Chapter 5
Non-Bt Soil Microbe-Derived Insecticidal Proteins

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5.1 Introduction

Although soil microbes have contributed immensely to world agriculture through their potential biochemical attributes in the areas of insect pest management, crop nutrient enhancement [increasing nutrient availability by plant growth-promoting rhizobacteria (PGPR), phosphate-solubilizing bacteria (PSB)], and soil fertility management [through metal detoxification, mycorrhizal-helping bacteria (MHB), and arbuscular mycorrhizal fungi (AMF)] (Khan 2005), innovations in biotechnology have opened new vistas for enhancing the contribution of the soil microbial diversity to agricultural productivity. For many decades, pest control programs in agriculture and public health have relied heavily on the use of broad-spectrum chemical insecticides. However, the use of chemical insecticides came under scrutiny since the early 1960s when the environmental classic *Silent Spring* was published (Carson 1962), leading to a paradigm shift in insect pest management strategies and emphasizing the need to identify non-chemical pest control strategies that are insect-specific and environmentally safe. Naturally occurring microbial entomopathogens, such as bacteria, fungi, viruses, and nematodes, are effective non-chemical alternatives for the suppression and management of insect pests causing economic losses in different crops (Lacey et al. 2001; Lacey and Kaya 2007; Shahid et al. 2012). Characterization of the genes and genomes of these entomopathogens has facilitated the identification and deployment of novel insecticidal genes for crop protection. Of all entomopathogens, bacteria have been the most extensively used organisms to date, and overwhelming commercial success was achieved with *Bacillus thuringiensis* (*Bt*) (Firmicutes: *Bacillaceae*) toxins.

Lepidoptera, the second largest insect order, comprised of moths and butterflies, represents a diverse and important group of insect pests that affect commercial

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agriculture, causing widespread economic damage on food and fiber crop plants, fruit trees, forests, and stored grains. The larval stage of the moths is detrimental to an array of economically valuable crops including cotton, tobacco, tomato, corn, sorghum, pulses, and wheat (Srinivasan et al. 2006). Some examples of lepidopteran pests include: the cotton bollworms, *Helicoverpa armigera* (Hübner) and *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae); the gypsy moth, *Lymantria dispar* (Linnaeus) (Lepidoptera: Lymantriidae), a voracious defoliator of Palaearctic and Nearctic forests (Reineke et al. 1999); the diamondback moth (DBM), *Plutella xylostella* (Linnaeus) (Lepidoptera: Plutellidae), a pest of cole crops (Talekar and Shelton 1993); brinjal shoot and fruit borer, *Leucinodes orbonalis* (Guenée) (Lepidoptera: Crambidae); and okra shoot and fruit borer, *Earias vittella* (Fabricius) (Lepidoptera: Noctuidae) which causes 69 % loss in marketable yield in okra (Radake and Undirwade 1981). Until recently, the control of pests in agriculture has mostly relied on the intensive application of broad-spectrum synthetic insecticides, with about 40 % targeted to the control of lepidopteran insects (Brooke and Hines 1999). Over the years the application of insecticides has led to the development of insecticide-resistant insects, destruction of natural enemies as well as harmful effects on humans and the environment. Therefore an urgent need was felt for alternative control strategies that reduce dependence on conventional insecticides. The interest in biopesticides started growing significantly, as a result of the withdrawals of many synthetic pesticides and the high cost of developing new ones. In this scenario, it is important to note that the global pesticide market is growing at a compound annual growth rate (CAGR) of 3.6 % and the value is expected to reach $51 billion in 2014. The biopesticide segment which represents a strong growth arena in the global pesticide market is expected to grow at a 15.6 % CAGR from $1.6 billion to $3.3 billion in 2014 (BCC Research 2010; Ruiu et al. 2013). Thus, the direct application of entomopathogens as biological control agents or deploying GM crops developed using novel entomotoxic proteins provides a good market opportunity that can be captured by the industry. Agriculture was perceived to benefit from futuristic eco-friendly strategies such as the use of natural enemies, autocidal control methods such as sterile insect technique (SIT) and F1 sterility, and transgenic plants expressing entomotoxic proteins (Fitt 1994; Gatehouse et al. 1994; Haq et al. 2004; Saour 2014).

B. thuringiensis (Bt) is a gram-positive bacterium that is found in a variety of ecological niches such as soil, water, plant surfaces, stored cereals and dead insects (Federici and Siegel 2008). The bacteria form spores containing proteinaceous crystals known as Cry or Cyt proteins (also known as δ-endotoxins), as well as VIPs (vegetative insecticidal proteins) exhibiting potent insecticidal activity (Sanahuja et al. 2011). Different strains of *Bt* produce different types of insect-toxic virulence factors, and the activity of these virulence factors toward Lepidoptera, Diptera, Coleoptera, Hymenoptera, Homoptera, Orthoptera, and Mallophaga insect orders have been reported (Schnepf et al. 1998). Bt was released as a biopesticide (ICP and viable spores) since 1951 (Steinhaus 1951) and formulations based on Bt (67 registered products and more than 450 formulations) occupied the key position accounting for nearly 90 % of the total biopesticide sales worldwide (Neale 1997). Bt products used for managing lepidopteran pests were primarily

derived from Bt *Kurstaki* HD-1 strain (e.g., Biobit, Dipel, and Thuricide) and to a lesser extent from *Kurstaki* SA-11 and *Kurstaki* SA-12 strains (Kaur 2000). However, Bt had limited use as a foliar insecticide due to the short window of effectiveness as a result of which multiple sprays had to be undertaken which led to increase in the amount of product for application and fuel needed for spraying. The sprays also had little impact on cryptic pests (Sanchis 2011). These inherent limitations of topical Bt pesticides were overcome by introducing Bt *cry* genes into target crops thereby enhancing plant health and conferring plant protection (Sanahuja et al. 2011).

Transgenic crops protected from insect pests have become an integral part of insect pest management (IPM) with over 58 Mha planted worldwide in 2010 (James 2010; Baum et al. 2012). A number of transgenic crops including corn, cotton, rice and soybean harboring *Bt* genes are cultivated commercially since 1996 (Huang et al. 2007; Sanahuja et al. 2011). *Bt* cotton, in particular, has provided effective control of several lepidopteran pest species including tobacco budworm, *Heliothis virescens* (Fabricius) (Lepidoptera: Noctuidae), pink bollworm, *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae), and the cotton bollworm, *H. armigera* (Hübner) (Lepidoptera: Noctuidae), resulting in increased yield, reduced frequency of insecticide applications, and area-wide suppression of the same primary insect pest in other crops (Perlak et al. 2001; Carrière et al. 2003; Jackson et al. 2003; Wu et al. 2008; James 2010). The widespread cultivation of Bt cotton varieties can contribute to a resurgence in beneficial arthropod populations necessary for successful IPM (Head et al. 2005; Naranjo 2005; Whitehouse et al. 2005). Although Bt-derived biopesticides used as foliar sprays or expressed in plants through genetic engineering are environmentally safe and effective, their use is still restricted due to problems of limited host range and the potential for the development of resistance and cross resistance in key pests due to continuous use, thus necessitating the discovery of novel insecticidal genes with improved activity and host range. Field evolved resistance to Bt crops has been reported for populations of several insect pests (Gassmann et al. 2011).

Insecticidal nematodes in the genera *Steinernema* and *Heterorhabditis*, each carrying a specific genus of bacteria, are the only insect-parasitic nematodes possessing an optimal balance of biological control attributes (Poinar 1979; Bedding et al. 1993), and they have been used for the biological control of soil-dwelling pests that include weevils and lepidopteran insects (Wang and Li 1987; Klein 1990). However, factors such as cost, shelf life, handling, mixing, compatibility, and profit margins to manufacturers and distributors have contributed to the failure of entomopathogenic nematodes (EPN) especially for large-scale agriculture applications, as they failed to penetrate many markets or gain significant market share in the current markets (Lacey and Georgis 2012). The widespread adoption and success of Bt crops and the associated risk of resistance development to Bt protein(s) have stimulated the research and development of more environmentally responsible alternatives. Thus, a significant research need was centered toward the characterization of insecticidal proteins, called the toxin complex (Tc) proteins and other virulence factors from bacteria that are symbionts of EPNs as they can be used

for the development of a new generation of GM crops that are protected against a wider spectrum of insect pests (Bowen and Ensign 1998).

Sequencing, annotation, and screening of the genomes of entomopathogenic bacteria such as *Photorhabdus*, *Xenorhabdus*, and *Pseudomonas* spp. have begun to reveal previously unidentified insecticidal toxins. The presence of orthologues of a multitude of insecticidal genes (*tc*, *mcf1* and *mcf2*, *xaxAB*) in different species of insect-pathogenic bacteria indicates that a large amount of genetic transfer occurs between these species, presumably within a shared environmental niche (Hinchliffe et al. 2010), thus providing an excellent source of novel candidates, which can be used as potential alternatives to the insecticidal proteins derived from *B. thuringiensis*. The toxin complex proteins from *Photorhabdus luminescens* have also been transferred into plants and tested for their activity on different insect pests. The intent of this book chapter is to provide a comprehensive review of virulence factors produced by gram-negative entomopathogenic bacteria that demonstrate potential toxicity toward lepidopteran pests so that they could be successfully exploited for plant protection.

5.2 A Vast Arsenal of Insecticidal Toxins Derived from Gram-Negative Bacteria

Although deployment of crops expressing insecticidal proteins has led to effective control of insect pests and reduced the use of chemicals for insect control, very few candidate proteins have been commercially used for crop protection. These proteins typically control limited ranges of pest species and are predominantly from gram-positive bacteria and derived mostly from *B. thuringiensis*. Entomopathogenic gram-negative bacteria also produce toxins that are harmful to insects and can be used to augment the list of genes used in developing pest control products. Members in the Enterobacteriaceae such as *Photorhabdus*, *Xenorhabdus*, *Serratia*, *Pseudomonas*, and *Yersinia* spp. produce insecticidal toxins with toxicity similar to that of Bt toxins. Initial studies showed that these bacteria were highly effective when fed to a range of pest species belonging to at least three orders of insects including the Lepidoptera. Based on their targeted tissue, these toxins can be categorized into three types: (a) cytotoxins, (b) digestive toxins, and (c) neurotoxins (Castagnola and Stock 2014). This section describes the virulence factors associated with different gram-negative bacteria, their activity spectrum, and mode of action.

5.2.1 Insect Virulence Factors Produced by *Photorhabdus* Species

The *Photorhabdus* genus currently consists of three species: *P. luminescens*, *Photorhabdus temperata*, and *Photorhabdus asymbiotica*, found in a symbiotic association with an insect-pathogenic soil nematode of the genus *Heterorhabditis*. Several subspecies are recognized (Fischer-Le Saux et al. 1999). The genus *Photorhabdus* and the two species *P. luminescens* and *P. temperata* have been the subject of intensive study by entomologists and agricultural scientists in view of their insect pathogenicity and their potential for the development of novel biopesticides and insect-resistant transgenic plants. *P. asymbiotica* is a human pathogen that has been recovered from human clinical specimens from the USA and Australia and is currently considered an emerging human pathogen model system (Gerrard et al. 2004; Gerrard et al. 2006).

