

Strong mosquitocidal *Bacillus thuringiensis* from Mt. Everest

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Abstract

Bacillus thuringiensis strains were isolated from soil samples collected from Khumbu Base Camp of the Everest region and characterized by standard microbiological techniques viz. colonial and morphological characteristics, and biochemical tests. Insect bioassay of each isolate was performed by standard method using mosquito larva. Among ten randomly selected isolates, one isolate showed the highest insecticidal activity against Dipteran insects.

Keywords: Insect-bioassay, Isolates, Khumbu region, Mosquitocidal, Mosquito larva

Introduction

Microbial insecticides are especially valuable as their toxicity to non-target animals and humans is extremely low compared to other commonly used chemical insecticides. They are safe for both the pesticide user and consumers of pesticide treated crops (Neppel, 2000). The soil bacterium, *Bacillus thuringiensis*, fulfills the requisites of a microbiological control agent against agricultural pest and vectors that cause massive crop destruction (Ben-Dov *et al.*, 1999). The main target pest of *B. thuringiensis* insecticides include various Lepidoptera (butterfly), Diptera (flies and mosquitoes), and individual Coleopteran (Beetle) species and some strains kill off nematodes (Schnepf *et al.*, 1998) where as *B. thuringiensis* var. *kurstaki* HD1 is highly

potent strain due to its wide spread insecticidal properties (Dulmage, 1970).

Methodology

Soil sampling, isolation and biochemical characterization

Soil samples were collected from Sagarmatha National Park (SNP) and Phereche of Khumbu Base Camp of Everest region and were transported to RLABB, where the study was carried out from March 2005 to December 2005 (Shrestha *et al.*, 2006). *B. thuringiensis* were isolated by acetate selection method (Travers *et al.*, 1987). The isolated organisms were identified by standard microbiological techniques including colonial and morphological characteristics, and biochemical tests (Claus and Berkeley, 1986).

Collection of Mosquito larvae

Mosquito (Diptera) larvae were collected from the ditches in local area of Bode, Bhaktapur, Nepal for insect bioassay. The larvae were identified as *Culex* spp. by zoologists at the Central Department of Zoology, Tribhuvan University, Kirtipur and bioassay was performed as described by Pang (1994).

Insect bioassay

Larvae, collected from the ditches in local area of Bode, Bhaktapur, Nepal, were reared in a jar containing 100 ml of sterilized water containing 0.3 ml of 5% Brewer's Yeast and 5 ml of *B. thuringiensis* stationary phase culture and allowed to stand for 3 days. The number of deaths was recorded for one, two and three days. The crystal protein from the stationary culture of the selected individual strain was partially purified by Alkanine method (Dulmage, 1970) followed by Native-PAGE (Blackshear, 1984). The purified crystal protein was bio-assayed (30µg/ml per assay) from each band (Pang, 1994).

Results

Out of 86 δ -endotoxin positive isolates, 10 randomly selected ones were used for insect bioassay. Although all isolates tested were effective against the larvae, isolate S₆ was the most effective of all (Figure 1).

Discussion

Due to high toxicity of chemical pesticide to human beings, animals and beneficial insects, the use of chemical pesticides is being replaced by environment friendly bio-pesticides. The crystal proteins of *B. thuringiensis*, as bio-control agent, have been extensively studied worldwide. Under

the present investigation, most of the bacterial strains were highly mosquitocidal. As the bacterial strains were collected from high altitude (above 4000m), where mosquitoes are not expected, the crystal proteins from the bacteria may be novel ones. In Nepalese context, though isolation and characterization of *B. thuringiensis* from different soil samples and their insect toxicity have been studied (Shrestha *et al.*, 2006) and tested from elsewhere, the characterization of crystal proteins for mosquitocidal properties haven't yet been explored from extreme environment. In an attempt to find novel crystal protein residing *B. thuringiensis* in high altitude, the bacteria were isolated from soil samples collected from Khumbu region of Mt. Everest base camp. The isolates showing potent insecticidal property tested against dipterans need to be studied further in larger trials so that it can have applicability to reduce the mosquitoes and different diseases caused by these vectors (Malaria, Filariasis, Kala-azar etc).

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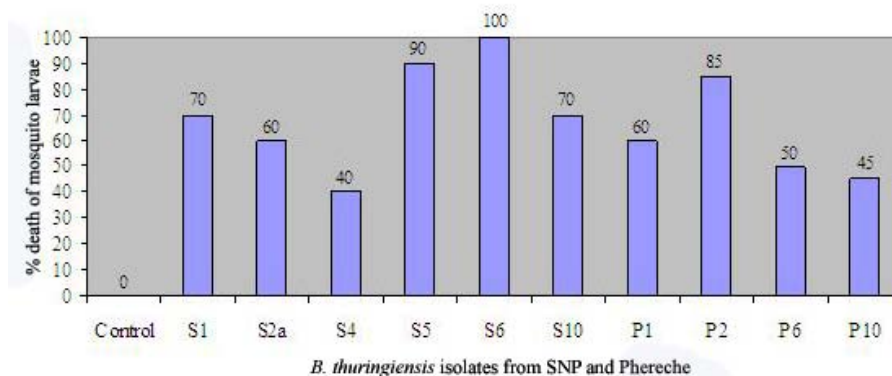


Figure 1. Insect bioassay of SNP and Phereche *B. thuringiensis* isolates