2 Entomopathogenic Bacteria

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Abstract

Bacteria are well known and extensive in the environment. They have developed a variety of interactions with pests, including essential symbiosis. The term biocontrol applies to the process where living organisms are used to check growth in the population density of specific pest to the extent that ecological balance is maintained and without making them extinct. Bacteria have been used as biocontrol agents for the biocontrol of pests for over a century. The scientific communities have also looked at them as an important component of integrated

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pest management to develop ecofriendly pest management system for crop protection and improvement. It became more pertinent in view of problems posed with synthetic chemicals as they induced harmful changes in nontarget insects and pests.

Microbial pesticides, as living organism or their products or byproducts, offer an environment-friendly alternative with target specificity. They control pests through their nontoxic mechanisms, which are pathogenic to them. The most studied bacterium belonging to the family *Bacillaceae* is *Bacillus thuringiensis* (BT). Besides that, some more bacteria have been reported as potent biocontrol agents, viz. *B. sphaericus, Bacillus popilliae, Bacillus lentimorbus, Paenibacillus popilliae, Serratia entomophila, Brevibacillus laterosporus, Chromobacterium subtsugae,* and *Yersinia entomophaga.*

Bacterium, as the active ingredient in living form, their products or byproducts can control many different kinds of pests. *Aedes aegypti* can easily be controlled by *Bacillus thuringiensis.* On the other hand, to check the population of *Culex quinquefasciatus* and other mosquitoes, *B. sphaericus* showed promising results. The host range of *Bacillus lentimorbus* is larvae (grubs) of Japanese beetle. However, specificity is also a limiting factor, as single application can be effective to a single pest species. Some physical factors, namely heat, desiccation, and radiation, and storage procedures reduce the effectiveness of these bioinsecticides. Despite that, bioinsecticides present great promise to develop better and environmentally friendly pest control programs. The aim of this chapter is to give a holistic and concise picture of insect pathogenic bacteria and their use as bioinsecticides in pest management programs.

Keywords

Bioinsecticides · Biocontrol · Bacteria, · *Bacillus* · Integrated pest management

Learning Objectives

The chapter has the following learning outcomes:

1. The majority of entomopathogenic bacteria belong to the families Bacillaceae, Pseudomonadaceae, Enterobacteriaceae, Streptococcaceae, and Micrococcaceae.
2. The entomopathogenic bacteria enter in insects by ingestion, where they produce toxins, disrupting epithelium of midgut that later cause death of insects.
3. Use of microbes in pest management is an ecofriendly approach to control pests in agricultural crops and to reduce the use of synthetic pesticides.

2.1 Introduction

In the agricultural fields, the farmers have always encountered huge crop damages as well as losses due to pests and diseases. Pesticides that include insecticides, herbicides, and fungicides are employed in modern agriculture to control pests and diseases, and to increase the crop yield. In both developed and developing countries, the use of synthetic pesticides has increased dramatically during the last few decades, and the control of pests with synthetic chemicals results in several problems (Gamliel et al. 1997). The residues of these synthetic insecticides cause toxic effects on humans and wildlife (e.g., birds, beneficial insects like honeybees). Another environmental concern is the contamination of groundwater (Lacey and Siegel 2000).

Despite many years of effective control of pests by insecticides, the continuous use of these chemicals has threatened their effectiveness. It includes the development of insecticide resistance in the target pest species and the deregistration of insecticides due to human health and environmental issues (Nicholson 2007). Therefore, environment-friendly options are the demand of the present time. Improvement in pest control strategies and efficacies leads to higher quality and a greater quantity of agricultural produce. Therefore, there is a great need to develop effective, biodegradable, and environment-friendly biopesticides.

Thus, because of the hazardous effects of insecticides, one of the environment-friendly methods is developed to protect the plant from plant pathogens recently, that is, the use of antagonistic microorganisms called biological control agents or referred to as "biopesticides" (Mazid et al. 2011). Various researches and facts have proved that biological control is a powerful plant disease management tool that can bring huge benefits. Microorganisms that can cause disease in an insect pest and, to some extent, in other arthropods are known as entomopathogens (Tanzini et al. 2001). Many beneficial microorganisms, such as *Pseudomonas fluorescens*, *Trichoderma* spp., *Bacillus subtilis*, and *Fusarium* nonpathogen, have been studied and tested for their efficacy against various plant pathogens. Some of them have been released and marketed as biopesticides (EPA 2011).

2.2 Biological Pesticides

Biopesticides are microorganisms or their products used in pest management and are not related to synthetic pesticides. They are derived from natural enemies, such as animals, plants, bacteria, and certain minerals (EPA 2015), and can be cultured and applied. For example, rapeseed oil and baking soda have insecticidal effects and are considered biopesticides. However, Sudakin (2003) and Gupta and Dikshit (2010) pointed out a broader definition of biopesticides, that is, biopesticides are biochemical pesticides, naturally occurring substances that can control pests through their nontoxic mechanisms. They are living organisms, like viruses, bacteria, fungi, protozoans, and nematodes or their byproducts (phytochemical products and microbial products) that can be used to manage harmful pests of crops.

The unique mode of action of biopesticides makes them different from synthetic pesticides. Biopesticides are usually effective in small amounts and degrade quickly, thereby reducing environmental exposure and avoiding pollution problems. Therefore, they are safer for the environment and human health; and have not found any residual impact on humans (Gupta and Dikshit 2010). According to the US Environmental Protection Agency (USEPA 2010), to classify a substance or a mixture of substances as a biopesticide active ingredient, three conditions are essential: (i) it must be naturally occurring, (ii) must have a nontoxic mode of action against the target pest, and (iii) must have a history of nontoxic exposure to humans and the environment.