Photorhabdus are gram-negative, bioluminescent, motile bacteria of the family Enterobacteriaceae which live in an obligate mutualistic association with insect-parasitic *Heterorhabditis* nematodes, which invade and kill insects in the soil (Waterfield et al. 2009). The infective juvenile (IJ) nematode exists as a free-living, non-feeding individual in the soil and actively seeks out and colonizes the insect prey in the soil. The IJ nematode enters a potential victim either through respiratory spiracles, the mouth, or the anus. The symbiotic bacteria vectored by these IJs are then regurgitated from the nematode intestine into the open circulatory system of the insect prey. The bacteria colonize the anterior midgut of the insect initially, undergo rapid multiplication, and subsequently kill the insect within 1–2 days. As the bacterial population reaches a high level, the insect cadaver becomes red in color and visibly bioluminescent (Bowen and Ensign 1998). During the growth in the insect prey, the bacteria release a plethora of virulence factors to kill the insect and produce antibiotics with antifungal and antibacterial activities that probably prevent the invasion of the cadaver by other microorganisms resulting in ideal conditions for the growth and reproduction of the nematode (Paul et al. 1981; Akhurst 1982).

P. luminescens appears to encode numerous putative (ffrench-Constant et al. 2000) and proven (Bowen et al. 1998; Waterfield et al. 2001) insect virulence factors in its genome. The *Photorhabdus* genome is organized into genomic islands relating both to pathogenicity and to symbiosis. Genomic islands involved in pathogenicity are called "pathogenicity islands" (PAIs). PAIs are unstable regions that are present in the pathogen but absent from non-pathogens (Hacker and Kaper 2000) and are often inserted next to tRNA genes and have differing GC content from the rest of the genome (Waterfield et al. 2002; ffrench-Constant et al. 2003). Functional analysis of genomic islands facilitated the identification of a diversity of anti-invertebrate virulence factors from *P. luminescens* (Daborn et al. 2002; Waterfield et al. 2002). Multiple copies of "toxin complex" (*tc*) genes (Bowen et al. 1998; Waterfield et al. 2001) inserted at an AspV tRNA were detected in the first unique island. The *tc* genes encode high molecular weight, multi-subunit,

orally active insecticidal toxins first characterized in insect pathogens *Photorhabdus* and *Xenorhabdus* spp. (Bowen et al. 1998; Waterfield et al. 2001), but now seen in a range of pathogens, including those of humans. Some Tc's have demonstrated oral toxicity to insects making them potential candidates for insect pest control. A second island inserted at a Phe tRNA was found to encode the novel toxin "makes caterpillars floppy" or Mcf, a large toxin with little similarity to known proteins (Daborn et al. 2002). A third island with a skewed GC content contains a gene encoding a cytotoxic necrotizing factor (CNF)-like toxin, designated *Pnf*. The specific role of *Pnf* in *Photorhabdus* is unknown (Buetow et al. 2001; Waterfield et al. 2002). Two copies of a macrophage-toxin-like encoding gene similar to that found in pathogenic strains of *Escherichia coli* are carried by a fourth island that also contains an *rhs* element and a CP4-like integrase gene. This island is linked to the *phlAB* hemolysin locus and, probably forms a part of larger region involved in pathogenicity. Lastly, a fifth island encodes a type III secretion system (TTSS), and the order of genes in the TTSS island is similar to that in *Yersinia pestis*, and these genes are probably important in the interaction of *Photorhabdus* with its invertebrate hosts (ffrench-Constant et al. 2000; Waterfield et al. 2002; Silva et al. 2002).

5.2.1.1 The Toxin Complexes (Tc's) of *Photorhabdus*

The Tc proteins produced by *P. luminescens* are an important class of secreted toxins, with an estimated molecular weight of 1,000,000 and with no detectable protease, phospholipase, or hemolytic activity but showing a trace lipase activity. The Tc is a large, multimeric complex comprising of several protein subunits ranging in size from 30 to 200 kDa, some of which are found to be lethal when fed to or injected into the hemolymph of *Manduca sexta* larvae and several other insect species (Bowen and Ensign 1998). Purification of the active protein complex revealed the presence of four distinct protein "toxin complexes" which were termed Tca, Tcb, Tcc, and Tcd, and the genes corresponding to these Tc proteins were cloned from strain W14 (Bowen et al. 1998). The different Tc's are encoded at discrete PAIs in the *Photorhabdus* genome where multiple *tc* gene copies are found (Wilkinson et al. 2009). Although all Tc proteins show injectable toxicity to *M. sexta*, majority of the oral toxicity of *tc* genes toward lepidopteran insect pests was found to be mediated by *tca* and *tcd* genes as shown via gene knockout studies (Bowen et al. 1998). Sequence analysis of the *tca*, *tcb*, *tcc* and *tcd* loci revealed a high degree of similarity between loci, and despite the apparent complexity of the loci, the individual genes within these loci could be grouped into three basic types of genetic elements: the *tcdA*-like or [A], the *tcaC*-like or [B], and the *tccC*-like or [C] (ffrench-Constant and Waterfield 2005). These groupings suggest similar roles of their encoded proteins within the assembled toxin complex, and a representative of [A], [B], and [C] was required for full toxicity (Waterfield et al. 2005a). The *tca* locus of *P. luminescens* W14 consists of three open reading frames (ORFs), *tcaA*, *tcaB*, and *tcaC* with the [A] subunit of the Tc encoded by *tcaA* and *tcaB*, the

[B] subunit encoded by *tcaC*, and the [C] subunit encoded by *tccC* gene from the *tcc* locus. A fourth ORF the *tcaZ* associated with the *tca* locus is encoded in the opposite orientation, and the function of this protein is not yet known. The *tcb* locus consists of a single [A] gene, *tcbA* and the *tcc* locus consists of an [A] encoded by *tccA* and *tccB*, and a [C] encoded by *tccC*. The *tcd* locus is the largest of the four *tc* loci and consists of four [A] genes, *tcdA1-A4*; two [B] genes, *tcdB1–tcdB2*; and four [C] genes, *tccC2–tccC5* (Hinchliffe et al. 2010). Orthologues of *tc* encoding genes are widespread in gram-negative bacteria (*Xenorhabdus*, *Serratia*, and *Yersinia*) and also present in some gram-positive bacteria (*Paenibacillus*). The association of these loci with transposase-like or bacteriophage-like genes, indicates that they are highly mobile and can be transferred between species (Hinchliffe et al. 2010).

The role of proteins encoded by the *tc loci* in *Photorhabdus* biology is ambiguous despite efforts undertaken to understand the structure, function, genetics, and mode of action of these proteins. The Tc's of *Photorhabdus* spp. appear to be very undiscriminating in their activity, with demonstrable toxicity toward a wide spectrum of insect species (Hinchliffe et al. 2010); therefore, their use as candidates for crop protection may be limited unless their effects on non-target organisms (NTO), especially the beneficial arthropods are investigated. The insecticidal *tc's* have been shown to be preferentially expressed at low temperatures (<15 °C). Analysis of the expression and insecticidal activity of the protein subunits of the *P. luminescens* W14 *tcd* locus revealed that the [A] subunit itself possessed a low level of toxicity which is potentiated by [BC], a complex formed by [B] and [C] when expressed together. The [BC] complex demonstrated mild oral toxicity toward *M. sexta*, whilst the [B] and [C] subunits individually were not orally toxic. The [C] subunit appeared to play an important role in the complex as its presence was observed to be necessary for oral toxicity of Tca and Tcd. A thorough understanding of specific protein interactions is therefore very crucial for these proteins to be successfully used for insect pest control.

The mechanism of action of the Tc's is not well understood. Although the toxicity of the Tc proteins has been demonstrated on specific model insects and cultured cells, comprehensive information on how these effects are mediated by the proteins is currently unavailable. The ingestion of purified *P. luminescens* Tca by *M. sexta* led to the complete destruction of the midgut epithelium leading to cessation of feeding and eventual starvation of the insect host. Tca also showed characteristic, midgut-specific histopathology in *M. sexta* that included the apical swelling of the columnar cells in the epithelium of the anterior midgut and blebbing of the vesicles into the gut lumen (Blackburn et al. 1998). No pathological effects were observed on any other tissues indicating the gut specificity of the toxin. Liu et al. (2006) reported that a *P. luminescens* Tca-like toxin (PL toxin) caused channel formation in the midguts and permeabilized unilamellar lipid vesicles of *M. sexta* in a pH-dependent manner. However, structural studies of XptA1 indicated that the protein binds to the brush border membrane vesicles but does not form pores in the membrane. It was therefore hypothesized that the [BC] probably aids in the insertion of the [A] tetramer into the membrane (Hinchliffe et al. 2010). Toxin A protein (283 kDa) was expressed in *Arabidopsis thaliana* using a synthetic plant-codon-optimized variant of *tcd*A, and the insecticidal efficacy of the protein was

tested for control of feeding insects (Liu et al. 2003). Transgenic plants expressing more than 700 ng/mg of extractable protein were found to be highly toxic to *M. sexta*, and the toxin A purified from transgenic plants had a strong inhibitory effect on the growth of southern corn rootworm. In the best transgenic *Arabidopsis* line, high toxin A expression and insect resistance were found to be consistent for at least five generations in all progeny (Liu et al. 2003). These results indicate that the tc proteins from *Photorhabdus* may open a new route to transgenic pest control.

5.2.1.2 *Photorhabdus* Insect-Related Binary Toxins, PirA/B

Two genetic loci (*plu4093–plu4092 and plu4437–plu4436*) sharing significant sequence similarity with a putative juvenile hormone esterase (JHEs) of *Leptinotarsa decemlineata* (Vermunt et al. 1997; Duchaud et al. 2003) were identified from the genome sequence of Pl TT01. JHEs regulate metamorphosis by inactivating the juvenile hormones involved in maintaining the insect in a larval state. The inappropriate activation of the insect endocrine machinery by JHE-like proteins may therefore be an effective strategy for insect control (Bonning and Hammock 1996). The *plu4093–plu4092* and *plu4437–plu4436* genetic loci were renamed as "*Photorhabdus* insect-related" (Pir) proteins, with PirA referring to products of *plu4093/4437* homologs and PirB to products of *plu4092/4436* homologs (Waterfield et al. 2005b). Pir proteins are binary toxins having both injectable (Waterfield et al. 2005b) and oral toxicity (Blackburn et al. 2006) toward insects from the orders Diptera and Lepidoptera. Waterfield et al. (2005b) demonstrated that each of the genes in the *P. luminescens* loci was required for toxicity when injected into larvae of *Galleria mellonella* L. (Lepidoptera: Pyralidae), but the combination was not sufficient to cause mortality in *M. sexta* L. (Lepidoptera: Sphingidae) by either injection or oral administration. The oral activity of Pir A/B tested against the diamondback moth, *P. xylostella*, demonstrated that the midgut is the primary site of action. The pathology observed was similar to those seen with other gut-active toxins, but consistent effects were noticed in the posterior midgut. *P. xylostella* was found to be 300-fold more susceptible to Pir toxins than other insect species tested (Blackburn et al. 2006). However, these proteins had no effect on the growth or mortality of *H. virescens* F. (Lepidoptera: Noctuidae), *M. sexta* L. (Lepidoptera: Sphingidae), or *L. dispar* L. (Lepidoptera: Lymantriidae) larvae on oral delivery (Blackburn et al. 2006). Based on insect bioassays, it can be concluded that the Pir A/B proteins may not be broadly useful as insecticidal proteins. Although the PirB protein shares some sequence similarities with the δ-endotoxins from *B. thuringiensis*, no significant difference was observed in the responses of the susceptible *P. xylostella* larvae (lab colony) and the commercially available Cry 1A-resistant strain to the PirB protein. The PirA/B proteins have been shown to lack the esterase activity and evidence presented by Crosland et al. (2005) suggested that these proteins are related to leptinotarsin, a neurotoxic protein present in the hemolymph of several *Leptinotarsa* species (Hsiao and Fraenkel 1969). Consequently the mechanism for the potential insecticidal activity appears

to be the destructive effects on the neural tissue upon injection (Castagnola and Stock 2014).

