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Entomopathogenic Bacteria: A Potential Biological Weapon against Insect-Pests Management

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Abstract

Food security and safety are major concerns in the world's ever-growing human population. Insect-pests have a huge impact on agricultural output every year. The majority of farmers utilised chemical pesticides to control them, which are harmful to the environment and human health. The loss in term of productivity and high cost of chemical pesticides enhance the production cost also. Biological management of agricultural pests and diseases has been discovered to have an important role in reducing dependency on chemical pesticides. The control of pests by entomopathogenic and plant associated bacteria is an alternative that may contribute to reduce or eliminate the pesticide use. Entomopathogenic bacteria, which cause illnesses in insects, kill the host through septicaemia and toxin production. Plant-associated bacteria, such as rhizospheric, endophytic, and phylloplane bacteria, play a role in bio control by producing defence compounds that induce systemic resistance. Among the entomopathogenic groups of bacteria *Bacillus thuringiensis*, *B. popilliae*,*B. Sphaericus* and *B. cereus* are used as biocontrol agents nowadays.Families of bacteria having the properties of pathogenesis comprise Bacillaceae, Enterobacteriaceae, Micrococcaceae, Pseudomonadaceae and Streptococcaceae. Some of these bacterial families are highly lethal to the various insect pests. Among these virulent families, Bacillaceae contain the genus *Bacillus* having *B. popilliae* species, which is responsible for causing the milky spore disease in the scarab beetles. Even one more species *B. sphaericus* is highly infectious to mosquitoes. Pathogen city of *B. thuringiensis* is also well known. They are virulent against a wide range of insect pests mainly lepidopterans and are world widely distributed as bio control agents. This article describes different types of bio-insecticides and the potential use for the control of insect-pests at global level.

Keywords: Entomopathogenic bacteria, mode of action, cry toxin, target pest management

Introduction

Indian agriculture sector accounts for 18% of India's gross domestic product (GDP), and about 58% population of India is dependent upon agriculture for its livelihood. The Indian population is expected to exceed 1.5 billion by 2050. There will be a huge pressure on the agricultural sector to feed such a gigantic population by increasing food production in an environmentally sustainable manner. Nowadays, agriculture supports two-thirds of the world's population, yet crop production is becoming increasingly susceptible owing to insect assault. Chemical pesticides are mostly used in agriculture to control insect pests. Farmers frequently use enormous volumes of chemical insecticides to reduce crop loss caused by pests. In spite of heavy insecticide application, crop loss rises due to a variety of factors such as resistance development, pest resurgence, and pest replacement, as well as negatively impacting the environment and human health by leaving harmful residues. Hence there is a need to evolve eco-friendly management strategies. Biological pest and disease control of cultivated plants has received a lot of attention during the last two decades as a strategy to reduce the use of conventional pesticides in agriculture. Natural enemies like as predators and parasitoids are used to manage pests, whereas helpful microorganisms such as insect pathogenic bacteria, fungi, viruses, protozoa, and nematodes are used to control pests.

Bacterial populations pathogenic to insect pests can cause major damage to the target insect population and are known as entomopathogenic bacteria. As the potential of entomopathogenic bacteria, particularly *Bacillus* species, was revealed, the biological control paradigm shifted. Initially, the species *Bacillus popilllae*Dutky was introduced for the management of the Japanese beetle *Popillia japonica* Newman, but more concrete results were achieved with the discovery of new *Bacillus thuringiensis* (*Bt*) strains showing high toxicity against specific insects at a competitive level in terms of efficacy and production costs. The bacteria *Bacillus thuringiensis* (*Bt*) was the first biological control agent developed for control of insects (Burges, 1982).Bt is a spore forming, entomocidal, gram-positive soil bacterium. The protein, delta toxin, formed during sporulation of the bacterium causes the insecticidal activity. These protein crystals dissolve in alkaline conditions of the insect midgut (Gill, 1992). Till now several bacterial insect pathogens and their insect hosts have been identified as bio-control agents and are highly pathogenic to arthropods (Table 1). The different strains of commercial formulations of bacterium are active against insect management (Table 2).Bacterial insecticides are the most common type of microbial pesticide. Bacterial-based bio-insecticides have been utilised to manage a variety of insect-pests. Both spore-forming and non-spore-forming bacteria are employed to manage insects.Bacteria disrupt the digestive system of insects by creating endotoxins (insecticidal proteins) that are particular to the insect. *Bacillus thurigiensis*, sometimes known as *Bt*, is the most widely utilised bacterium. *B. thuringiensis* has been shown to be effective against several insect pests in forestry, agriculture and other areas. Besides the entomopathogenic bacteria, there exist certain bacteria that can effectively colonise plants by occupying different sites such as roots, leaves or as inter or intracellular colonisers. Pest management with entomopathogenic and plant-associated bacteria is an option that may help to decrease or eliminate the usage of pesticides.

