Benevolent Microbial Warriors: Exploring the Role of Entomopathogenic Bacteria in Pest Control

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Various biological control methods, including specific insecticides, have been explored, with a focus on entomopathogenic microbes like bacteria, viruses, protozoa, fungi, microsporidia, and nematodes (Vega and Kaya, 2012). Notably, entomopathogenic bacteria, both spore-forming (e.g., *Bacillus*, *Paenibacillus*, *Clostridium*) and non-spore-forming (e.g., *Pseudomonas*, *Serratia*, *Yersinia*, *Photorhabdus*, *Xenorhabdus*), are successful and commercially valuable, initiating infection upon ingestion by susceptible insects. Integrated pest management (IPM) utilizes inundative methods to release these bacteria and fungi effectively. Bacillus thuringiensis, crucial for lepidopteran control, faces limitations due to UV light inactivation, necessitating repetitive releases and increased costs. Meanwhile, bacteria like *Pseudomonas* spp. exhibit favourable properties for mass production and environmental stability.

Type of Entomopathogenic Bacteria

Bacterial pathogens used for insect control are spore forming, rod-shaped bacteria in the genus Bacillus. Pathogenic bacteria of insects which have potential for use in biological pesticides are limited to three species of spore forms, i.e., *Bacillus thuringiensis*, *B. sphaericus* and *B. papillae.*

Spore forming (Obligate)

Bacillus popillae attack only beetles of family Scarabaediae. It causes milky disease in Japanese beetle, *Popillia japonica*. It produces endospores which upon ingestion by the susceptible host, germinate in the gut and the vegetative cells invade into the haemocoel where they multiply and sporulate. At this point the blood becomes milky white, hence the term “milky disease”. After the death, host disintegrates and spores are released into the soil.

Spore forming (Facultative)

In addition to endospores, produces a proteinaceous parasporal Crystal in the Sporangium at the time of sporulation. The crystals contain an endotoxin capable of paralyzing the gut of most lepidopteran larvae. The toxin is known as delta-endotoxin. It is the most widely exploited microbial control agent. Lepidoteran larvae with a gut pH ranging from 9.0 to 10.5 are most susceptible.

Spore forming (Facultative)- Non-crystalliferous

Bacillus cereus a common spore former and soil inhabitant, effective against Coleoptera, Hymenoptera and Lepidoptera.

Mode of Action I

Bacterial life cycle involves vegetative cell division under optimal growth conditions and sporulation in response to environmental changes. Sporulation produces endotoxin, and when conditions improve, spores germinate, initiating another round of vegetative cell division. For bacterial insecticides to be effective against target insects, ingestion is crucial as they don't act as contact poisons.

The crystal contains a protoxin protein (130 kDa), which, when solubilized in the larval midgut's alkaline pH, is enzymatically cleaved to form an active toxin (60-70 kDa). This toxin diffuses through the peritrophic membrane, binds to midgut receptors, paralyzing the gut and halting feeding. The toxin disrupts the membrane, creating pores that lead to potassium imbalance, microvilli swelling, cell lysis, and potential septicemia, allowing bacterial invasion into the haemolymph.

Mode of Action II

Upon ingestion, Bt toxins are activated in alkaline insect gut conditions, forming pores in midgut cells. This disrupts osmoregulation, causing midgut paralysis and cell lysis. The leakage of gut contents into the hemocoel and hemolymph leads to pH imbalance, septicemia, and eventual insect death. Responses vary based on crystal proteins, receptor sites, exotoxin production, and spore requirements, influencing caterpillar susceptibility to Bt toxins.

Mode of action of Bt, an Entomopathogenic bacteria, adopted from Glare et al., 2017 Basic and applied research: entomopathogenic bacteria. In Microbial control of insect and mite pests.