5.2.1.3 The Makes Caterpillars Floppy (Mcf1 and Mcf2) Toxins

The Mcf toxin is a high molecular weight protein (324 kDa) that was found to facilitate the persistence of *E. coli* expressing this gene within the insect host and kill the insects (Daborn et al. 2002). The predicted amino acid sequence of the 8.8-Kb *mcf1* gene fragment cloned from *P. luminescens* subsp. *akhurstii* strain W14 showed only partial homology to known proteins; however, it carried a BH3 domain, a domain found in pro-apoptotic proteins (Budd 2001). The Mcf toxins are potent toxins that are active upon injection and induce apoptosis via the mitochondrial pathway in insect phagocytes, helping the bacteria avoid phagocytosis. These proteins destroy the columnar and goblet cells of the insect midgut epithelium, causing the caterpillar to lose body turgor due to impaired osmoregulation, and become “floppy” (Daborn et al. 2002). This toxin also promotes apoptosis in mammalian tissue culture cells (Dowling et al. 2004). A second Mcf1-like ORF (*mcf2*) which also caused loss of body turgor when injected into *M. sexta* larvae was identified during end sequencing of Pl W14 cosmid library (Waterfield et al. 2003). The two mcf proteins are 77.5 % identical across the majority of their lengths only differing in their N-terminal regions (Hinchliffe et al. 2010). While Mcf1 contained a long 900 amino acid N-terminal region with no similarity to other proteins in the database, a shorter 300 amino acid N-terminal region (a HopA1-like region) containing a domain showing similarity to several type III secreted proteins was found in Mcf2. The region of similarity between Mcf1 and Mcf2 contains a BH3-like domain, two domains found in RTX-like toxins, and a large domain found in the *Clostridium difficile* binary toxins (Hinchliffe et al. 2010). Comparisons of available sequence data from different *Photorhabdus* strains revealed that copies of *mcf1* are always present, suggesting that it may be the dominant insect-killing toxin. However, it is very likely that *mcf2* is only present in a subset of strains. Toxins like Mcf which act on both the gut and insect immune system represent a promising, yet underexploited avenue for future insecticide development (Daborn et al. 2002).

5.2.1.4 Txp40 Toxin

A novel 42 kDa secreted protein encoded by the toxin gene *txp40* (t$xp40_{V16}$, identified from *P. luminescens* strain V16) and initially identified in *Xenorhabdus nematophila* (*A24tox*, $txp40_{A24}$) was found to be part of a genomic island involved in pathogenicity and highly conserved and widespread among the *Photorhabdus* strains (Brown et al. 2006). Txp40 protein was found to have hemolymph toxicity and was effective against a range of lepidopteran species (*G. mellonella*, *H. armigera*, and *Plodia interpunctella*) and the dipteran species *Lucilia cuprina*. The protein exhibited significant cytotoxicity in vitro against two dipteran cell lines

(*Aedes aegypti* and *Drosophila melanogaster* cell line S2) and two lepidopteran cell lines (*Spodoptera* cell lines Sf9 and Sf21), but not against a mammalian cell line. The broad insecticidal activity of the Txp40 toxin suggests that the toxin has a target that is common to many different insects (Brown et al. 2006). Gut histology studies of *H. armigera* showed that the midgut and fat body are the targets and the toxin caused a significant decrease in midgut intercellular adhesion, degradation of the peritrophic matrix lining of the midgut cells, and degradation of the fat body nuclei. Although the selective toxicity of Txp40 against a broad spectrum of lepidopteran insect pests, and the lack of toxicity against mammalian cell lines makes it a good candidate for pest control, the protein has to retain its insect-toxic properties (it should not be degraded by the insect gut enzymatic machinery) upon oral delivery for it to be a potential option for developing transgenic crops.

5.2.1.5 An Array of Other Insecticidal Proteins from *Photorhabdus*

Photorhabdus Insecticidal Toxin

Photorhabdus insecticidal toxin (Pit), a probable toxin from *P. luminescens* showing 30 % amino acid sequence similarity to a fragment of a 13.6 kDa insecticidal crystal protein gene of *B. thuringiensis*, demonstrated injectable toxicity to the larvae of *G. mellonella* and *Spodoptera litura*. However, ingestion of purified Pit protein caused an inhibition of growth of *S. litura* and *H. armigera* larvae, but did not cause larval mortality. The hemocoel insecticidal activity of Pit was comparable with other hemocoel toxins such as Txp40 of *Photorhabdus* (Li et al. 2009).

Photorhabdus Virulence Cassettes

Photorhabdus virulence cassettes (PVCs) are phage-like loci found as repetitive cassettes in the genome of *Photorhabdus* and contain putative toxin effector genes. PVCs are functional homologues of the prophage-like locus on the pADAP plasmid of *Serratia entomophila* (Yang et al. 2006). Recombinant expression of various PVC loci from *P. luminescens* and *P. asymbiotica* demonstrated that they have differing toxicities toward *G. mellonella* upon injection with PVC product derived from the human pathogen *P. asymbiotica* (Gerrard et al. 2004) having greater toxicity for insects than PVC product from the insect pathogen *P. luminescens* TT01 (Duchaud et al. 2003). Although the PVC products showed structural similarity to an antibacterial R-type pyocin, they had no conspicuous antibacterial activity but triggered rapid destruction of insect phagocytes, thus allowing the persistence of recombinant bacteria in wax moth, *G. mellonella* larvae. Comparison of the genomic organizations of PVCs in different *Photorhabdus* species revealed that they have a conserved phage-like structure with a variable number of putative anti-insect effectors encoded at one end. Expression of these putative effectors

directly inside cultured cells showed that they are capable of rearranging the actin cytoskeleton (Yang et al. 2006).

Hemolysins or Hemagglutinin-Related Proteins

Hemolysins are extracellular toxic proteins that function as virulence factors and derive their name because of their activity toward red blood cells (Brillard et al. 2002; Cowles and Goodrich-Blair 2005). Hemolysins are produced by a wide spectrum of bacterial species that include the gram-positive (e.g., *Listeria* spp., *Streptococcus* spp.) and the gram-negative (e.g., *E. coli*, *Vibrio* spp., *Photorhabdus* spp., *Xenorhabdus* spp., *Serratia* spp.) bacteria, and these proteins frequently target the immune cells and may aid in evading insect immune responses during infection (Konig et al. 1987; Swihart and Welch 1990; Cowles and Goodrich-Blair 2005). *P. luminescens phlBA* operon, a locus encoding a hemolysin, shows similarities to the pore-forming, calcium-independent hemolysins from *S. marcescens*, and *Proteus mirabilis* type of hemolysins, and belongs to the two-partner secretion (TPS) family of proteins (Brillard et al. 2002). Hemolysins target red blood cells to provide access to iron and may mediate the successful occupation of the different host environments (nematode and insect) it encounters during its life cycle. In case of *X. nematophila*, XhlA (**X**. nematophila **h**aemo**l**ysin) was observed to be necessary for full virulence against *M. sexta* larvae (Cowles and Goodrich-Blair 2005).

5.2.2 Insect Virulence Factors Produced by Xenorhabdus Species

Xenorhabdus species are motile gram-negative bacteria of the family Enterobacteriaceae that are mutualistic symbionts of the soil-dwelling nematodes from the family *Steinernematidae*. The life cycle of *Xenorhabdus* is similar to that described for *Photorhabdus*, with the *Steinernema* nematodes playing a key role in vectoring these bacteria from one host to another (Hinchliffe et al. 2010; Castagnola and Stock 2014). Although both types of bacteria are mutualists with nematodes and are entomopathogens, they use distinct, functionally different approaches for these roles (Poinar 1993; Griffin et al. 2001; Goodrich-Blair and Clarke 2007), suggesting that *Xenorhabdus* and *Photorhabdus* underwent divergent evolution that arrived at convergent lifestyles (Chaston et al. 2011). The ingestion of hemolymph was found to trigger the release of *Xenorhabdus* through the anus of nematode host, *S. carpocapsae*. Five species (*Xenorhabdus beddingii*, *Xenorhabdus bovienii*, *Xenorhabdus japonicus*, *Xenorhabdus nematophilus*, and *Xenorhabdus poinarii*) were recognized in the genus *Xenorhabdus* after initial reclassification, and a total of 15 new species have been identified from *Steinernema* nematode collections since then (Lengyel et al. 2005; Somvanshi et al. 2006; Tailliez et al. 2006).

Of these, the best studied nematode-bacterial associations are those of *X. nematophila*–*S. carpocapsae*, and it has been demonstrated that certain *Steinernema*–-*Xenorhabdus* associations are exclusive and non-cognate pairs will not associate during experimental mixing (Akhurst 1983; Sicard et al. 2004). The complete genomes of *X. nematophila* ATCC 19061 and *X. bovienii* SS-2004 have been sequenced (Chaston et al. 2011).

Xenorhabdus overcomes the insect's defense systems and produces an array of virulence factors (proteases, lipases, hemolysins, immunosuppressants, and toxins) that participate in suppressing insect immunity and killing the host (Forst and Nealson 1996). Two types of hemocytes (the granulocytes and plasmatocytes) comprise greater than 70 % of the cells found in the lepidopteran larval hemolymph (Gillespie et al. 1997). During *X. nematophila* infection, the overall numbers of circulating insect hemocytes are drastically reduced (da Silva et al. 2000). Two factors (C1 and C2) that are produced in liquid cultures and target the insect hemocytes have been identified in *Xenorhabdus* (Brillard et al. 2001). Two cytotoxins, the αX and Xax, having identical biological effects on insect hemocytes and associated with C1 factor have been characterized from *Xenorhabdus* (Ribeiro et al. 2003; Vigneux et al. 2007). The toxin complex genes (*xpt*) encoding high molecular weight insecticidal proteins have also been observed in *X. nematophila*. *Xenorhabdus xpt* genes exist on a pathogenicity island (PAI) like the *tc* genes of *Photorhabdus*, and it has been demonstrated that the PAIs of strains of *Xenorhabdus* are nearly identical, indicating that the presence of PAIs corresponds to either an evolutionary advantage or increased fitness (Sergeant et al. 2006).