2.3 Microbial Pesticides

Biopesticides are further divided into three major categories: microbial pesticides, plant-incorporated protectants (PIPs), and biochemical pesticides (Pathak et al. 2017). Microbial pesticides belong to naturally occurring bacteria, fungi, viruses, etc. Plant-incorporated protective agents (PIP) are the pesticides produced inside the plants from their genetic material. For example, scientists have incorporated *Bt* insecticidal protein genes into plant genomes. However, biochemical pesticides are natural substances, such as plant extracts and fatty acids, from which pests can be controlled through nontoxic mechanisms.

Microbial pesticides can be effectively used as a substitute for chemical pesticides. They are biological pesticides derived from microorganisms, like bacteria or fungi (Pathak et al. 2017). The pathogenic effects of these microorganisms on target pests are host-specific. The mode of action of microbial insect pathogens is by invasion through the outer skin or intestinal tract of insects, which leads to the reproduction of pathogens inside the host. Further, these pathogens produce insecticidal toxins and cause the death of the host. These toxins are identified as peptides, but their structure, toxicity, and specificity greatly differ (Burges 1981).

Moreover, microbial pesticides can be used alone or in combination with other pest management tools (Rathod et al. 2014). Therefore, these microbial pesticides can effectively replace synthetic pesticides with target specificity. A comprehensive pest management plan, also termed as integrated pest management (IPM), evaluates various control measures, cultural operations, weather, potential interactions between pests and crops, and considers all available pest control measures (Flint and Van den Bosch 1981). They leave little or no residue on food crops, thus are efficient and safe for humans and nontarget animals. Since microbial pesticides are species-specific, so other natural enemies are not threatened. They are, therefore, ecologically safe and capable of maintaining biodiversity in the ecosystem.

Microbial pesticides also promote the survival of beneficial insects in treated crops (Hangay et al. 2008). For example, the bacterium, *Paenibacillus popilliae,* is used in biological control. It causes milky spore disease and is useful for controlling

"Japanese beetles." It is very specific to the host species and harmless to other natural enemies. That is why microbial insecticides have been widely used as biocontrol agents in the past three decades (Lacey et al. 2001).

2.4 Bacterial Insect Pathogenesis

Microorganisms that are pathogenic to arthropods, especially to insects, are called entomopathogens. Several naturally occurring bacteria, nematodes, fungi, and viruses infect various insects, mites, and ticks and play an important role to manage them (Kalha et al. 2014). Entomopathogenic bacteria are single-celled prokaryotes, whose size ranges from less than 1μm to several μm. The mode of action of pathogenesis by different bacteria is mentioned below.

2.4.1 Mode of Action

To infect any host, the first task of a pathogen is to enter the host's cells and cavities of its body. This can take place through three main mechanisms: through a lesion, attack of the body cavity through the nematode vector, and the consumption of infected food by the host (Fig. 2.1, Vallet-Gely et al. 2008). On infection of the host, the pathogen enters the hemolymph (Evans et al. 2006). An example of infection by nematode vector is *Photorhabdus luminescens* and *Xenorhabdus nematophila*,

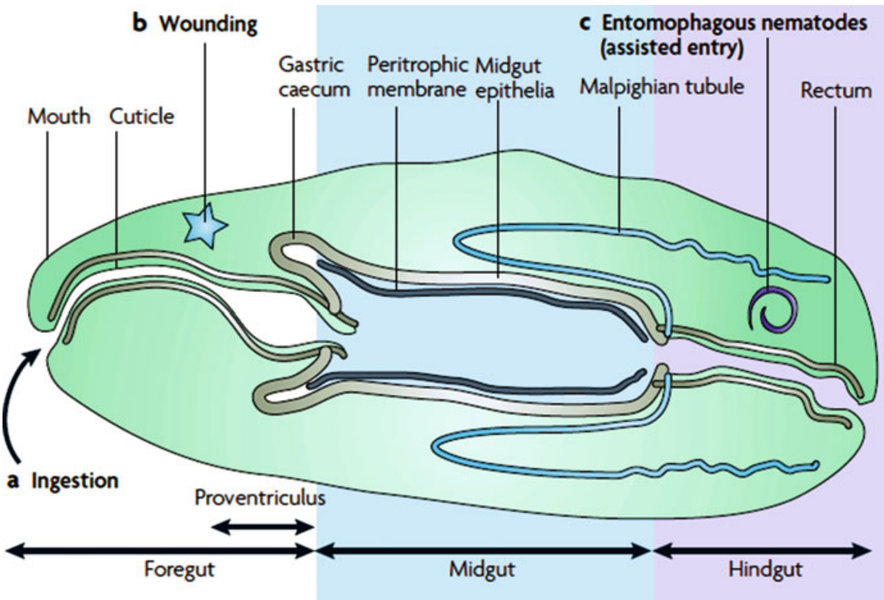

Fig. 2.1 Main routes of bacterial infection in the host. (Vallet-Gely et al. 2008)

which first colonize the gut of the nematode in a symbiotic manner. The nematode then infects the insect and allows the bacteria to colonize inside the insect. Finally, the bacteria infect and kill the insects (Forst et al. 1997).