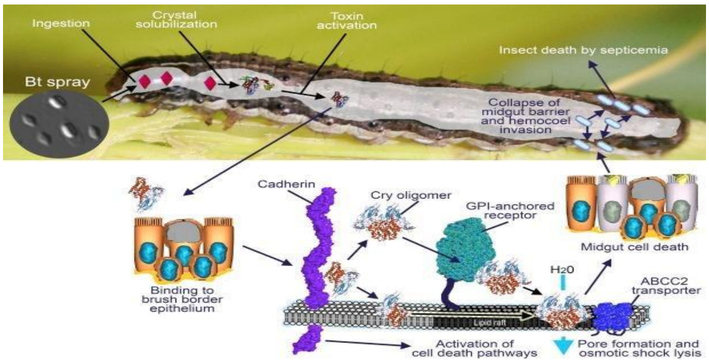

Fig. 1: Mode of action of Bt, an Entomopathogenic bacteria, (Glare *et al*., 2017)

Table 1. Entomopathogenic bacteria and target insects

Entomopathogenic bacteria	Target Insect(s)
Bacillus thuringiensis subspecies *kurstaki*	Caterpillars (larvae of moths and butterflies)
Bacillus thuringiensis subspecies israelensis	Larvae of *Aedes* and *Psorophora* mosquitoes, black flies, and fungus gnats
Bacillus thuringiensis subspecies *tenebrinos*	larvae of Colorado potato beetle, elm leaf beetle adults
Bacillus thuringiensis subspecies *aizawai*	wax moth caterpillars
Bacillus popilliae and *Bacillus lentimorbus*	larvae (grubs) of Japanese beetle
Bacillus sphaericus	larvae of *Culex*, *Psorophora*, and *Culiseta* mosquitos, larvae of some *Aedes* spp
Bacillus thuringiensis subspecies japonensis	Coleoptera: Scarabaeidae
Paenibacillus popilliae	Coleoptera: Scarabaeidae, *Popillia japonica*

(Source: Usta, 2013)

Bacillaceae

Bacillus thuringiensis

Bacillus thuringiensis (Bt), discovered in 1901, is a well-studied entomopathogenic bacterium known for crystal-producing strains crucial in microbial pest control. Its parasporal bodies contain Cry and Cyt toxins, with Cry proteins causing insect midgut disruption and death. Integration of cry genes into genetically modified plants enhances crop resistance to specific pests (Jurat-Fuentes and Jackson, 2012).

Lysinibacillus sphaericus

Lysinibacillus sphaericus, exhibits a distinctive phenotype characterized by the production of spherical spores within swollen sporangia. Entomopathogenic strains within this species group produce equimolar ratio binary protein toxins (BinA and BinB) in parasporal crystals, targeting midgut cells in host larvae. Mosquitocidal activity depends on Bin and/or Mtx toxins, akin to *B. thuringiensis*, causing damage to midgut microvillar epithelial cells. While non-mosquitocidal insecticidal toxins exist, *L. sphaericus* is mainly employed in commercial pesticides targeting mosquitoes, blackflies, and non-biting midges. Activity varies based on toxin specificity and mosquito species sensitivity, with Culex mosquitoes being the most susceptible, followed by Anopheles, Mansonia, and some Aedes species (Nishiwaki *et al*., 2007).

Paenibacillus spp.

The genus *Paenibacillus* comprises various species, including the pathogenic *Paenibacillus* larvae subsp. larvae responsible for American Foulbrood (AFB) in honeybees. However, due to their limited potential as microbial pesticides, the discussion excludes these causative agents of bee diseases. Instead, focus is on spore-forming species like *P. popilliae* and *P. lentimorbus*, which cause milky disease in coleopteran larvae. *P. popilliae,* used successfully in the first documented bacterial entomopathogen control program, produces parasporal inclusions, showing homology with Bt Cry toxins. Upon ingestion, spores germinate in the host's midgut, leading to insect control (Genersch, 2010).

Brevibacillus laterosporus

Brevibacillus laterosporus is a versatile pathogen with broad-spectrum antimicrobial properties, targeting various invertebrates including insects (Coleoptera, Lepidoptera, Diptera), mollusks, nematodes, and phytopathogenic bacteria and fungi. Its genome, recently published, indicates the potential to produce diverse toxins, with certain strains exhibiting insecticidal secreted proteins (ISPs) like Bt's Vip proteins, effective against corn rootworms and other coleopteran larvae. Additionally, strains toxic to mosquitoes produces parasporal inclusion bodies akin to *B. thuringiensis*, with reported mosquitocidal action.

Notably, a crystal-lacking strain demonstrates high toxicity to house flies, causing histopathological changes in the midgut. (Ruju *et al.*, 2012).