5.2.2.1 The Toxin Complexes (Tc's) of *Xenorhabdus*

X. nematophila contains only a single *tc* locus encoding all three subunits with two [A] genes, *xptA1* and *xptA2*; a single [B] gene, *xptC*; and a single [C] gene, *xptB*. These show the greatest levels of identity to the *P. luminescens* genes tcdA, tcaC, and tccC, respectively (Hinchliffe et al. 2010). The native toxin complex (toxin complex 1) from *Xenorhabdus* is composed of three different proteins XptA2 [284 kDa], XptB1 [110 kDa], and XptC1 [158 kDa], representing class A, B, and C proteins that were found to interact in a 4:1:1 (XptA2:XptB1:XptC1) stoichiometry (Sheets et al. 2011), while *Xenorhabdus tc2* contains XptA1 [287 kDa], in addition to XptB1 and XptC1 where the two separate [A} genes, XptA1 and XptA2, have been shown to be responsible for different host species specificity within the *tc's* (Lee et al. 2007). XptA1 protein confers specificity toward *Pieris brassicae* and *Pieris rapae*, and the XptA2 protein confers specificity toward *H. virescens* (Sergeant et al. 2003). This indicates that these [A] subunits must interact with some kind of specific receptor in order for the complex to cause toxicity. The *tc* gene products can be categorized into toxins and potentiators. The potentiators synergize with their Tc toxin counterpart for full insecticidal activity (Waterfield et al. 2005a). The A component of Tc complexes has toxin activity potentiated by the [BC] components, and this has been demonstrated for the Tc's of *X. nematophila*

as well (Sergeant et al. 2003). All three components were found essential for the formation of a biologically active toxin complex. Although the Tc's have often been thought of as being possible pore-forming toxins, Lee et al. (2007) observed that purified XptA1 binds specifically to brush border membrane vesicles (BBMV) from *P. brassicae* and to *Sf*21 cells but does not form pores in the membranes. It is likely that the [A] tetramer alone cannot form pores, whilst a mature complex containing [BC] subunits can, thereby suggesting that [BC] is probably mediating the insertion of [A] into membranes and thus "potentiating" the toxicity of [A] (Hinchliffe et al. 2010).

5.2.2.2 Txp40 Toxin

Brown et al. (2004) described a novel 42 kDa toxin, A24tox, from *X. nematophila* strain A24 that had a lethal with effect on lepidopteran larvae such as *G. mellonella* and *H. armigera* when injected at doses of 30 to 40 ng/g larvae. Injection of the A24tox protein into lepidopteran larvae caused the larvae to cease feeding almost immediately, indicating that the midgut may be the primary site of action for the toxin. Detection, characterization and alignment of the *txp40* gene sequences from several strains of *Xenorhabdus* and *Photorhabdus* highlighted the conserved nature of the gene and its ubiquitous occurrence within this group. (Brown et al. 2006; Castagnola and Stock 2014). The insecticidal activity of the Txp40 toxin and the histopathology of larvae treated with the toxin are similar to those observed with the *Photorhabdus* Txp40 protein and discussed in detail in the Sect. 5.2.1.4.

5.2.2.3 Insecticidal Pilin Protein

All gram-negative bacterial pathogens have been shown to secrete their virulence factors enclosed in outer membrane vesicles (OMVs) (Beveridge 1999). The naturally secreted OMVs of *X. nematophilus* contained a number of proteins and showed larvicidal activity when they were incorporated into the diet of neonatal larvae of *H. armigera* (Khandelwal and Banerjee-Bhatnagar 2003). A 17 kDa pilin subunit protein present in the *X. nematophila* OMV was found to be cytotoxic to the cultured larval hemocytes of *H. armigera*, causing agglutination, and subsequent release of the cytoplasmic enzyme lactate dehydrogenase (Khandelwal et al. 2004). The 17 kDa pilin subunit demonstrated oral toxicity to the fourth or fifth instar larvae of *H. armigera* in a dose-dependent manner, causing the breakdown of the gut epithelial lining, thereby affecting the integrity of the cellular lining, resulting in the sloughing of the cell debris into the lumen (Khandelwal et al. 2004).

5.2.2.4 Insecticidal GroEL Protein

XnGroEL is a ~58 kDa OMV protein secreted by *X. nematophilus* that belongs to a highly conserved family of molecular chaperones and is required for the proper folding of cellular proteins. XnGroEL has chitin-binding property and interacts with the larval peritrophic lining, and oral ingestion of this protein caused inhibition of the growth and development of *H. armigera* larvae. While all three domains (apical, intermediate, and equatorial) of the protein were found to be necessary for optimal insecticidal activity, two surface-exposed residues Thr-347 and Ser-356 in the apical domain were found to be vitally important for binding to the gut epithelium and insect-toxicity (Joshi et al. 2008). The oral toxicity of XnGroEL against *H. armigera*, evaluated by transgenic expression of the protein in tobacco, showed 100 % reduction in the larval survival on transgenic plants (Kumari et al. 2014).

5.2.2.5 *Xenorhabdus* Alpha-Xenorhabdolysin (Xax) Toxin

The XaxAB cytotoxin produced by *X. nematophila* is encoded by two genes *xaxA* and *xaxB* and appears to be the prototype of a new family of binary toxins based on molecular characterization of the locus, gene and amino acid sequences. Xax triggers apoptosis in both insect (*Spodoptera littoralis*) hemocytes and mammalian cells (Vigneux et al. 2007). Active protein was produced when the two genes were expressed in recombinant *E. coli*. However optimum hemolytic activity was observed when these proteins were added to cells in vitro in a specific order (Xax A and then Xax B) and at equal concentrations. *xax* genes A and B were found to be present in the genome sequences from various bacterial pathogens of insects (*Xenorhabdus*, *Photorhabdus*, *Pseudomonas entomophila*), plants (*Pseudomonas syringae*), and humans (*Yersinia enterocolitica* and *P. mirabilis*) (Vigneux et al. 2007).

5.2.3 *Insect Virulence Factors Produced by Pseudomonas Species*

Pseudomonas spp. (Enterobacteriaceae) are metabolically versatile gram-negative bacteria that are ubiquitous in their distribution and have been recovered from a wide variety of ecological niches, including soil and plants (Vodovar et al. 2006). The root-associated bacteria of the genus *Pseudomonas* exhibiting inhibitory activity toward fungal plant pathogens have been extensively used for crop protection. The insecticidal properties of *P. entomophila*, *P. syringae*, and *Pseudomonas fluorescens* were discovered thereafter (Castagnola and Stock 2014).

P. entomophila is highly pathogenic to *Drosophila melanogaster* and also exhibits considerable insecticidal potency against other insects (e.g., *Bombyx mori*, *Anopheles gambiae*) upon ingestion. The precise mechanism of death remains unclear, but the invading bacteria were found to be resistant to the immune response triggered in the insect after oral ingestion (Vodovar et al. 2005). It is speculated that *P. entomophila* evades the insect immune response by making proteases (alkaline protease AprA) and exotoxins (hemolysins and lipases) that punch holes in the cell membranes of the insect phagocytes. *P. entomophila* genome encodes several gut-specific toxin complexes (Tc's) with multiple copies of C-like elements and one copy of a B-like gene that are not organized as a single operon but are scattered across the genome. The genome lacks a gene encoding an A toxin which determines the host range, suggesting that the insecticidal activities of toxin complexes from *P. entomophila* may be fairly restricted. *P. entomophila* genome encodes the apparatus required to produce hydrogen cyanide, the precise role of which in bacterial biology remains to be established (ffrench-Constant and Waterfield 2006). *P. entomophila* has a large repertoire of potential virulence factors such as insecticidal toxins, proteases, putative hemolysins, hydrogen cyanide, and novel secondary metabolites that are envisaged to be important for virulence toward insects. The two-component regulatory system GacS/GacA was observed to play a key role in *P. entomophila* pathogenicity by regulating the expression of many virulence factors (Vallet-Gely et al. 2010).

P. fluorescens produces a proteinaceous insecticidal toxin Fit that demonstrated hemocoel-based toxicity in *M. sexta* and *G. mellonella* (Pechy-Tarr et al. 2008) and caused complete loss of turgor pressure and melanization. Fit has been shown to be orally toxic to *S. littoralis*, *H. virescens*, and *P. xylostella*. *P. chlororaphis* also expresses a Fit toxin and has oral insecticidal activity (Ruffner et al. 2013). Certain strains of *P. fluorescens* contain genes encoding δ-endotoxins, Mcf toxins, *tc* genes encoding B and C components only, lipases, and exotoxins with hemolytic activity. The genome of *P. syringae pv. syringae* contains an intact toxin complex encoding ABC complement, suggesting that this species which was formerly thought to be only plant pathogenic may have an association with insects (ffrench-Constant and Waterfield 2006).

5.2.4 Insect Virulence Factors Produced by Serratia Species

Serratia spp. (Enterobacteriaceae) are commonly isolated from grassland soils, and they often exist as endophytic rhizobacteria possessing antifungal activity. However, several species within the genus *Serratia* are often found associated with insects of many orders (Grimont and Grimont 1978; Lamelas et al. 2011) and nematodes (Rae et al. 2008; Abebe et al. 2011) in a facultative manner. *S. plymuthica*, isolated from the intestine of *Neombius fasciatus* (Steinhaus 1941) caused no infection in the insect host; however, *S. marcescens* and *S. liquefaciens* were regarded as facultative pathogens. *S. marcescens* was found to infect lepidopteran hosts such as poorly reared *H. virescens* (Sikorowski et al. 2001). Contrarily,

S. entomophila and *S. proteamaculans*, the causal agents of amber disease of the New Zealand grass grub, *Costelytra zealandica* (Coleoptera: Scarabaeidae), are considered as true entomopathogenic bacteria (Jackson et al. 2001).

S. entomophila and *S. proteamaculans* colonize and propagate in the grass grub crop; consequently, the infected larvae cease to feed, clear their gut, and become amber-colored (Jackson et al. 1993; Jackson 1995). Eventually the bacteria invade the hemolymph, causing general septicemia and death (Nuñez-Valdez et al. 2008). The pathogenicity determinants in *S. entomophila* are encoded at two regions on a 153-kb plasmid designated as pADAP (amber disease-associated plasmid). The *afp* (anti-feeding prophage) gene cluster, encoding an R-type pyocin structure, mediates the transport of toxins to a target site and causes a cessation of feeding by the grass grub larvae (Hurst et al. 2004; 2007a). The *sep* virulence-associated region which comprises of three genes designated as *sepA* (tcdA-like), *sepB* (tcdB-like), and *sepC* (tccC-like) mediate for the amber disease symptoms of gut clearance and amber coloration of the larvae (Hurst et al. 2000). Both the *sep* genes and *afp* are needed for full virulence of *Serratia* in grass grubs. Amber disease was found to be chronic in nature and despite widespread testing; no other insect species have been shown to be susceptible to *S. entomophila*. The host-specific nature of insecticidal proteins from *Serratia* therefore limits their use in crop protection as these proteins can be deployed against only specific species of pest insects. The *sepABC* genes show homology to the components of the Tc proteins from *P. luminescens* (Bowen et al. 1998) and *xpt* genes identified from *X. nematophilus* (Morgan et al. 2001). However, while Tc toxins have shown cytotoxic effects, direct toxicity of the Sep proteins is unknown. Sep gene orthologues were found to be plasmid-borne in *S. entomophila*, *S. proteamaculans*, *S. liquefaciens*, and *Yersinia frederiksenii*. The *sepA* and *sepB* genes of *S. entomophila* show high nucleotide identity to *Y. frederiksenii* genes thus suggesting a horizontal gene transfer between the two species (Dodd et al. 2006).

