Microbial Pathogenesis 158 (2021) 105093

Contents lists available at ScienceDirect

Microbial Pathogenesis

journal homepage: www.elsevier.com/locate/micpath

Characterization and mosquitocidal potency of a *Bacillus thuringiensis* strain of rice field soil of Burdwan, West Bengal, India

Milita Roy [a,b], Soumendranath Chatterjee [b,*], Tushar Kanti Dangar [c]

[a] Bejoy Narayan Mahavidyalaya, Itachuna, Hooghly, West Bengal, 712147, India
[b] Parasitology and Microbiology Research Laboratory, Department of Zoology, The University of Burdwan, Burdwan, West Bengal, 713104, India
[c] Microbiology Laboratory, Division of Crop Production, National Rice Research Institute, Cuttack, Odisha, 753006, India

ARTICLE INFO

Keywords:
Japanese encephalitis
Culex tritaeniorhynchus
Bacillus thuringiensis
Cry proteins
Bioassay
LC_{50}

ABSTRACT

Bacillus thuringiensis is the most popular mosquitocidal bacteria, strains of which are effective against almost all mosquito larvae. It has host specificity and thus, has no adverse effect on non-target species of the ecosystem. *Culex tritaeniorhynchus*, a vector of Japanese encephalitis (JE), breeds in vast area of rice fields in Burdwan district of West Bengal, India, which has already confronted JE epidemic.

Entomological investigation and ecological studies on this vector mosquito showed that JE epidemic may reoccur anytime in the area. A strain of Bt (BU55) was isolated from rice field soil, efficacy was tested against *Cx. tritaeniorhynchus* and mosquitocidal role was confirmed against *Cx. quinquefascistus* also. The LC_{50} of *Bacillus thuringiensis* BU55 against *Cx. tritaeniorhynchus* and *Cx. quinquefascistus* after 72 h was 8.59 ml (final dose 2.49 $x10^7$ CFU/ml) and 7.52 ml (final dose 2.20 x 10^7 CFU/ml), respectively. Insecticidal crystal protein profile of BU55 produced 136.89, 64.80, 43.45, 33.65 and 26.98 kDa bands. Among them 136.89, 64.29, 26.98 kDa proteins are comparable to actual toxins viz. Cry1Ac (138.3 kDa, Lepidoptera specific), Cry4D (68.0 kDa, Diptera specific) and Cyt (27.4 kDa, Diptera specific). The results clearly showed that the Bt strain is a potent dipteran larvicide and can be used against the JE vectors to control the disease.

1. Introduction

Bacillus thuringiensis (Bt) is the most popular mosquitocidal bacteria, which has high larvicidal activity and is used for decades against mosquito larvae as one of the most powerful biocide [1–6]. Bt is a Gram positive, rod shaped, motile bacterium that occurs commonly in soil, root surface, leaf litter, insect faeces or part of flora of many insect gut etc. [7–14]. Bt is fermentation friendly and therefore, commercially exploitable [15]. Broadly, the Bt strains are species or host specific or have narrow host range [1,2,4,5]. The Cry4A, Cry4B, Cry4D, Cry10A, Cry11A crystal proteins of *B. thuringiensis* have insect spectra limited to mosquitoes, as well as, blackflies [5,16]. Another Cry unrelated dipteran toxin, CytA (27 kDa) binds with the lipids of the membrane and acts synergistically with Cry toxin to enhance the toxicity [5,12,17]. Bt strains and toxins show outstanding diversity which may be due to its high degree of genetic plasticity [13–15,18]. Prolonged use throughout the world is now a days causing emergence of resistance against different Bt strains [19], though in a very slow rate. The resistance drift found in mosquitoes made scientists keen to find new strains of *B. thuringiensis* or other mosquitocidal bacteria. Thus, aim of the proposed study was to search new biocidal strains of Bt for biocontrol of *Cx. tritaeniorhynchus*, the predominant vector of JE in Burdwan, W.B., India.

2. Materials and methods

2.1. Isolation of the bacteria from soil

Nine (9) plots of rice fields were randomly chosen from the University Farm House; Tarabag (23°15′7″ N, 87°50′35″ E) for bacteria isolation. Sub-surface soil (up to 1 cm) were excavated and samples were collected in sterile plastic bags, brought to the laboratory for further processing for isolation of different strains of Bt [20].

Five (5) samples, 10 g each, were collected from each location from 5 spots of each plot. Collected soils were mixed thoroughly, air dried up to 20% moisture level, powdered, sieved (200 mesh), put in the polythene bags and stored in desiccators. Soil sample (1g) was suspended in 100 ml

* Corresponding author. Parasitology and Microbiology Research Laboratory, Department of Zoology, The University of Burdwan, Burdwan, West Bengal, 713104, India.
E-mail address: soumen.microbiology@gmail.com (S. Chatterjee).

https://doi.org/10.1016/j.micpath.2021.105093
Received 10 June 2020; Received in revised form 25 June 2021; Accepted 9 July 2021
Available online 13 July 2021
0882-4010/