PUBLICATIONS
“It doesn’t matter, what you get by the end of the day but what matters is how did you get that.”
Mohan Chandra Joshi

PUBLICATIONS


AN INSECTICIDAL GROEL PROTEIN WITH CHITIN BINDING ACTIVITY FROM *XENORHABDUS NEMATOPHILA*

Mohan Chandra Joshi 1,4, Animesh Sharma 2, Ajanta Birah 3, Gorakh Prasad Gupta 3, Sharik R. Khan 1, Rakesh Bhatnagar 4 and Nirupama Banerjee 1

From 1 International Center for Genetic Engineering and Biotechnology (ICGEB), New Delhi, India 110067. 2 Fuzzylife, Open Source Bioinformatics Education, New Delhi, India. 3 Division of Entomology, IARI, New Delhi, India. 4 School of Biotechnology, Jawaharlal Nehru University, New Delhi, India.

Address correspondence to: Nirupama Banerjee, International Center for Genetic Engineering and Biotechnology (ICGEB), New Delhi, India 110067. Fax: 91-11-26162316; E mail: nirupama@icgeb.res.in

Summary

*Xenorhabdus nematophila* secretes insecticidal proteins to kill its larval prey. We have isolated a ~58 kDa GroEL homolog, secreted in the culture medium through outer membrane vesicles. The protein was orally insecticidal to a major crop pest *Helicoverpa armigera* with LD50 ~3.6 µg. All the three domains of the protein—apical, intermediate and equatorial were necessary for optimum insecticidal activity. The apical domain alone was able to bind to the larval gut membranes and manifest low-level insecticidal activity. At equimolar concentration the apical domain contained ~1/3 and apical-intermediate domain, ~1/2 bioactivity of the full-length protein. Interaction of the protein with the larval gut membrane was specifically inhibited by N-acetyl glucosamine. Based on the 3D structural model, mutational analysis demonstrated that surface exposed residues S347 and S356 in the apical domain were crucial for both, binding to the gut epithelium and insecticidal activity.

A double mutant S347A, S356A was 80% less (P<0.001) toxic than the wild type protein. Treatment of the larval gut membranes with chitinase abolished protein binding. The GroEL homolog showed chitin binding activity with Kd ~0.636 µM and Bmax ~4.683 µmol/gram chitin. The variation in α-chitin binding activity of the mutant proteins was in good agreement with membrane binding characteristics and insecticidal activity. The less toxic double mutant XnGroEL showed ~8 fold increase of Kd in chitin binding assay. Our results demonstrate that *X. nematophila* secretes an insecticidal GroEL protein with chitin binding activity.

Introduction

*Xenorhabdus nematophila*, a gram-negative bacterium resides as symbiont in the gut of a soil nematode of the genus *steinernema* (1-3). The bacteria-nematode association is highly toxic to many insect species, causing rapid larval death. The bacterium has a complex life cycle, encompassing symbiotic and pathogenic stages. The symbiotic phase is spent in the nematode gut while pathogenicity is manifested in the insect larval body. The bacterium is released in the insect hemocoel (2), or gut (4), where it produces a variety of effector molecules including toxic proteins to kill the prey. The larval carcass provides a nutrient rich environment for growth and development of both the nematode and the bacteria. The bacterium alone is also able to kill the insect host when grown axenically in the laboratory medium. Earlier *X. nematophila* was shown to produce outer membrane vesicle (OMV) during growth in the broth culture (5).