After entry into the insect's body, the next step is to maintain and colonize the insect. A variety of enzymes are secreted for this process, which work against the gut products of the insect, start producing biofilm, and modify the host gut (Jarrett et al. 2004; Vallet-Gely et al. 2008). AFP (antifading propagase) gene present in *Serratia* spp. is a good example of this. It has toxic effects on host gut epithelial cells and favors bacterial colonization (Hurst et al. 2007).

The bacteria have to escape from the host immune system while living in the gut of the insect. They do this either by avoiding recognition by the immune system or by suppressing the immune response (Vallet-Gely et al. 2008). Host insects use a mechanism to prevent itself from infection by producing reactive oxygen species (ROS) and antimicrobial peptides (AMPs) (Hinnebusch et al. 2002; Parsek and Singh 2003) (Fig. 2.2). On the contrary, bacteria produce proteins that either protect them from the effects of these compounds or degrade these compounds. For example, *Pseudomonas entomophila* produces zinc metalloprotease AprA that acts against host AMP's (Liehl et al. 2006). After evading the host's defense mechanism, the bacteria start killing the host.

Although several factors involved in the pathogenesis of bacteria have been described, it is still unclear what exactly causes the insect's death. It seems that either excessive bacterial proliferation or the production of toxic factors cause damage to the host insect and eventually causes its death (Fig. 2.3). The different stages of infection and bacterial response are outlined in Fig. 2.4. Various degradative enzymes, such as lipases, proteases, and hemolysin, are produced by bacteria inside the host and have harmful effects on the host. For example, the metalloproteinases produced by *Serratia marcescens* inactivate host AMP and cause host tissue degradation (Miyoshi and Shinoda 2000; Ffrench-Constant et al. 2003).

2.5 Entomopathogenic Bacteria

Soil flora and fauna make a very closely net microbial community that includes bacteria, fungi, algae, and nematodes (Sims 1990; Jones et al. 2010). To maintain general balance, microbial communities existing in soil have various roles assigned to them, viz., decomposers, nitrogen binders, and pathogens (Waldrop et al. 2000).

Most of the entomopathogenic bacteria are 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, Lysinibacillus sphaericus, Paenibacillus* spp., *Brevibacillus laterosporus, Clostridium bifermentans, Saccharopolyspora spinosa,* and *Streptomyces* spp..

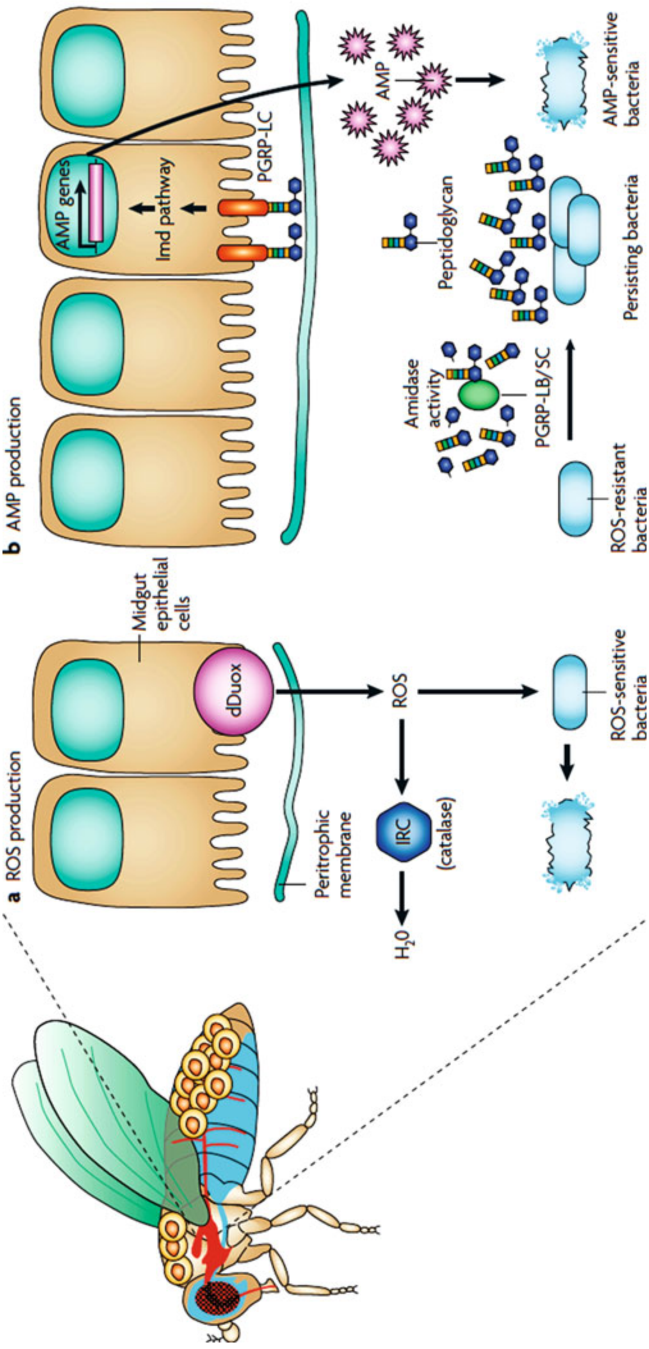

Fig. 2.2 Overview of the local immune response in Drosophila. (Vallet-Gely et al. 2008)