5.2.5 *Insect Virulence Factors Produced by* Yersinia *Species*

Members of the genus *Yersinia* (Enterobacteriaceae) have undergone extensive diversification during the course of their evolution and are represented by pathogenic species such as *Y. pestis* and *Yersinia ruckeri*, the causative agent of bubonic plague (Perry and Fetherston 1997) and the causative agent of enteric redmouth disease in salmonid fish, respectively (Ewing et al. 1978), while other species (e.g., *Y. aldovae*) have diverged into nonpathogenic organisms (Sulakvelidze 2000). *Y. entomophaga* is a non-sporulating entomopathogenic bacterium that was isolated from diseased larvae of the New Zealand grass grub, *Costelytra zealandica* White (Coleoptera: Scarabaeidae) (Hurst et al. 2011a). The pathogenicity island of *Y. entomophaga*, termed PAI^{Ye96}, is composed of the multi-subunit toxin complex (Yen-Tc) showing homology with toxin complexes produced by *Photorhabdus* spp.

and insecticidal chitinases. The Tc complex includes three protein families termed A (YenA1, Yen A2), B (YenB), and C (YenC1, YenC2) and two chitinases (Chi1 and Chi2) with high endochitinase activity (Hurst et al. 2011b). The 3D structures of the Tc complex showed that subunits YenA1 and YenA2 form the basis of a fivefold symmetric assembly, while subunits B and C form a surface accessible region and are the main toxicity determinants. The structure of the chitinases that adorn the surface of the TcA scaffold has been analyzed and different hypothesis have been proposed to explain their role in mediating Tc toxicity (Landsberg et al. 2011; Busby et al. 2012). Tc protein complex from *Y. entomophaga* exhibits broad host range oral insecticidal activity, causing rapid mortality in many insect pests belonging to the orders Coleoptera, Lepidoptera, and Orthoptera. The culture supernatants of *Y. entomophaga* were found to be toxic to a variety of coleopteran species, including the New Zealand grass grub, *C. zealandica* (Coleoptera: Scarabaeidae); the redheaded cockchafer, *Adoryphorus couloni* (Coleoptera: Scarabaeidae); the blackheaded pasture cockchafer, *Acrossidius tasmaniae* (Coleoptera: Scarabaeidae); and the diamondback moth, *P. xylostella* (Lepidoptera: Plutellidae) (Hurst et al. 2011b; Castagnola and Stock 2014). In *P. xylostella*, initial apical swelling of gut columnar cells occurred after ingestion of purified Tc from *Y. entomophaga*, followed by complete dissolution of the gut lining (Hurst et al. 2011b). The orally active nature and the broad-spectrum insecticidal activity of the Tc protein derived from *Yersinia* species indicates that it may be a potential alternative to *B. thuringiensis* toxins for use in insect control (Bravo and Soberon 2008).

5.3 Toxins Shared by Gram-Negative Bacterial Pathogens of Invertebrates: New Insights from the Comparative Genomics of Entomopathogens

The proliferation of genomic information of invertebrate pathogens (*P. luminescens*, *X. nematophilus*, *P. entomophila*, etc.) and functional analysis of genome data has revealed that the composition of bacterial genomes is dynamic and susceptible to many changes through the process of genome reduction (Moran 2002), gene duplication and divergence (Ohno 1970), vertical inheritance (Woese 1987), and horizontal gene transfer (Ochman et al. 2000) that occur due to multiple pressures, including the environment, mutation, and competition (Chaston et al. 2011). Therefore, comparative genomics will provide an excellent opportunity to elucidate the genetic similarities and differences in different species that will have an impact on the innovations in crop protection technologies, in addition to providing a fundamental understanding of evolutionary relationships and changes contributing to pathogenesis in bacteria. Bacterial genome can be subdivided into “core genome” and “flexible genome.” Core genome of bacterial communities is common to all bacterial strains in a defined set of species and contributes to basic cellular functions.

The genomic regions variably present between individual strains constitute "flexible genome" component, and these regions are organized principally into polymorphic strain-specific segments called regions of genome plasticity (RGPs) that play an important role in bacterial adaptation to special growth conditions, such as those involved in the colonization of new ecological niches, symbiosis, host-cell interaction, and pathogenicity. Flexible gene pools act as a site for inter-genomic and intra-genomic rearrangements (Frost et al. 2005; Gaudriault et al. 2008). RGPs (underlining the continuous gene transfer among the bacterial genomes), insertion sequences (IS), putative transposons, and the presence of phage remnants are the key indicators of the transfer of genetic elements among different microbes, especially in the bacterial genomes. The flexible genome of the *Xenorhabdus* and *Photorhabdus* genera accounted for 52.6 to 61.5 % of the entire genome, and this region was found to be larger in *Photorhabdus* than in *Xenorhabdus* (Ogier et al. 2010).

A comparison between entomopathogenic bacteria in the genera *Photorhabdus* and *Xenorhabdus* revealed that despite their similar lifestyles the species within the two genera use functionally different approaches to achieve successful host interactions. *Photorhabdus* spp. encode a dedicated type three secretion system (TTSS) which can suppress phagocytosis and nodule formation by injection of effectors, such as LopT and SctC directly into hemocyte cells (Brugirard-Ricaud et al. 2004; Brugirard-Ricaud et al. 2005). *Xenorhabdus* spp. lack a TTSS and associated effectors; however, they do encode several other cytotoxic strategies in order to evade the host cellular responses (Hinchliffe et al. 2010). The TTSS of *Photorhabdus* is highly similar to the plasmid encoded system of *Y. pestis* (Wolters et al. 2013). However, in *Yersinia*, the effector protein YopT is a cytotoxic cysteine protease, whereas the homolog in *P. luminescens*, called LopT, has been shown to prevent phagocytosis (Brugirard-Ricaud et al. 2004).

Insecticidal toxin complexes (Tc's) were first identified in *P. luminescens* and have been studied extensively by independent research groups (Bowen et al. 1998; ffrench-Constant et al. 2000, 2003). Tc toxins were subsequently identified in the genomes of other gram-negative insect pathogens such as *X. nematophila* (Morgan et al. 2001), *S. entomophila* (Hurst et al. 2007b), and *Y. entomophaga* (Hurst et al. 2011b) and have even been reported in the gram-positive *Paenibacillus* (Hinchliffe et al. 2010). The genome of *Y. pestis* contains a locus encoding the Tc protein homologues *yitA* (TcaA-like), *yitB* (TcaB-like), and *yitC* (TcaC-like) and *YipA* and *YipB* (two TccC-like proteins). The *tcaB* and *tcaC* genes of *Y. pestis* contain a frame shift mutation and internal deletion, respectively, which is indicative of a loss of function (Parkhill et al. 2001; Spinner et al. 2012). The nomenclature of the Tc proteins has been revised (ffrench-Constant and Waterfield 2005), and the ABC designation is adopted currently to describe the components of the Tc complex. The Tc-Bs and Tc-Cs are known to make the Tc-As more toxic. The *tc*-like genes identified in *P. luminescens*, *X. nematophila*, and *Y. pestis* are chromosomally borne, while the *sep* genes of *S. entomophila* and *tc*-like genes of *Y. frederiksenii* strain 49 are plasmid-borne. A toxin-encoding operon similar to the *tca* of *P. luminescens* W14 was found to be present in an isolate of *Bt* (*Bt*-IBL200)

from the Invasive Insect Biocontrol and Behavior Laboratory (IIBBL, Beltsville, MD, USA) (Blackburn et al. 2011). All three components of *tc* (A, B, and C) were present in the *Bt* IBL200 isolate. The genomic organization and diversity of Tc proteins among different species of insect-pathogenic bacteria increases the likelihood of tc gene transfer between species via plasmids, and suggests that the chromosomally located *tc* genes could also once have been plasmid-borne or mobile in other bacteria (Dodd et al. 2006). Tc toxins are active against different tissues within individual hosts, namely, Tcb against hemocytes and Tcd and Tca against cells of the insect gut. The Tc toxins reside as multiple but dissimilar orthologues throughout the *P. luminescens* TT01 genome with different insecticidal activities attributed to a different Tc cluster (Duchaud et al. 2003; Hey et al. 2006). Plasmid-borne Sep proteins of *S. entomophila* are host-specific. The *xpt* genes of *Xenorhabdus* exist on a PAI like the *tc* genes of *Photorhabdus*; however, PAIs are nearly identical in *Xenorhabdus*. The insecticidal genes *tcdB1*, *yitC*, and *spvB* of *P. luminescens*, *Y. pestis*, and *Salmonella*, respectively, have regions of homology, viz., the N-terminal 367 amino acids of *yitC* are similar to the N-terminus of the putative effector *spvB* of *Salmonella* (Browne et al. 2002; Castagnola and Stock 2014). The *Yersinia* spp. contain islands harboring insecticidal *tc*-like genes; however, insecticidal activity was observed only when a low-growth temperature was used to culture the bacteria and produce a protein extract (Bresolin et al. 2006).

Chitinases having antimycotic activity have been found in both *Xenorhabdus* and *Photorhabdus* (Chen et al. 1996). Interestingly, the assembled Tc toxin of *Y. entomophaga* was found to have endochitinase activity, which was attributed to putative chitinase subunits associated with TcA scaffold. This has not previously been reported in a Tc (Hurst et al. 2011b; Landsberg et al. 2011). *S. marcescens* was found to produce orally active insecticidal toxins and chitinases (Jeong et al. 2010; Brurberg et al. 1996). *S. marcescens* cultures and *Bt* Cry1C toxin exhibited a synergistic insecticidal effect against *S. litura* (Asano et al. 1999).

The apoptotic binary toxin Xax is found in *P. luminescens* and *X. nematophila*. *X. nematophila* XaxA and XaxB showed the strongest similarity to *plu3075* (61 %) and *plu1961* (56 %), respectively, from *P. luminescens*. The putative hemolysin loci, containing two closely linked genes, *xaxA* and *xaxB*, are found together in genome sequences from various bacterial pathogens of plants (*P. syringae*), insects (*Photorhabdus*, *Xenorhabdus*, *P. entomophila*), and humans (*P. mirabilis*, *Y. enterocolitica*). The *xaxAB* homologues in *X. nematophila* are found in a unique genomic context that does not show characteristic features of genome flexibility, such as genomic islands, transposon-related structures, or phages (Vigneux et al. 2007). Interestingly, the *xax* hemolysin locus was found to be present in *Y. enterocolitica* and not in *Y. pestis* even though the latter, like *X. nematophila*, spends part of its life cycle in an insect.