Major groups of entomopathogenic bacteria

Entomopathogenic bacteria are unicellular prokaryotic organisms (without nucleus) having size ranging from less than 1 μm to several μm in length. Bacteria with rigid cell walls are cocci, rod-shaped and spiral while bacteria without cell walls are pleomorphic.There are more than 100 species of bacteria, found to be pathogenic to insect pests. Among the most entomopathogenic groups of bacteriaare both gram-negative as well as gram-positive and are soil-borne. Some important gram-negative entomopathogenic bacteria include *Photorhabdus* spp., *Xenorhabdus* spp., *Serratia* spp., *Yersinia entomophaga*, *Pseudomonas entomophila, Chromobacterium* spp., and *Burkholderia* spp. Gram-positive bacteria include *Bacillus thuringiensis*, *Lysinibacillussphaericus*, *Paenibacillus* spp., *Brevibacilluslaterosporus*, *Clostridium bifermentans*, *Saccharopolysporaspinosa*, and *Streptomyces* spp.The bacterial familiesBacillaceae, Pseudomonadaceae, Enterobacteriaceae, Streptococcaceae, and Micrococcaceaehave so far found vast majority of arthropod infections. The majority of these bacteria are mild pathogens that infect insects under stress, but a small number are very virulent. These families often contain epiphytes, however certain infections have been demonstrated to be extremely harmful to their hosts.A small number of entomopathogenic bacteria have been commercially developed for control of insect pests. These include several

Bacillus thuringiensis sub-species, *Lysinibacillus* (*Bacillus*) *sphaericus*, *Paenibacillus* spp. and *Serratiaentomophila*. *B. thuringiensis* sub-species *kurstaki* is the most widely used for control of insect-pestsof crops and forests, and *B. thuringiensis* sub-species *israelensis*and *L. sphaericus* are the primary pathogens used for control of medically important pests (Sand flies, black flies, stable fly, Red imported fire ants, southern fire ants, Aedes, Anopheles, Culex, Honey bees, bumble bees, cicada killer wasps)including dipteran vectors. There is dozen recognize subspecies of *Bacillus thuringiensis*but most common species and their target pests are *Bacillus thuringiensis* sub-species*kurstaki* (moths and butterflies), *Bacillus thuringiensis* sub-species*israelensis*(flies and mosquitoes), *Bacillus thuringiensis* sub-species*aizawai*(wax moth larvae), *Bacillus thuringiensis* sub-species*tenebrionis* (potato beetle and cotton ball weevil) and *Paenibacilluspopilliae*(Japanese beetle *Popillia japonica*).

Family Bacillaceae

Bacillaceaefamily bacteria are Gram-positive, heterotrophic, rod-shaped bacteria with the ability to produce endospores. Members of this family include *Bacillus thuringiensis, B. sphaericus, B. popillae, B. pumilus, Brevibacilluslaterosporus* etc.