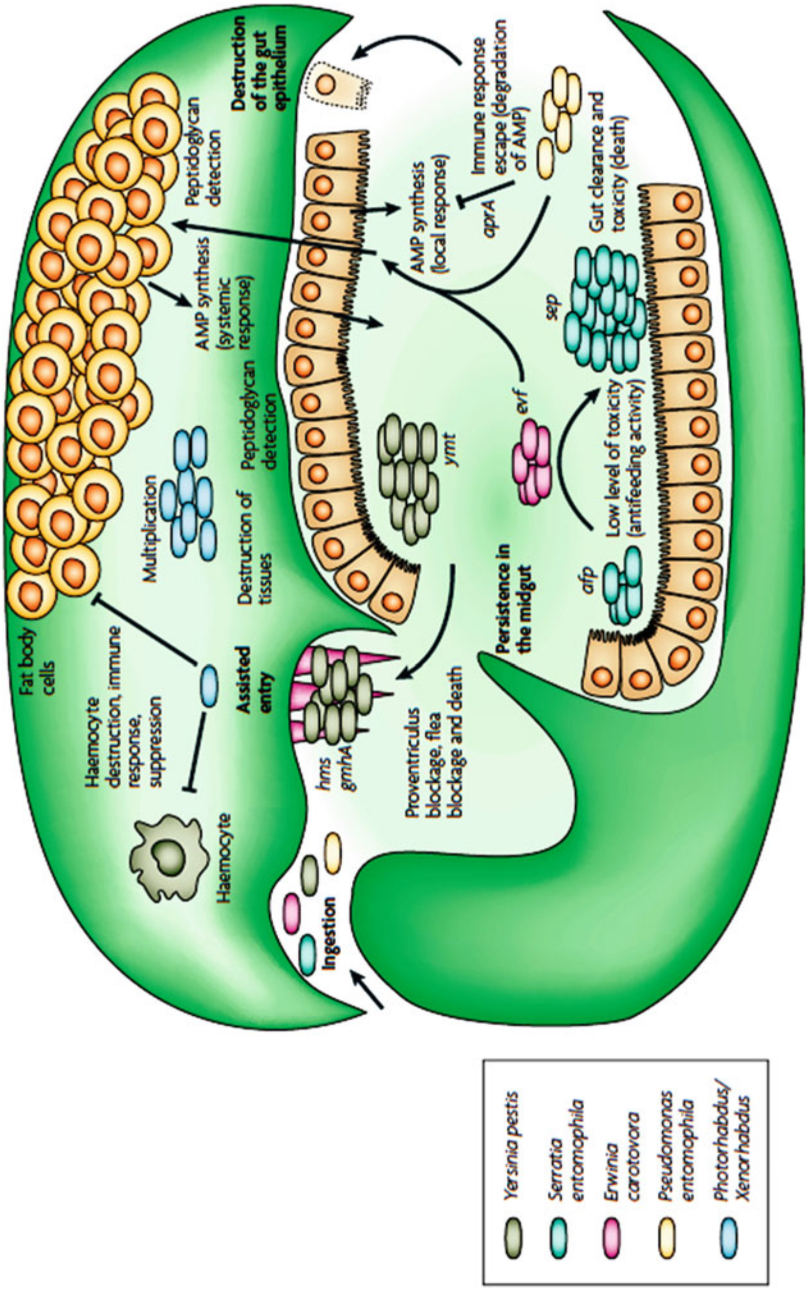

Fig. 2.3 Schematic diagram of different bacterial infections in the host. (Vallet-Gely et al. 2008)