The *mcf* (makes caterpillar floppy) gene, which encodes a large proapoptotic multidomain protein, is present in *P. luminescens*, *P. temperata*, and *P. asymbiotica* (Daborn et al. 2002; Forst and Goodner 2006). Two *mcf* paralogous genes (*mcf1* and *mcf2*) are found in the strains Pl W14 and Pl TT01. Genome sequencing has revealed the presence of other Mcf-like proteins in other bacterial species. The *FitD* gene of *P. fluorescens* encodes a Mcf1-like protein and shows 73.5 % identity

to *P. luminescens mcf1* (Pechy-Tarr et al. 2008). The *fitD* locus of *P. fluorescens* is associated with a TolC-family outer membrane efflux protein (*fitE*), two response regulators (*fitF*, *fitH*), and a LysR-like regulator (fitG). However, paralogues of *fitE–H* are not present in *Photorhabdus* and may be involved in the specific regulation of *fitD* in *P. fluorescens* (Hinchliffe et al. 2010).

RTX, the repeats in toxin family possessing different enzymatic activities including cytolytic, protease, or lipase activity are observed to be dramatically expanded in *P. luminescens* (eight *rtxA* genes), but this gene has not been found in *Y. pestis*. Four of the eight *rtxA* genes of *P. luminescens* were disrupted by either insertion sequence (IS) elements or inactivated by frameshift mutations. An RTX-like alkaline protease has recently been isolated from *P. luminescens* W14 and *P. temperata* (Bowen et al. 2003; Forst and Goodner 2006). Phage-related loci are found in both *Serratia* and *Photorhabdus*. The PVCs of *Photorhabdus* are homologous to a prophage-like locus on the pADAP plasmid of *S. entomophila* (Yang et al. 2006). The PVCs have injectable toxicity toward *G. mellonella* hemocytes, whereas the pADAP locus has been associated with anti-feeding effects (Hurst et al. 2004). The *txp40* gene, encoding a 42 kDa protein with injectable cytotoxic activity, was identified in several strains of *Xenorhabdus* and *Photorhabdus*, indicating that it is both highly conserved and widespread among these bacteria (Brown et al. 2006). Proteins similar to the δ-endotoxin from *B. thuringiensis* have also been identified in *P. luminescens*. The *pir* gene of *Photorhabdus* shows a 30 % amino acid sequence similarity to part of the insecticidal crystal protein of *B. thuringiensis* (Li et al. 2009).

The genome of *P. luminescens* was found to be ~1 Mb larger than closely related bacteria such as *Xenorhabdus* and *Yersinia spp*. The average genome size of most members of the Enterobacteriaceae family is approximately 4.6 Mb (Forst and Goodner 2006). The emerging human pathogen *P. asymbiotica* has a smaller genome than that of *P. luminescens* genome. A reduction in the genome size of *P. asymbiotica* was found to coincide with a reduction in different classes of anti-insect virulence factors. Unlike *P.luminescens* strains, the *P. asymbiotica* strains carry a plasmid related to pMT1 from *Y. pestis* that promotes deep tissue invasion, and several PAIs including a novel TTSS; these features suggested that human pathogenicity in *P. asymbiotica* was acquired through the acquisition of pMT1-like plasmid and specific effectors. Despite these molecular changes, the pathogenicity toward insects was found to remain intact in *P. asymbiotica* illustrating a lifestyle superior to *P. luminescens*, which is pathogenic only to insects (Wilkinson et al. 2009).

Comparative genome analysis, gene profiling, functional genomics, and the newly developed genetic approaches like microarrays and software tools like RGP finder will be helpful for several unresolved, mechanistic and evolutionary questions about members of soil bacteria in future. Whole-genome sequencing approaches and comprehensive analysis undertaken by the rapid virulence annotation (RVA) technique (Waterfield et al. 2008) have begun to reveal previously unidentified insecticidal toxins, uncharacterized secondary metabolites possessing toxic activities, putative lipases, and type VI secretion systems with insecticidal activity. Thus, a significant challenge of comparative genomics is to interpret the interrelationships between anti-invertebrate and anti-vertebrate virulence factors

and assign biological roles to the candidate virulence factors (Hinchliffe et al. 2010).

5.4 Strategies for Enhancing Transgenic Resistance to Lepidopteran Pests: A Dynamic Landscape

Undoubtedly, *B. thuringiensis* and its insecticidal toxins have been overwhelmingly successful for agronomical pest control for decades. Transgenic crops protected from the damage of lepidopteran and coleopteran insect feeding express insecticidal proteins derived from the entomopathogenic bacteria, *B. thuringiensis* (Huesing and English 2004). The first generation of genetically modified (GM) crops conferring insect protection has been extraordinarily successful, and GM crops are considered as the fastest adopted crop technology in the history of modern agriculture (James 2012). Insect-resistant products expressing *Bt*-derived proteins have been available for corn and cotton since 1996. *Bt* cotton, in particular, has provided effective control of several lepidopteran pest species including tobacco budworm, *H. virescens* F. (Lepidoptera: Noctuidae); pink bollworm, *P. gossypiella* Saunders (Lepidoptera: Gelechiidae); the cotton bollworm, *H. armigera* Hübner (Lepidoptera: Noctuidae); and *Spodoptera* spp. (Lepidoptera: Noctuidae) (Sanahuja et al. 2011; Baum et al. 2012). The second-generation GM varieties generated by stacking and pyramiding resistance genes were also approved for commercialization (Marra et al. 2010). *Bt* cotton planted in more than 18.8 million hectares in 13 countries was the third most dominant crop grown in 2012, which is equivalent to 11 % of the global biotech area (James 2012). Despite the commercial success of the *Bt* technology, there are concerns over the development of resistance by insect species, the problem of efficacy, and narrow spectrum of activity of *Bt* proteins (de Maagd et al. 2001; Pereira et al. 2008; Sayyed et al. 2008). Therefore, efforts are ongoing for the discovery of potent *Bt* strains expressing novel toxins with improved and broad-spectrum activity as well as for the characterization of genes exhibiting biopesticidal properties from other entomopathogens (Christou et al. 2006; Crickmore 2006).

Whole-genome sequencing and screening of genomes of soil-dwelling gram-negative entomopathogenic bacteria and gram-negative bacterial symbionts of soil-dwelling EPNs has yielded a gold mine of potential novel insect toxins that augment a growing list of candidates for use in crop protection (Hinchliffe et al. 2010). The insect virulence factors from gram-negative bacteria that can be used as biocontrol agents against Lepidoptera include a host of insecticidal toxins, proteases, putative hemolysins, and other previously unidentified proteins. Although a surge in the patent applications has been observed with the discovery, functional annotation, and insecticidal efficacy evaluation of novel genes/proteins, data on how far many of these potential candidates have progressed toward commercial field applications is ambiguous (Hinchliffe et al. 2010).

Photorhabdus, *Xenorhabdus*, and other gram-negative entomopathogens have provided novel candidates having both oral and hemolymph-based toxic activity. Proteins that are active upon ingestion and damage the insect midgut are good candidates for developing transgenic crops. The "toxin complexes" are a prime candidate fulfilling these criteria. However, the orally toxic nature of the Tc proteins produced by an insect pathogen that is directly delivered into the insect hemolymph is intriguing. The presence of tc genes in many organisms which are not directly delivered into the insect hemolymph may somewhat explain the oral toxicity of Tc proteins (Castagnola and Stock 2014). The histopathological effects on lepidopteran larvae of toxins that include Tc proteins, the *mcf* gene product, the PirAB binary toxins, the 17 kDa *pilin* subunit, and the Txp40 proteins from *Photorhabdus* and *Xenorhabdus* demonstrated a distinct damage to the midgut. The *tc* genes have been studied extensively by researchers in academia and industry and were considered suitable for commercial product development. One of the Tc proteins, the *tcdA* protein expressed in *A. thaliana* to sufficient levels, was found to be toxic to *M. sexta* (Liu et al. 2003). The demonstration that the large toxin genes such as *tc* can be engineered and expressed in transgenic plants makes them suitable alternative toxins to *B. thuringiensis* (Schnepf et al. 1998). Application of Pir toxins in insect control may be limited, as they were found to be effective only against the diamondback moth, *P. xylostella*. The limited activity spectrum and the possible relation of the Pir toxins to the leptinotarsins may indicate problems with vertebrate toxicity. Toxins like Mcf which act on both the gut and insect immune system represent a promising, yet underexploited avenue for the development of insect-resistant crops in the future (Daborn et al. 2002). It can be speculated that there are many more toxins yet to be functionally characterized from the *Photorhabdus* genome which are responsible for hemolymph-based toxicity.

Stacking virulence factors like Mcf which have hemolymph-based toxicity with conventional orally active toxins would expand their application to IPM. However, only those hemolymph-based toxins which are toxic upon ingestion as well could be used in this case, as these proteins have to be stable in the insect gut. Alternatively, two proteins with different modes of action and diverse targets can also be used in developing novel combinations of genes for pest control, as it has the advantage of reducing the development of insect resistance (Gould 1998). Site-directed mutagenesis and domain-engineering have great potential to alter toxin-encoding genes particularly when sufficient structural information is available, these methods can thus be applied to produce novel recombinant toxins (Gatehouse 2008). Using a combination of insect-toxic genes derived from *B. thuringiensis* and gram-negative entomopathogens can also result in synergistic insecticidal activity (Asano et al. 1999).

The ability of *P. entomophila* to orally infect and kill larvae of insect species belonging to different orders makes it a promising model for the study of host–pathogen interactions and for the development of biocontrol agents against insect pests. *S. entomophila* was developed as a biopesticide and used for 15 years as a commercial product for grass grub control in New Zealand. This microbe was initially developed and applied as a liquid biopesticide Invade®, which is New Zealand's first registered, safety-tested, indigenous biological pesticide and

the first microbial control agent in the world to be based on a member of the gram-negative Enterobacteriaceae (Jackson et al. 1992). The pathogenic bacterium has recently been developed for application as a solid granule formulation, Bioshield™ (Young et al. 2010).

The important attributes of an insecticidal protein for successful commercial use include its efficacy and specificity. First and foremost the insecticidal toxin must be highly toxic to a wide range of potential pests, and it has to prevent crop damage by efficiently killing or deterring the insect pests. Delivery of toxic proteins to potential pests is either through their host plants via the expression of the toxin, or toxic subunit/domain, in transgenic crops or developing a pesticide formulation and coating them onto the crops in a stable form (Hinchliffe et al. 2010; Ruiu et al. 2013). Due to specific biological properties and technical reasons such as the specific mode of action (oral or injectable activity), target site of action, and stability, commercially available strains including *Bt* have their restrictions in terms of performance in the field. The toxin protein has to be orally active for developing insect-resistant GM crops. Although "toxin complex" proteins described in different gram-negative entomopathogens are orally active, the Tc's consist of large protein subunits which are inherently difficult to express transgenically in crop plants. Therefore, only individual proteins having limited toxicity can be expressed with the technology available currently. As both toxins and potentiators have to be co-expressed in order to harness the full toxic potential of *tc* genes, either the proteins have to be cropped down to smaller active domains or the transgenic technology has to be improved to allow all subunits to be expressed to achieve the desired level of efficacy (Hinchliffe et al. 2010). Transgenic expression of ingestible insecticidal proteins confers certain degree of pest specificity as only insects actually feeding on the crop will ingest the toxin directly. The toxicity of insecticidal Tc proteins of *P. luminescens* to cultured mammalian cells may attract criticism particularly with regard to their use in crop protection and the associated concerns on biosafety (Waterfield et al. 2005a; Hares et al. 2008). The bioecological compatibility of *P. luminescens* biopesticide against two species of the beneficial insect *Trichogramma* was investigated by Mohan and Sabir (2005). Their study demonstrated that there was a significant reduction of up to 84 % in the emergence of *Trichogramma* adults from the host, *Corcyra cephalonica* eggs, as 65 % of the eggs exposed to either *P. luminescens* cells or their toxins became flaccid. In some species of pathogenic yersiniae viz., *Yersinia pseudotuberculosis* and *Yersinia pestis* the *tc* genes are not insecticidal but have evolved to show mammalian pathogenicity. These data suggest that the biological activity of the toxins against target species and nontarget species should be thoroughly investigated before being considered for crop protection. However, appropriate evaluation of the effects of the toxin on the target pests should be undertaken so that the protein is active toward the target species and does not cause any harm to the non-pest species (beneficial insects, bystander insect species, predatory species which feed off the intoxicated pests, humans) upon exposure.