Photorhabdus spp.* and *Xenorhabdus spp.

Photorhabdus and *Xenorhabdus*, endosymbionts of entomopathogenic nematodes, primarily associated with *Heterorhabditis* and *Steinernema* species, respectively, employ a pathogenic strategy upon entering insect hosts. After nematode entry, symbiotic bacteria are released into the insect hemocoel, where they proliferate and produce antimicrobial compounds, creating a favourable environment for nematode development. Bacterial virulence factors, including insecticidal toxins complex (Tc) and *Photorhabdus* insect related (Pir) proteins, mimic B. thuringiensis delta-endotoxins, affecting insect development regulation. Additionally, these endosymbionts employ diverse mechanisms, such as lipopolysaccharide modifications and interference with host antimicrobial peptide expression, to counteract the insect immune response (Waterfield *et al*., 2005; Ji *et al*., 2004)

Enterobacteriaceae

***Serratia* spp**

Serratia spp. exhibit associations with insects and entomopathogenic nematodes, with *S. entomophila* identified during population collapses in field larvae. Various species in this genus produce toxin complexes resembling those of *Xenorhabdus* spp. and *Photorhabdus* spp. *S. entomophila*, targeting the grass grub *Costelytra zealandica*, produces Sep toxins (SepA, SepB, SepC) akin to *P. luminescens*. The sep gene cluster, found in *S. proteamaculans*, *S. liquefaciens,* and *Yersinia frederiksenii* plasmids, suggests horizontal mobility (Hurst *et al.,* 2011). While *S. marcescens* has limited research in human infections, a new strain demonstrates activity against the diamondback moth, and *S. nematodiphila*, associated with *Heterorhabditinoides* sp., hints at further microbial control agents. S. marcescens Bizio's pathogenicity is enhanced by a secreted serralysin metalloprotease, suppressing insect host cellular immunity through decreased adhesive properties of immunosurveillance cells (Castagnola *et al*., 2014).

Yersinia entomophaga

Yersinia entomophaga, a non-spore-forming bacterium within the Enterobacteriaceae family, exhibits substantial diversification in its evolutionary history. Derived from the New Zealand grass grub, *C. zealandica*, this entomopathogenic bacterium produces an insecticidal toxin complex (Yen-Tc), akin to those found in *Photorabdus* spp. (Hurst *et al.,* 2011). With potential as a microbial control agent, *Y. entomophaga* demonstrates activity against various pests including *C. zealandica*, diamondback moth (*Plutella xylostella*), small white butterfly (*Pieris rapae*), locust (*Locusta migratoria*), and cotton bollworm (*Helicoverpa armigera*). The toxin complex comprises three Yen protein families (A, B, and C) and two chitinases (Chi1 and Chi2). Upon ingestion by *C. zealandica* larvae, *Y. entomophaga* prompts a swift halt in feeding, subsequent regurgitation of gut contents, and degradation of gut epithelial membranes, leading to hemocoel invasion, septicemia, and death within 2–5 days (Marshall *et al.,* 2012).

Pseudomonadaceae

Pseudomonas entomophila

Pseudomonas entomophila, a widespread entomopathogenic bacterium, induces extensive gut cell damage in insect larvae across multiple orders. Host-pathogen interactions were elucidated through experiments with *Drosophila melanogaster*, revealing a distinctive post-ingestion immune response (Vodovar *et al*., 2006). Genome sequencing of *P. entomophila* uncovered a unique secretion system and toxins, likely underpinning its entomopathogenic properties (Dieppois *et al*., 2015).

Clostridiaceae

Clostridium bifermentans

Clostridium bifermentans, a gram-positive, anaerobic, spore-forming bacillus, isolated in Malaysia exhibits high toxicity against mosquitoes and black flies, particularly through the production of three major proteins during sporulation. Notably, the strain, identified as *C. bifermentans* subsp. malaysia, demonstrates insecticidal activity with the mosquitocidal protein Cbm71, akin to B. thuringiensis delta endotoxins, and utilizes a four-toxin operon (Cry16A, Cry17A, Cbm17.1, and Cbm17.2) for specific toxicity against Aedes spp. and heightened susceptibility in Anopheles mosquitoes (Edagiz *et al.*, 2015; Qureshi *et al.,* 2014).

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