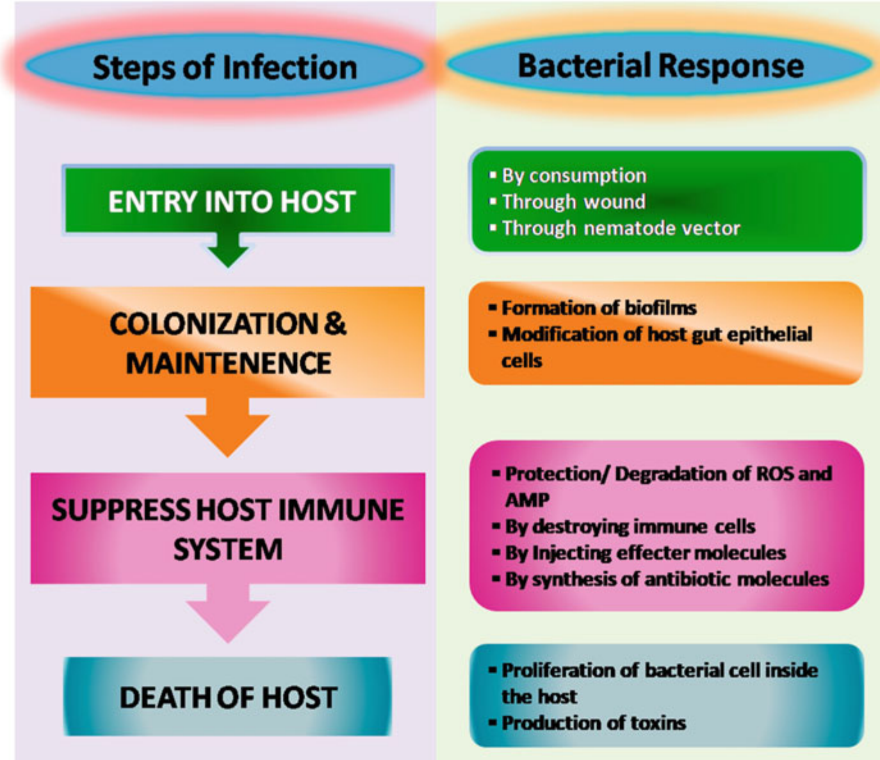

Fig. 2.4 Different stages of bacterial infection and responses in the host

2.5.1 Types of Entomopathogenic Bacteria Other than BT

Several arthropod pathogens have been identified by the bacterial families Bacillaceae, Enterobacteriaceae, Pseudomonadaceae, Micrococcaceae, and Streptococcaceae so far. Usually, these families contain epiphytes, yet some pathogens have been shown to have very toxic effects on their hosts. The two genera (*Photorhabdus* and *Xenorhabdus*) carried by nematodes have been used for agricultural purposes in various ways (Toh et al. 2006; Shigenobu et al. 2000; Akman et al. 2002). They live symbiotically in the alimentary canal of entomopathogenic nematodes and produce a series of toxins (Forst et al. 1997; Ffrench-Constant et al. 2007). These toxins can be applied over leaves of crp plants as extracts or cell suspensions to control insects (Munson et al. 1991). Also, genes that encode toxins can be used to develop transgenic plants to protect crops (Ffrench-Constant and Bowen 2000).

Among entomopathogenic bacteria, the family Bacillaceae has attracted a lot of attention. *Bacillus thuringiensis* (*Bt*) is a soil-borne bacterial species and a series of many deadly pathogens. It is most widely used as a biocontrol agent for insect pests (Pigott and Ellar 2007; Bravo et al. 2007; Kalha et al. 2014). Some other bacterial

Table 2.1 Bacterial insect pathogens and their insect hosts (Vallet-Gely et al. 2008)

Bacteria	Type of interaction	Mode of interaction	Host
Erwinia aphidicola	Pathogen	Ingestion	Pea aphid
Dickeya dadantii	Pathogen	Ingestion	Pea aphid
Pseudomonas entomophila	Pathogen	Ingestion	Drosophila, Bombyx, galleria
Yersinia pestis	Pathogen	Ingestion	Rat flea
Serratia entomophila	Pathogen	Ingestion	Grass grub
Serratia marcescens	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
Paenibacillus lentimorbus	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
Erwinia carotovora	Infectious	Ingestion	Drosophila larvae
Shigella spp.	Passive	Ingestion	Vector house fly
Rickettsia spp.	Vector	Ingestion	Cat flea
Bartonella spp.	Vector	Ingestion	Cat flea

species of *Bacillus* and *Paenibacillus* are also found pathogenic for coleopteran, dipteran, and lepidopteran insects. For example, *B. sphaericus* is highly toxic to mosquitoes, whereas *Bacillus popilliae* and *Paenibacillus popilliae* cause milk spore disease and are used against Japanese beetle larvae (Baumann et al. 1991; Charles et al. 1997; Davidson et al. 1975; Zhang et al. 1997).

Till now more than 100 species of *Clostridium* spp., *Paenibacillus* spp., and *Bacillus* spp. have been identified as biocontrol agents and are highly pathogenic to arthropods (Table 2.1 and Fig. 2.5). Besides, it is reported that biopesticides based on heat-inactivated *Burkholderia rinojensis* and *Chromobacterium subtsugae* can target different types of mites, as they have multiple modes of action (Burkhead et al. 1994; Janisiewicz and Roitman 1988; Martin et al. 2007). Some nonspore-forming ones that belong to the genus *Pseudomonas, Xenorhabdu, Photorhabdus, Yersinia,* and *Serratia* have also received great attention as microbial agents (Waterfield et al. 2001; Zhang et al. 2009; Marshall et al. 2012; Vodovar et al. 2005).

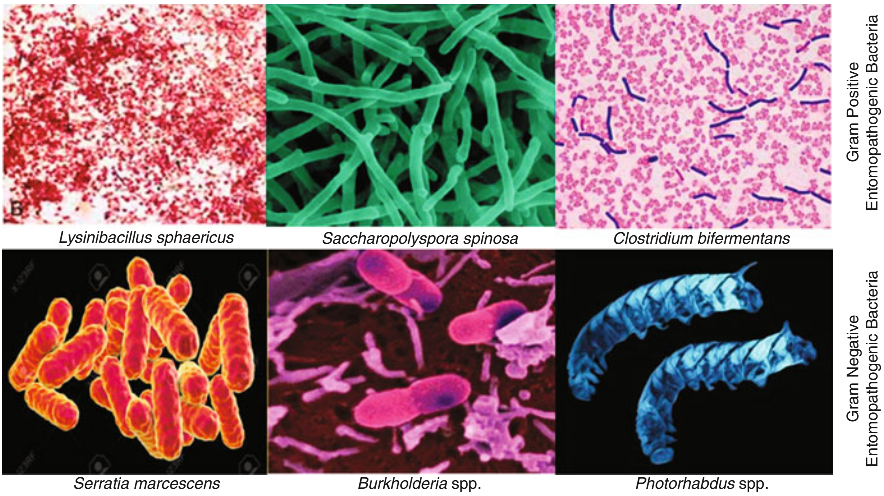

Fig. 2.5 Diagrams of different gram-positive and gram-negative entomopathogenic bacteria

2.5.2 Insect Pathogenic Bacteria Belong to Different Groups/Classes

The four bacterial phyla to which entomopathogens belong are Firmicutes, Actinobacteria, Proteobacteria, and Tenericutes (Fig. 2.6). And family includes Neisseriaceae, Enterobacteriaceae, Paenibacillaceae, and Bacillaceae. Plenty of data on entomopathogens are related to the genus *Bacillus*.