5.5 Conclusion and Perspectives

Insecticidal toxins are an important option for the biological control of lepidopteran insect pests. Their use in genetic engineering of plants could provide a new generation of insect-resistant crops that can help in maintaining crop yields. A majority of the toxic proteins expressed by gram-negative bacteria have been tested only against model insects (*M. sexta*, *G. mellonella*, *S. litura*, *P. xylostella*, *H. armigera*), and besides few reports on mammalian toxicity, there is very limited information on the effects of these proteins on beneficial fauna (predators, parasitoids, and pollinators). Therefore, a significant research need is centered on understanding the specific effects of the insecticidal proteins, their activity spectrum, and their effect on nontarget organisms in the ecological sphere.

Insect pest control has entered the genomic era with the recent sequencing and functional analysis of the genomes of agricultural pests and entomopathogenic bacteria. This has enabled the discovery of novel targets in the pests and novel proteins in the entomopathogens and has provided a comprehensive understanding of invertebrate pathology by providing critical insights into evolutionary patterns of bacterial pathogens. Genome analysis has also raised several pertinent questions about the complex life cycles of these pathogens and their association with various invertebrate hosts and vectors. Ultimately a relevant challenge of comparative genomics is to understand the interrelationships between pathogenic mechanisms targeted to invertebrates and vertebrates as such insight may help us understand the evolution and the probable invertebrate origins, of emerging human pathogens, in addition to using the insecticidal genes derived from these bacteria for large-scale, commercial agricultural applications.

References

Abebe E, Abebe-Akele F, Morrison J (2011) An insect pathogenic symbiosis between a *caenorhabditis* and *serratia*. Virulence 2:158–161

Akhurst RJ (1982) Antibiotic activity of *Xenorhabdus* spp, bacteria symbiotically associated with insect pathogenic nematodes of the families *Heterorhabditidae* and Steinernematidae. J Gen Microbiol 128:3061–3065

Akhurst RJ (1983) Taxonomic study of *Xenorhabdus,* a genus of bacteria symbiotically associated with insect pathogenic nematodes. Int J Syst Bacteriol 33:38–45

Asano S, Suzuki K, Hori H (1999) Synergistic effects of the supernatants from *Serratia marcescens* culture on larvicidal activity of *Bacillus thuringiensis* Cry1C toxin against common cutworm, *Spodoptera litura*. J Pestic Sci 24:44–48

Baum JA, Sukuru UR, Penn SR, Meyer SE, Subbarao S, Shi X, Flasinski S, Heck GR, Brown RS, Clark TL (2012) Cotton plants expressing a hemipteran-active *Bacillus thuringiensis* crystal protein impact the development and survival of *Lygus hesperus* (Hemiptera: Miridae) nymphs. J Econ Entomol 105:616–624

Bedding RA, Akhurst RJ, Kaya HK (1993) Nematodes and the biological control of insect pests. CSIRO Publications, Melbourne, p 178

Beveridge TJ (1999) Structures of gram-negative cell walls and their derived membrane vesicles. J Bacteriol 181:4725–4733

Blackburn M, Golubeva E, Bowen D, ffrench-Constant RH (1998) A novel insecticidal toxin from *Photorhabdus luminescens*, Toxin Complex a (Tca), and its histopathological effects on the midgut of *Manduca sexta*. Appl Environ Microbiol 64:3036–3041

Blackburn MB, Farrar RR, Novak NG (2006) Remarkable susceptibility to the diamondback moth (*Plutella xylostella*) to ingestion of Pir toxins from *Photorhabdus luminescens*. Entomol Exp Appl 121:31–37

Blackburn MB, Martin PA, Kuhar D, Farrar RR Jr, Gundersen-Rindal DE (2011) The occurrence of *Photorhabdus*-like toxin complexes in *Bacillus thuringiensis*. PLoS One 6. doi:10.1371/journal.pone.0018122

Bonning BC, Hammock BD (1996) Development of recombinant baculoviruses for insect control. Annu Rev Entomol 41:191–210

Bowen DJ, Ensign JC (1998) Purification and characterization of a high-molecular-weight insecticidal protein complex produced by the entomopathogenic bacterium *Photorhabdus luminescens*. Appl Environ Microbiol 64:3029–3035

Bowen D, Rocheleau TA, Blackburn M, Andreev O, Golubeva E, Bhartia R, ffrench-Constant RH (1998) Insecticidal toxins from the bacterium *Photorhabdus luminescens*. Science 280: 2129–2132

Bowen D, Rocheleau TA, Grutzmacher CK, Meslet L, Valens LM, Marble D, Dowling D, ffrench-Constant RH, Blight MA (2003) Genetic and biochemical characterization of PrtA, an RTX-like metalloprotease from *Photorhabdus*. Microbiology 149:1581–1591

Bravo A, Soberon M (2008) How to cope with insect resistance to Bt toxins? Trends Biotechnol 26:573–579

Bresolin G, Morgan JA, Illgen D (2006) Low temperature-induced insecticidal activity of *Yersinia enterocolitica*. Mol Microbiol 59:503–512

Brillard J, Ribeiro C, Boemare N, Brehélin M, Givaudan A (2001) Two distinct hemolytic activities in *Xenorhabdus nematophila* are active against immunocompetent insect cells. Appl Environ Microbiol 67:2515–2525

Brillard J, Duchaud E, Boemare N, Kunst F, Givaudan A (2002) The PhlA hemolysin from the entomopathogenic bacterium *Photorhabdus luminescens* belongs to the two-partner secretion family of hemolysins. J Bacteriol 184:3871–3878

Brooke E, Hines E (1999) Viral biopesticides for *Heliothine* control-fact of fiction? Today's Life Sci 11:38–45

Brown SE, Cao AT, Hines ER, Akhurst RJ, East PD (2004) A novel secreted protein toxin from the insect pathogenic bacterium *Xenorhabdus nematophila*. J Biol Chem 279:14595–14601

Brown SE, Cao AT, Dobson P, Hines ER, Akhurst RJ, East PD (2006) Txp40, a ubiquitous insecticidal toxin protein from *Xenorhabdus* and *Photorhabdus* bacteria. Appl Environ Microbiol 72:1653–1662

Browne SH, Lesnick ML, Guiney DG (2002) Genetic requirements for salmonella-induced cytopathology in human monocyte-derived macrophages. Infect Immun 70:7126–7135

Brugirard-Ricaud K, Givaudan A, Parkhill J, Boemare N, Zumbihl R, Duchaud E (2004) Variation in the effectors of the *Photorhabdus* type III secretion system among species revealed by genomic analysis. J Bacteriol 186:4376–4381

Brugirard-Ricaud K, Duchaud E, Givaudan A, Girard PA, Kunst F, Boemare N, Brehelin M, Zumbihl R (2005) Site-specific antiphagocytic function of the *Photorhabdus luminescens* type III secretion system during insect colonization. Cell Microbiol 7:363–371

Brurberg MB, Nes IF, Eijsink VG (1996) Comparative studies of chitinases A and B from *Serratia marcescens*. Microbiology 142:1581–1589

Budd RC (2001) Activation-induced cell death. Curr Opin Immunol 13:356–362

Buetow L, Flatau G, Chiu K, Boquet P, Ghosh P (2001) Structure of the Rho-activating domain of *Escherichia coli* cytotoxic necrotizing factor 1. Nat Struct Biol 8:584–588

Busby JN, Landsberg MJ, Simpson R, Jones SA, Hankamer B, Hurst MRH, Lott JS (2012) Structural analysis of Chi1 chitinase from Yen-Tc: The multisubunit insecticidal ABC toxin complex of *Yersinia entomophaga*. J Mol Biol 415:359–371

Carrière Y, Ellers-Kirk C, Sisterson M, Antilla L, Whitlow M, Dennehy TJ, Tabashnik BE (2003) Long-term regional suppression of pink bollworm by *Bacillus thuringiensis* cotton. Proc Natl Acad Sci USA 100:1519–1523

Carson R (1962) Silent spring. Houghton, Mifflin

Castagnola A, Stock P (2014) Common virulence factors and tissue targets of entomopathogenic bacteria for biological control of lepidopteran pests. Insects 5:139–166. doi:10.3390/insects5010139

Chaston JM, Suen G, Tucker SL, Andersen AW, Bhasin A, Bode E, Bode HB, Brachmann AO, Cowles CE, Cowles KN, Darby C, de Léon L, Drace K, Du Z, Givaudan A, Herbert Tran EE, Jewell KA, Knack JJ, Krasomil-Osterfeld KC, Kukor R, Lanois A, Latreille P, Leimgruber NK, Lipke CM, Liu R, Lu X, Martens EC, Marri PR, Médigue C, Menard ML, Miller NM, Morales-Soto N, Norton S, Ogier JC, Orchard SS, Park D, Park Y, Qurollo BA, Sugar DR, Richards GR, Rouy Z, Slominski B, Slominski K, Snyder H, Tjaden BC, van der Hoeven R, Welch RD, Wheeler C, Xiang B, Barbazuk B, Gaudriault S, Goodner B, Slater SC, Forst S, Goldman BS, Goodrich-Blair H (2011) The entomopathogenic bacterial endosymbionts *Xenorhabdus and Photorhabdus*: convergent lifestyles from divergent genomes. PLoS One 6:e27909

Chen G, Zhang Y, Li J (1996) Chitinase activity of *Xenorhabdus* and *Photorhabdus* species, bacterial associates of entomopathogenic nematodes. J Invertebr Pathol 68:101–108

Christou P, Capell T, Kohli A, Gatehouse JA, Gatehouse AM (2006) Recent developments and future prospects in insect pest control in transgenic crops. Trends Plant Sci 11:302–308

Cowles KN, Goodrich-Blair H (2005) Expression and activity of a *Xenorhabdus nematophila* haemolysin required for full virulence towards *Manduca sexta* insects. Cell Microbiol 7: 209–219

Crickmore N (2006) Beyond the spore – past and future developments of *Bacillus thuringiensis* as a biopesticide. J Appl Microbiol 101:616–619