1. *Bacillus thuringiensis*

Bacillus thuringiensis (Bt) is the most effective insect pathogen used for pest control, accounting for 2% of the overall insecticidal market. Bt is an aerobic spore forming, gram-positive, soil-dwelling bacteria known for its ability to produce crystalline inclusions (Cry poisons) containing insecticidal proteins known as δ-endotoxin (delta-endotoxin) during sporulation. These toxins damage the midgut of the pest causing septicaemia and death within 2-3 days (Ruiu, L., 2015, Mampallil*et al.,* 2017).*B. thuringiensis* subsp. *kurstaki* (Btk) is generally used against young Lepidopteran larvae and includes different strains with significant commercial interest like HD-1, SA-11, SA-12, PB 54, ABTS-351 and EG2348. Strains of *B. thuringiensis* subsp. *aizawai*(Bta) (i.e., ABTS-1857) are also used against armyworms and diamondback moth larvae. Besides, strains belonging to the*Bacillus thuringiensis* subsp. *israelensis* (Bti) andBacillus*thuringiensis*subsp. *tenebrionis* (Btt) have been employed for the management of mosquitoes and simulids, and against coleoptera, respectively Glare and O'Callaghan, 2000).

2. *Bacillus popilllae*

The spore-former *B. popillae* (Dutky) is the causal agent of milky disease in phytophagous coleopteran larvae. The production of parasporal inclusions within the sporangial cells has been observed in *B. popillae*, even if they are not directly responsible for the insecticidal action. After the spores are ingested by the host, they germinate in the midgut (Mampallil*et al.*, 2017).

3. *Lysinibacillussphaericus (Bacillus sphaericus)*

Entomopathogenic strains belonging to the *L. sphaericus* (formerly *Bacillus sphaericus*) species group are featured by the production of spherical endospores closely associated with parasporal crystals containing an equimolar ratio of binary protein toxins (BinA and BinB). The insecticidal mechanism of action involves midgutmicrovillar epithelial cell destruction similar to that seen with B. thuringiensis. Also, certain strains' vegetative cells release mosquitocidal poisons (Mtx proteins).The main targets of commercial formulations based on *L. sphaericus* strains are mosquitoes, blackflies and non-biting midges(Ruiu, L., 2015, Mampallil*et al.*, 2017).

4. *Bacillus pumilus*

Bacillus pumilus strain 15.1 has recently been shown to be poisonous to larvae of the Mediterranean fruit fly, *Ceratitiscapitata*, one of the most devastating pests to fruits and vegetables globally. During sporulation, this strain produces parasporal crystals that resemble those formed by *Bacillus thuringiensis* cry proteins. Several genes in the *B. pumilus*15.1 genome encode well-known entomopathogenic factors such chitinases, metalloproteases, and cytolysins (Mampallil*et al.*, 2017).

5. *Brevibacilluslaterosporus*

So many *B. laterosporus* strains have been shown to be insecticidal against insects of many orders, including coleoptera, lepidoptera, and diptera. It has the ability to create a variety of poisons. Some strains that generate insecticidal secreted proteins (ISPs) that function as binary toxins in the insect midgut and have high similarity with *B. thuringiensis* vegetative insecticidal proteins cause toxicity against maize rootworms (*Diabrotrica* spp.) and other coleopteran larvae(Mampallil*et al.*, 2017).

Family Pseudomonadaceae

Pseudomonadaceaefamily members are strictly aerobic, gram-negative, straight or curved rods with polar flagella. Many species are pathogens, whereas others are frequent commensals in insect digestive systems. A variety of *Pseudomonas* species are present in the digestive tracts of insects, either as pathogens or as commensals. Despite the fact that *Pseudomonas aeruginosa* is one of the most regularly isolated bacteria from insects, it seldom causes epizootics in field populations (Mampallil*et al.*, 2017).