2.5.3 Gram-Positive Entomopathogenic Bacteria

Bacillaceae

Bacillus thuringiensis

Bacillus thuringiensis (BT) is a soil-borne naturally occurring bacterium that has been widely studied and used for pest control naturally. During the last decades, some of its crystal-producing strains have been popularized as the main active substances used in microbial pest management programs (Vega et al. 2012). The pathogenic action of this bacterium is generally followed by spores and crystalline inclusions containing insecticidal δ-endotoxins. The δ-endotoxins interact with receptors present in the midgut epithelial cells of insects and cause cell lysis followed by gut paralysis and death (Pigott and Ellar 2007; Bravo et al. 2007).

B. thuringiensis subsp. *kurstaki* (*Btk*) is commonly used to control lepidopteran larvae. Different strains of *Btk* with important commercial value are ABTS-351, EG2348, HD-1, PB 54, SA-11, and SA-12. Another strain of *B. thuringiensis* subsp. *aizawai* (*Bta*) (ABTS-1857) is also used to suppress the populations of armyworms

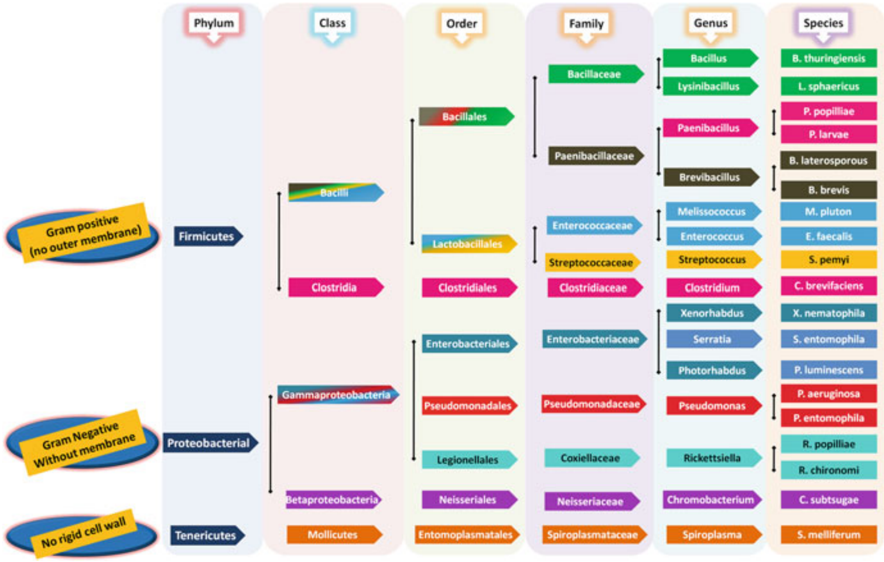

Fig. 2.6 Broad classification of entomopathogenic bacteria

and diamondback moth larvae. Besides, strains belonging to subspecies *israelensis* (*Bti*) have been used to manage mosquitoes and simulids, and of tenebrionids (*Btt*) to fight against Coleoptera (Glare and O'Callaghan 2000).

Lysinibacillus Sphaericus

Entomopathogenic strains belonging to the group of *Lysinibacillus sphaericus* species are characterized by the production of spherical endospores. The endospores are closely related to the parasporal crystals with equivalent proportions of binary protein toxins (BinA and BinB) (Baumann et al. 1991). The insecticidal mode of action involves the lysis of the microvilli epithelial cells of the insect midgut (Charles et al. 1997). Also, the vegetative cells of certain strains produce toxins (Mtx proteins) that kill mosquitoes. Therefore, the main targets of commercially prepared *L. sphaericus* strains are mosquitoes, blackflies, and nonbiting midges.

Paenibacillaceae

Paenibacillus Spp.

Various species of the genus *Paenibacillus* cause pathogenicity to pests, such as American Foulbrood disease in honeybees caused by the spore-forming bacterium *P. larvae* subsp. *larvae* (Davidson 1973). Similarly, *P. popilliae* and *P. lentimorbus* cause milky diseases in phytophagous coleopteran larvae. Even though *P. popilliae* is not directly responsible for the insecticidal effect, the production of parasporal inclusions within the sporangial cells has been reported. After entering the host, the spores begin to germinate in the host's midgut. The pathogenicity of this bacterium can be found as septicemia.

Brevibacillus laterosporus

Brevibacillus laterosporus is a pathogen of invertebrates with a wide range of antimicrobial effects (Ruiu 2013). During the sporulation process, it produces the typical canoe-like parasporal body, which is tightly combined with the sporecoat and gives the species unique morphological characteristics. The insecticidal effects of different strains of *B. laterosporus* on different classes of insects including Coleoptera, Diptera, and Lepidoptera, as well as phytopathogenic bacteria, nematodes, fungi, and molluscs have been reported.

Recently, the whole genome of *B. laterosporus* has been deciphered, revealing its ability to produce various toxins (Djukic et al. 2011; Sharma et al. 2012). Some strains that are toxic to corn rootworms (*Diabrotrica* spp.) and other coleopteran larvae produce insecticidal secreted proteins (ISPs). The protein acts as a binary toxin in the insect midgut and has a high degree of homology with the plant insecticidal protein produced by *B. thuringiensis* (Warren 1997).

