review. *Agronomy for Sustainable Development*, *28*(1), 11–20.

Sharma, S., Tiwari, S., Thapa, R. B., Pokhrel, S., & Neupane, S. (2022). Laboratory bioassay of fall armyworm (Spodoptera frugiperda) larva using various insecticides. *Journal of Agriculture and Forestry University*, 133–138.

Syed, T., Askari, M., Meng, Z., Li, Y., Abid, M., Wei, Y., Guo, S., Liang, C., & Zhang, R. (2020). Current Insights on Vegetative Insecticidal Proteins (Vip) as Next Generation Pest Killers. *Toxins*, *12*(8), 522.

Tabashnik, B. E. (1994). Evolution of Resistance to Bacillus Thuringiensis. *Annual Review of Entomology*, *39*(1), 47–79.

Travers, R. S., Martin, P. A. W., & Reichelderfer, C. F. (1987). Selective Process for Efficient Isolation of Soil *Bacillus* spp. *Applied and Environmental Microbiology*, *53*(6), 1263–1266.

Van Rie, J., & Ferré, J. (2000). Insect resistance to Bacillus thuringiensis insecticidal crystal proteins. In J.-F. Charles, A. Delécluse, & C. N.-L. Roux (Eds.), *Entomopathogenic Bacteria: From Laboratory to Field Application* (pp. 219–236). Springer Netherlands.

Wang, J. B., & Cheung, W. W. K. (1994). Histopathological Effects of Bacillus thuringiensis o-endotoxin on the Malpighian Tubules of Pieris canidia Larva. *Zoological Studies*, *33*(3), 192-199.

Washim, M., Manda, P., & Das, R. (2024). Pesticide resistance in insects: Challenges and sustainable solutions for modern agriculture. *International Journal of Agriculture and Food Science*, *6*(2), 121–127.

Watkins, P. R., Huesing, J. E., Margam, V., Murdock, L. L., & Higgins, T. J. V. (2012). Insects, nematodes, and other pests. In *Plant Biotechnology and Agriculture* (pp. 353–370). Elsevier.

Yan, X., Lu, J., Ren, M., He, Y., Wang, Y., Wang, Z., & He, K. (2020). Insecticidal Activity of 11 Bt toxins and 3 Transgenic Maize Events Expressing Vip3Aa19 to Black Cutworm, Agrotis ipsilon (Hufnagel). *Insects*, *11*(4), 208.

Yu, C. G., Mullins, M. A., Warren, G. W., Koziel, M. G., & Estruch, J. J. (1997). The Bacillus thuringiensis vegetative insecticidal protein Vip3A lyses midgut epithelium cells of susceptible insects. *Applied and Environmental Microbiology*, *63*(2), 532–536.

Yu, X., Zheng, A., Zhu, J., Wang, S., Wang, L., Deng, Q., Li, S., Liu, H., & Li, P. (2011). Characterization of Vegetative Insecticidal Protein vip Genes of Bacillus thuringiensis from Sichuan Basin in China. *Current Microbiology*, *62*(3), 752–757.