Family Enterobacteriaceae

Several of the more recognisable diseases, such as *Salmonella, Escherichia coli, Yersinia pestis, Klebsiella*, and *Shigella*, are members of the Enterobacteriaceae family of gram-negative bacteria.*Serratia* and *Enterobacter* are the most commonly reported entomopathogenic bacteria in the Enterobacteriaceae.*Serratiamarcescens* is a facultative anaerobe that grows rapidly in the guts of many insect species, producing septicaemia and death. It is frequently isolated from dead and sick insects.Some species, such as *S. entomophila*, cause illnesses in pest insects when the bacterium is consumed.*Serratiaentomophila* and *Serratiaproteamacula*are used as effective biological pesticides against Newzeland grass grubs, *Costelytrazealandica*(Hurst, M.R.H. 2000, Mampallil*et al.*, 2017).

Mode/mechanism of action of Entomopathogenic bacteria

Bacteria cause illnesses in insects when they are eaten. They have developed a variety of techniques to penetrate the host, defeat its immune defences, infect, and kill it. They infect the host through the midgut epithelial cells, causing septicaemia and the generation of toxins. Entomopathogenic bacteria comprise both spore-forming and non-spore-forming bacteria. Endospores are produced by all spore-forming bacteria, allowing them to survive in a latent state outside of the host. Spores germinate in the intestines after consumption. Crystalliferous spore formers create parasporal crystals in addition to endospores in the sporangium. Insecticidal action is exhibited by non-spore formers by the synthesis of insecticidal poisons (Mampallil*et al.*, 2017).

When larvae consumesfoliage treated with Bt (spores and crystalline toxin) and it enters the gut, alkaline conditions (pH >8) activate the toxin protein (delta endotoxin), within a minutes, the toxin binds to receptor sites in the midgut wall and causes abrasions in midgut cells and the larvae/caterpillar stop feeding. Within hours, the gut wall breaks down. This results in ion loss due to leaching, midgut paralysis, and cell lysis. Midgut contents flow into insect blood (hemolymph) and body cavity (hemocoel), disturbing the pH equilibrium.

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Bacteria enter into the body cavity cause septicaemia and eventually death of the host insect (Yadav*et al.*, 2021). *Lysinibacillussphaericus* cause injury to the epithelial microvilli in the midgut of the insect. *Clostridium bifermentans* is highly pathogenic against black flies and mosquitoes as it has Cbm71 protein showing a similar effect like delta endotoxins of *B. thuringiensis*.

Table 1: Bacterial insect pathogens and their insect hosts(Vallet-Gely*et al.*, 2008)

EntomopathogenicBacteria	Type of interaction	Mode of interaction	Insect hosts
Erwiniaaphidicola	Pathogen	Ingestion	Pea aphid
Dickeyadadantii	Pathogen	Ingestion	Pea aphid
Pseudomonas entomophila	Pathogen	Ingestion	Drosophila, Bombyx, galleria
Yersinia pestis	Pathogen	Ingestion	Rat flea
Serratiaentomophila	Pathogen	Ingestion	Grass grub
Serratiamarcescens	Pathogen	Ingestion	Drosophila
Photorhabdus sp.	Pathogen	Assisted entry	Lepidopteran
Xenorhabdus sp.	Pathogen	Assisted entry	Lepidopteran
Vibrio cholera	Pathogen	Ingestion	Drosophila
Melissococcus pluton	Pathogen	Ingestion	Honey bee
Bacillus thuringiensis	Pathogen	Ingestion	Different orders
Bacillus papillae	Pathogen	Ingestion	Scarab larvae
Paenibacilluslentimorbus	Pathogen	Ingestion	Scarab larvae
Paenibacillus larvae	Pathogen	Ingestion	Honey bee larvae
Bacillus sphaericus	Pathogen	Ingestion	Mosquito
Bacillus laterosporus	Pathogen	Ingestion	Bee larvae, dipteran
Pseudomonas aeruginosa	Opportunistic	Ingestion	Caterpillar
Pseudomonas aeruginosa	Opportunistic	Direct injection	Drosophila
Bacillus cereus	Opportunistic	Ingestion	*Galleria mellonella*
Erwiniacarotovora	Infectious	Ingestion	Drosophila larvae

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Shigella spp.	Passive	Ingestion	Vector house fly
Rickettsia spp.	Vector	Ingestion	Cat flea
Bartonella spp.	Vector	Ingestion	Cat flea