Clostridiaceae

Clostridium bifermentans

Clostridium bifermentans strains are highly toxic to mosquitoes and black flies and produce three main proteins involved in insecticidal action (Nicolas et al. 1990). Among these mosquitocidal proteins, Cbm71 is found similar to *B. thuringiensis* delta endotoxins (Barloy et al. 1996).

Actinobacteria

Saccharopolyspora spinosa

During the screening procedure, *Saccharopolyspora spinosa,* a species of bacteria was found from a sugar mill rum and later revealed the insecticidal activity of its isolate "A83543" (Mertz and Yao 1990). The subsequent analysis emphasized the widespread toxicity of specific compounds isolated from the fermentation broth, which was later renamed "spinosyns." It is a new insecticide that contains a structurally unique glycosylated macrolactone that has selective activity against a wide variety of insect pests. Spinosyns have a unique mode of action, including the postsynaptic nicotinic acetylcholine and gamma-aminobutyric acid (GABA) receptors (Bond et al. 2004; Watson 2001).

The interaction of these receptors ultimately leads to the destruction of neuronal activity and the resulting paralysis and death of insects (Perry et al. 2007). Despite its widespread pathogenic activity, it has a lower risk with nontarget species than other pesticides (Sparks et al. 2001).

Streptomyces Spp.

Different *Streptomyces* spp. are related to herbivorous insects that use their cellulose decomposing properties for pathogenicity (Book et al. 2014). Other species and strains of this genus produce various metabolites as effective toxins that have high insecticidal activity (Copping and Menn 2000). Some of the insecticidal compounds produced by *Streptomyces* species are Antimycin A, Macrotetralides, Piericidins, Prasinons, and Flavensomycin (Ruiu 2015). Another substance "Avermectins" produced by *Streptomyces avermitilis* was discovered that had insecticidal and repellent activity (Turner and Schaeffer 1989). These compounds target GABA receptors present in the peripheral nervous system of the insect. The GABA binding to macrocyclic lactone derivatives produces a cascade of events that cause inhibition of neurotransmission and finally paralysis of the neuromuscular system (Bloomquist 1996).

2.5.4 Gram-Negative Entomopathogenic Bacteria

Gammaproteobacteria

Photorhabdus Spp. and *Xenorhabdus* Spp.

Entomopathogenic members of the genus *Photorhabdus* and *Xenorhabdus* are endosymbionts and are usually related to the genus *Heterorhabditis* and *Steinernema* species, respectively (Ffrench-Constant et al. 2007). Once the nematode actively enters the insect body, it releases symbiotic bacteria into the insect hemocoel under pathological action. Here, released bacteria produce and spread various antimicrobial compounds to combat the growth of other microorganisms. They also release various enzymes that contribute to the degradation of hemocoel and make an ideal environment for the development of the nematode population (Tailliez et al. 2006).

Serratia Spp.

The relationship of *Serratia* spp. with insects or insect-pathogenic nematodes has been fully proven (Zhang et al. 2009; Abebe et al. 2011; Torres-Barragan et al. 2011). The pathogen of the grass grub *Costelytra zealandica* produces a group of insecticidal toxins named Sep protein (SepA, SepB, SepC) that show similarities to the insecticidal toxins of *P. luminescens* (Jackson et al. 1992; Hurst et al. 2000). Different species of this genus produce multiple virulence factors. On the other hand, recent genome sequencing of *S. nematodiphila* has also highlighted other pathogenic factors of *Serratia* species (Kwak et al. 2015). It has recently been demonstrated that the pathogenicity of *Serratia marcescens* is increased by the action of serralysin metalloproteinase. It allows bacteria to suppress cellular immunity by reducing the adhesion properties of immune surveillance cells in the insect hosts (Ishii et al. 2014).

Yersinia entomophaga

Yersinia entomophaga is a nonspore-forming pathogenic bacterium with the characteristic of producing insecticidal toxin complex (Yen-Tc). It shows the similarity with *Photorabdus* spp. products (Hurst et al. 2011). These complexes include three Y protein families, A, B, and C, and two chitinases (Chi1 and Chi2) (Landsberg et al. 2011). Various studies on its wide range of toxins and its post-ingestion histopathological effects in the midgut epithelium of insects have been reported (Marshall et al. 2012). Promising research has been carried out under field conditions in which insecticides containing *Y. entomophaga* are used to combat the pasture pest porina (Ferguson et al. 2012).

Pseudomonas entomophila

A ubiquitous bacterium, *P. entomophila,* infects insect larvae orally in a different order and determines extensive intestinal cell damage. The host–pathogen interaction has been studied in *Drosophila melanogaster* that highlights specific immune responses after ingestion (Vodovar et al. 2005). The complete genome sequencing of *P. entomophila* recently highlighted a specific secretion system and related toxins, which may be responsible for its pathogenicity (Vodovar et al. 2006).

Betaproteobacteria

Chromobacterium Spp.

