Introduction
Pest damage is one of the major yield-destabilizing factor in the agriculture system. Every year farmers suffer huge losses due to crop pests. In spite of the extensive usage of chemical insecticides, the worldwide losses incurred by the pests with respect to edible crops amounts to Rs 98,000 million. However, indiscriminate use of chemical insecticides has brought serious consequences to the ecological balance of the environment and human health. This has fuelled the necessity to explore more ecofriendly, naturally occurring and biodegradable insecticides.

The current strategies for the control of insect pests in agriculture have relied on the development of transgenic crops expressing insecticidal protein toxins. To date, the most successful protein toxin applied to develop transgenic insect resistant crops is derived almost exclusively from the bacterium *Bacillus thuringiensis* (BT) that produces insecticidal crystalline toxins during sporulation. However, the widespread use of BT toxin and the development of transgenic plants expressing these toxin genes have raised concerns over the rapid evolution of resistant insects. Thus, the new recommended strategies are to use the mixture of active ingredients (pyramiding more than one toxin) or alternatively the use of one active ingredient with another or with conventional insecticides. For a sustained and effective pest management continuous use of bioinsecticides is required along with the conventional chemical pesticides, which could be slowly phased out in course of time.

Entomopathogenic nematodes and the insecticidal bacteria residing in them have long been used as biological control agents. *Xenorhabdus nematophila* and *Photorhabdus luminescens* are two related genera of bacteria associated with the soil nematodes. Members of both these genera produce insecticidal toxin proteins that are toxic to a wide variety of lepidopteron insects such as *Spodoptera litura*, *Helicoverpa armigera*, *Menduca sexta* and *Galleria mellonella*. The interest in these bacteria as bio control agents is attributed to their environmental safety and efficacy in the field.

The life cycle of *X. nematophila* is characterized by symbiotic association with a soil nematode and a pathogenic interaction with different insect hosts. *Xenorhabdus* resides as an endosymbiont in the foreguts of infective juvenile (IJ) larvae of a soil nematode *Steinerema carpocapsae*. The IJs enter the insect and transport the bacteria into the gut or hemocoel of the insect larvae. Bacterial multiplication and secretion of toxic proteins are
the primary causes of death of the insect host. The insect carcass provides a rich nutrient source on which both the bacteria and the nematode feeds grow and replicate. A successful nematode-bacterial symbiotic system is key to an effective pest control. However, direct use of *X. nematophila* as a biopesticide is severely limited due to their inability to survive outside their nematode host in the soil or water as a free-living form. Thus, it is important that the insecticidal proteins of the *Xenorhabdus* should be expressed in other bacteria, microorganisms and plants for useful exploitation of their insecticidal potential.

All gram-negative bacteria are known to produce spherical two-layer outer membrane (OM) blebs in culture media. Besides serving as a selective diffusion barrier permitting water-soluble nutrients to diffuse passively into the cell, outer membrane proteins of pathogenic bacteria have been demonstrated to perform several other critical functions under adverse environmental conditions such as recognition and interaction with the target host cells, transport of toxic proteins and secretion of virulence factors and antibacterial proteins.

Adherence to host tissue is a prerequisite for successful colonization of a microbial species to a growth-permissive niche. The pili or fimbriae are hair like, surface organelles, hetero polymeric in nature, with different subunit forming the shaft and tip fibril. The fimbriae mediate recognition and subsequent interaction of several bacterial pathogens with the host cells and are considered as important virulence factors.

In this background of insecticidal potential of *Xenorhabdus*, the present study was initiated to find insecticidal protein from *X. nematophila*, and investigate their role in the pathogenicity of the bacteria.

The following objectives were undertaken for this study:

1. **Purification and characterization of Outer Membrane Vesicles from *X. nematophila***.
2. **Isolation and characterization of native 17 kDa pilin protein from *X. nematophila***.
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- Cloning and expression of the gene encoding 17 kDa protein.
- Purification and characterization of recombinant 17 kDa pilin protein.
- Site directed mutagenesis of 17 kDa pilin gene in the N-terminal residues involved in head to tail interactions for oligomerization.
- Construction of a mutant strain lacking the fimbrial shaft protein by allelic exchange.
- Characterization of the mutant strain and evaluation of the role of fimbriae in pathogenesis and symbiosis.