Table 2: Commercial Entomopathogenic bacterial formulations and target pest management

Entomopathogenic bacterial formulation	Products name	Target pests
*Bacillus thuringiensis*subsp.*israelensis*5% WP, 5% AS	Tacibio/Vectobac®, Teknar®, Bactimos®, Skeetal®, and Mosquito attack®,VectoBac, Vectoba	Lepidopterous pests/Diptera pests/ Mosquito and black flies, house fly, stable fly or blow fly
*B.thuringiensis*subsp.*kurstaki*5% WP, 7.5% WP	Bio-Dart/ Biolep/Halt/Taciobio-Btk, Dipel®, Javelin®, Thuricide®, Worm Attack®, Caterpillar Killer®, Bactospeine® and SOK-Bt® Biobit, Cordalene, Costar-WG, Crymax-WDG, Deliver, Foray, Javelin-WG, Lepinox Plus, Lipel, Rapax	Lepidopteran pests/American ball worm in cotton, caster semilooper, diamond back moth on cruciferous vegetables, rice stem borer, *Heliothisarmigera* on various crops
*B.thuringiensis*subsp.*Kurstaki*plus beta-exotoxin	Javelin	Armyworm and other moths
*Bacillusthuringiensis*subsp.*sphaericus*1.3% FC	VectoLex, VectoMax	Diptera/Mosquito larvae
*Bacillusthuringiensis*subsp.*galleriae*	VectoLex, Spicturin	Lepidopteran larvae /Mosquito larvae, bollworms, Diamondback moth

Bacillus thuringiensis sub-species*aizawai*	Able-WG, Agree-WP, Florbac, XenTari, Certan, Agree	Lepidoptera/Wax moth larvae, Armyworms, diamondback moth
Bacillus thuringiensis sub-species*tenebrionsis*	M-One®, Novodor, Trident	Coleoptera/Potato beetle and cotton ball weevil
*Paenibacilluspopilliae*and *B. lentimorbus*	Doom®, Japidemic®, Grub attack®	Japanese beetle *Popillia japonica*
Bacillus moritai	-	Diptera
Bacillus popilliae	-	Coleoptera
*Pseudomonas fumosoroseus*0.5%, 1% WP	Nemato-Guard, Bio-Dart	Whitefly
Pseudomonas lilacinus	Yorker/ABTEC/Paceilomyces/Paecil/Pacihit/R OM biomite/Bio-Nematon	Whitefly
Saccharopolysporaspinosa	Tracer™ 120, Conserve	Insects
Chromobacteriumsubtsugae	Grandevo	Chewing and sucking insects and mites

Method of application of Bacterial bioagents:

1. **Foliar application:**1.0-1.5 gram/litre of water bacterial formulation (powder formulation) or 1.0-1.5 ml/litre of water (liquid formulation) with stickerspray uniformly in eveningor cloudy day condition for pest management (sucking pests, bug and beetles etc.), not during rains. 2-3 spray are required depending upon the intensity of the pests infestation at 5-10 days intervals. Scout fields 3-5 days after application to time of subsequent applications. The bacterial formulations are most effective when eaten by newly hatched or 1st and 2nd instar larvae. Scout for eggs and newly hatched larvae on terminals and squares to ensure proper application time.
2. **Soil application:** For control of soil inhabiting larvae, 2.0-2.5 kg bacterial formulation (powder formulation) or 500-1000 ml (liquid formulation) is added in 25-50 kg farm yard manure (FYM). Mixed thoroughly, cover with jute bag/sugarcane leaves/paddy straw and kept for 2-3 week in shade for proper multiplication. Maintain moisture and mix the mixture in every 3-4 days intervals before broadcasting in the

field. Maintain optimum moisture for better multiplication of bio-agents. Apply well decomposed bacterial based FYM to the field before 15 days of sowing. This mixture can be applied in furrow/pit/pot and at the time of transplanting/sowing. This mixture is sufficient for one acre of land.

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