The *Chromobacterium subtsugae* strain named PRAA4-1 T was isolated from soil samples in Maryland (USA). This strain has high insecticidal activity against different species of insects, viz., the Western corn rootworm, *Diabrotica virgifer*, the Diamondback moth, *Plutella xylostella* L., the Southern corn rootworm, and Diabrotica undecimpunctata (Martin et al. 2007). This strain has broad-spectrum activity by multiple pathogenic actions, possibly due to the production of different chemical compounds. Among the bacterial metabolites, *C. subtsugae* specifically synthesizes the violacein, a derivative of tryptophan, which gives its colonies of the characteristic purple color. Besides, various compounds produced by this species have been characterized and found its insecticidal effects (Asolkar et al. 2019). It is reported that the biologically active compound is related to the stationary growth phase and the thermal stability of the insecticidal toxin has also been confirmed (Koivunen et al. 2009).

Burkholderia Spp.

Different insect species carry symbiotic bacteria, of the genus *Burkholderia*, most of which are related to specific intestinal areas (Kim et al. 2013; Martinson et al. 2011). In addition to their mutual interactions with insects, it has recently been reported that *Burkholderia* spp. affect the oviposition and fecundity of bean bug, *Riptortus pedestris* (Kil et al. 2014). The ability of *Burkholderia* species against different plant pathogens as a biocontrol agent has also been reported (Burkhead et al. 1994; Janisiewicz and Roitman 1988). The broth culture of the bacterial strain, named A396, showed oral toxicity and contact effects on *Spodoptera exigua* and

Tetranychus urticae. Its insecticidal and acaricidal properties persist even after heat treatment. Therefore, its commercial formulations are mostly based on heat-killed cells against a variety of chewing and sucking insects and mites.

2.6 Advantages

Biocontrol agents are generally acknowledged as low-risk substances compared to traditional chemical pesticides. Various benefits are associated with the use of entomopathogenic bacteria as biocontrol agents. Like other natural enemies, insect pathogens, such as bacteria and viruses, can achieve considerable control over target populations. For example, their method of action is generally more complex than that of conventional chemicals. They target a wide variety of active sites, thereby reducing the chances of developing resistant pests. Although entomopathogenic bacteria are efficient enough for pest management in organic farming; nevertheless their rotational use and combination of chemicals are strongly promoted to achieve full efficacy and ecostability. Several studies have reported the mutual compatibility and synergistic effects of entomopathogenic bacteria and chemical substances (Morris 1972; Seleena et al. 1999; Musser et al. 2009).

The use of entomopathogenic bacteria in pest management programs has many more advantages. Organic pesticides are nontoxic and nonpathogenic during their handling, dilution, mixing, and application. This property makes them safe for the environment as well as for agricultural workers. The aggregates of these entomopathogenic bacteria and viruses spoil easily, so it leaves fewer residues on crops. Therefore, these can be used even when the crops are almost ready for the harvest. However, they are easy to incorporate into organic farming agreements. Nevertheless, the effectiveness of biopesticides is usually achieved through its correct application in the field. In the long term, organic pesticides are more effective than chemical pesticides. Therefore, it proves to be quite helpful in reducing the total load of chemical pesticides on food, feed, or fiber crops.

2.7 Disadvantages

Although these biopesticides have different benefits from chemical pesticides, their mass production is still a daunting challenge because they require a special substrate and even live host insects to grow. The special formulation and storage procedures increase its production cost and time. Thus, it makes these biopesticides more expensive and less readily available than conventional insecticides. Due to which farmers with large cropping areas may find it difficult to use biopesticides continuously.

One of the advantages of biological pesticides is its high specificity. However, this biggest advantage is also its biggest disadvantage because if the crops are attacked by nontargeted pests, they will have immunity. This means that multiple types of biological pesticides may be required to control all pests at one time.

Therefore, the potential market for these products becomes restricted. Microbial pesticides are dependent on environmental biotic and abiotic factors. It has a limited lifespan. The effectiveness of many microbial pesticides is reduced by exposure to heat, solution, or ultraviolet radiation. Thus, their efficacy shows variability against the population of target pests of different areas. Consequently, the correct timing and proper application of biopesticides are particularly important. Additionally, frequent exposure to toxins puts evolutionary pressure on pests to resist that toxin. Due to this, organisms develop and increase their resistance against control treatments.

2.8 Conclusions

Currently, there is a need to feed the growing human population and limit losses in the production of major crops. On the other hand, the land available for farming and cultivation on the Earth is also limited. It, therefore, requires the development of new technologies to support productivity improvement and its continuous progress in pest management systems. To reduce the health risks caused by the increased use of chemical pesticides in agriculture, ecofriendly pest and disease management methods are being developed and evaluated globally. In view of this, entomopathogenic bacteria and viruses have a wide range of biocontrol agents.

The targeted toxicity and their nontoxic effects on nontarget organisms make biological pesticides ideal tools for integrated pest management (IPM) programs. More attention should be paid to its use in combination with chemical pesticides and the integration of biological agents into production systems. At the same time, it is also necessary to encourage public funding schemes, pesticide companies, and commercial investors to establish biological pesticide companies. Thus, biopesticides can work effectively by combining performance along with minimal application and safety benefits to humans and the environment. It may be considered that their role in agriculture and horticulture can be enriched by encouraging their use at the government and nongovernment levels shortly.

2.9 Points to Remember

1. The synthetic insecticides and pesticides used in pest management cause hazardous effects on the environment and human health.
2. Use of broad-spectrum pesticides destroys natural enemies of pests.
3. Biological pesticides provide an ecofriendly measure for pest management.
4. Entomopathogenic bacteria other than *Bt* can also be used in insect pest management.
5. They are a valuable source of insect toxins for the biocontrol of pests.
6. Discovery of new biopesticides is critical in tackling environmental degradation and pest resistance development.

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