Crosland RD, Fitch RW, Hines HB (2005) Characterization of β-leptinotarsin-h and the effects of calcium flux agonists on its activity. Toxicon 45:829–841

da Silva CC, Dunphy GB, Rau ME (2000) Interaction of *Xenorhabdus nematophilus* (Enterobacteriaceae) with the antimicrobial defenses of the house cricket, *Acheta domesticus*. J Invertebr Pathol 76:285–292

Daborn PJ, Waterfield N, Silva CP (2002) A single *Photorhabdus* gene, makes caterpillars floppy (mcf), allows *Escherichia coli* to persist within and kill Insects. Proc Natl Acad Sci USA 99: 10742–10747

de Maagd RA, Bravo A, Crickmore N (2001) How *Bacillus thuringiensis* has evolved specific toxins to colonize the insect world. Trends Genet 17:193–199

Dodd SJ, Hurst MR, Glare TR, O'Callaghan M, Ronson CW (2006) Occurrence of sep insecticidal toxin complex genes in *Serratia* spp. and *Yersinia frederiksenii*. Appl Environ Microbiol 72: 6584–6592

Dowling AJ, Daborn PJ, Waterfield NR, Wang P, Streuli CH, ffrench-Constant RH (2004) The insecticidal toxin Makes caterpillars floppy (Mcf) promotes apoptosis in mammalian cells. Cell Microbiol 6:345–353

Duchaud E, Rusniok C, Frangeul L, Buchrieser C, Givaudan A, Taourit S, Bocs S, Boursaux-Eude C, Chandler M, Charles JF, Dassa E, Derose R, Derzelle S, Freyssinet G, Gaudriault S, Medigue C, Lanois A, Powell K, Siguier P, Vincent R, Wingate V, Zouine M, Glaser P, Boemare N, Danchin A, Kunst F (2003) The genome sequence of the entomopathogenic bacterium *Photorhabdus luminescens*. Nat Biotechnol 21:1307–1313

Ewing WH, Ross AJ, Brenner DJ, Fanning GR (1978) Yersinia ruckeri sp. nov., the Redmouth (RM) Bacterium. Int J Syst Bacteriol 28:37–44. doi:10.1099/00207713-28-1-37

Federici BA, Siegel JP (2008) Safety assessment of Bacillus thuringiensis and Bt crops used in insect control. In: Hammond BG (ed) Food safety of proteins in agricultural biotechnology. CRC Press Taylor and Francis Group, Chapter 3, pp 45–102

ffrench-Constant RH, Waterfield NR (2005) An ABC guide to the bacterial toxin complexes. Adv Appl Microbiol 58:169–183

ffrench-Constant RH, Waterfield NR (2006) Ground control for insect pests. Nat Biotechnol 24:660–661

ffrench-Constant RH, Waterfield N, Burland V, Perna NT, Daborn PJ, Bowen D, Blattner FR (2000) A Genomic sample sequence of the entomopathogenic bacterium *Photorhabdus luminescens* W14: Potential implications for virulence. Appl Environ Microbiol 66:3310–3329

ffrench-Constant RH, Waterfield NR, Daborn P, Joyce S, Bennet H, Au C, Dowling A, Boundy S, Reynolds S, Clarke D (2003) Photorhabdus: towards a functional genome analysis of a symbiont and pathogen. FEMS Microbiology Rev 26:433–456

Fischer-Le Saux M, Viallard V, Brunel B, Normand P, Boemare N (1999) Polyphasic classification of the genus *Photorhabdus* and proposal of new taxa: *P. luminescens* subsp. *luminescens* subsp. nov, *P. luminescens* subsp. *akhurstii* subsp. nov, *P. luminescens* subsp. *laumondii* subsp. nov, *P. temperata* sp. nov, *P. temperata* subsp. *temperata* subsp. nov. and *P. asymbiotica* sp. nov. Int J Syst Bacteriol 49:1645–1656

Fitt GP (1994) Cotton pest management: part 3; an Australian perspective. Annu Rev Entomol 39: 532–562

Forst S, Goodner B (2006) Comparative bacterial genomics and its use in undergraduate education. Biol Control 38:47–53

Forst S, Nealson K (1996) Molecular biology of the symbiotic-pathogenic bacteria *Xenorhabdus* spp. and *Photorhabdus* spp. Microbiol Rev 60:21–43

Frost LS, Leplae R, Summers AO, Toussaint A (2005) Mobile genetic elements: the agents of open source evolution. Nat Rev Microbiol 3:722–732

Gassmann AJ, Petzold-Maxwell JL, Keweshan RS, Dunbar MW (2011) Field-evolved resistance to Bt maize by western corn rootworm. PLoS One 6:e22629. doi:10.1371/journal.pone.0022629

Gatehouse JA (2008) Biotechnological prospects for engineering insect-resistant plants. Plant Physiol 146:881–887

Gatehouse AM, Hilder VA, Powell KS, Wang M, Davison GM, Gatehouse LN, Down RE, Edmonds HS, Boulter D, Newell CA et al (1994) Insect-resistant transgenic plants: choosing the gene to do the 'job'. Biochem Soc Trans 22:944–949

Gaudriault S, Pages S, Lanois A, Laroui C, Teyssier C, Jumas-Bilak E, Givaudan A (2008) Plastic architecture of bacterial genome revealed by comparative genomics of Photorhabdus variants. Genome Biol 9:R117. doi:10.1186/gb-2008-9-7-r117

Gerrard J, Waterfield N, Vohra R, ffrench-Constant RH (2004) Human infection with *Photorhabdus asymbiotica*: an emerging bacterial pathogen. Microbes Infect 6:229–237

Gerrard JG, Joyce SA, Waterfield NR (2006) Nematode symbiont for *Photorhabdus asymbiotica*. Emerg Infect Dis 12:1562–1564

Gillespie JP, Kanost MR, Trenczek T (1997) Biological mediators of insect immunity. Ann Rev Entomol 42:611–643

Goodrich-Blair H, Clarke DJ (2007) Mutualism and pathogenesis in *Xenorhabdus* and *Photorhabd*us: two roads to the same destination. Mol Microbiol 64:260–268

Gould F (1998) Sustainability of transgenic insecticidal cultivars: integrating pest genetics and ecology. Annu Rev Entomol 43:701–726

Griffin CT, O' Callaghan K, Dix I (2001) A self-fertile species of *Steinernema* from Indonesia: further evidence of convergent evolution amongst entomopathogenic nematodes? Parasitology 122:181–186

Grimont PAD, Grimont F (1978) The genus *Serratia*. Annu Rev Microbiol 32:221–248. doi:10.1146/annurev.mi.32.100178.001253

Hacker J, Kaper JB (2000) Pathogenicity islands and the evolution of microbes. Annu Rev Microbiol 54:641–679

Haq SK, Atif SM, Khan RH (2004) Protein proteinase inhibitor genes in combat against insects, pests, and pathogens: natural and engineered phytoprotection. Arch Biochem Biophys 431:145–159

Hares MC, Hinchliffe SJ, Strong PC, Eleftherianos I, Dowling AJ, ffrench-Constant RH, Waterfield N (2008) The *Yersinia pseudotuberculosis* and *Yersinia pestis* toxin complex is active against cultured mammalian cells. Microbiology 154:3503–3517

Head G, Moar W, Eubanks M, Freeman B, Ruberson J, Hagerty A, Turnipseed S (2005) A multiyear, large-scale comparison of arthropod populations on commercially managed Bt and non-Bt cotton fields. Environ Entomol 34:1257–1266

Hey TD, Meade T, Burton SL, Merlo DJ, Cai Q, Moon HJ, Sheets JJ, Woosley AT (2006) Insecticidal toxin complex fusion proteins. US Patent 2006/0168683

Hinchliffe SJ, Hares MC, Dowling AJ, ffrench-Constant RH (2010) Insecticidal toxins from the *Photorhabdus* and *Xenorhabdus* bacteria. The Open Toxicol J 3:101–118

Hsiao TH, Fraenkel G (1969) Properties of leptinotarsin, a toxic hemolymph protein from the Colorado potato beetle. Toxicon 7:119–130

Huang DF, Zhang J, Song FP, Lang ZH (2007) Microbial control and biotechnology research on *Bacillus thuringiensis* in China. J Invertebr Pathol 95:175–180

Huesing J, English L (2004) The impact of crops on the developing world. AgBio Forum 7:84–95

Hurst MR, Glare TR, Jackson TA, Ronson CW (2000) Plasmid-located pathogenicity determinants of *Serratia entomophila*, the causal agent of amber disease of grass grub, show similarity to the insecticidal toxins of *Photorhabdus luminescens*. J Bacteriol 182:5127–5138

Hurst MR, Glare TR, Jackson TA (2004) Cloning *Serratia entomophila* antifeeding genes-a putative defective prophage active against the grass grub *Costelytra zealandica*. J Bacteriol 186:5116–5128

Hurst MR, Beard SS, Jackson TA, Jones SM (2007a) Purification and characterisation of the *Serratia entomophila* Afp. FEMS Microbiol Lett 270:42–48

Hurst MR, Jones SM, Tan B, Jackson TA (2007b) Induced expression of the *Serratia entomophila* Sep proteins shows activity towards the larvae of the New Zealand grass grub *Costelytra zealandica*. FEMS Microbiol Lett 275:160–167

Hurst MR, Becher SA, Young SD, Nelson TL, Glare TR (2011a) *Yersinia entomophaga* sp nov., isolated from the New Zealand grass grub *Costelytra zealandica*. Int J Syst Evol Microbial 61: 844–849

Hurst MR, Jones SA, Binglin T, Harper LA, Jackson TA, Glare TR (2011b) The main virulence determinant of *Yersinia entomophaga* MH96 is a broad-host-range toxin complex active against insects. J Bacteriol 193:1966–1980

Jackson TA (1995) Amber disease reduces trypsin activity in midgut of *Costelytra zealandica* (Coleoptera, Scarabaeidae) larvae. Research note. J Invertebr Pathol 65:68–69

Jackson TA, Pearson JF, O'Callaghan M, Mahanty HK, Willocks M (1992) Pathogen to product – development of *Serratia entomophila* (Enterobacteriaceae) as a commercial biological control agent for the New Zealand grass grub (*Costelytra zealandica*). In: Glare TR, Jackson TA (eds) Use of pathogens in scarab pest management. Intercept Ltd., Andover, pp 191–198

Jackson TA, Huger AM, Glare TR (1993) Pathology of amber disease in the New Zealand grass grub *Costelytra zealandica* (Coleoptera: Scarabaeidae). J Invertebr Pathol 61:123–130

Jackson TA, Boucias DG, Thaler JO (2001) Pathobiology of amber disease, caused by *Serratia* spp, in the New Zealand grass grub, *Costelytra zealandica*. J Invertebr Pathol 78:232–243

Jackson RE, Bradley JR, Van DWJ (2003) Field performance of transgenic cotton expressing one or two *Bacillus thuringiensis* endotoxins against bollworm, *Helicoverpa zea* (Boddie). J Cotton Sci 7:57–64

James C (2010) Global status of commercialized biotech/GMCrops ISAAA Briefs No. 42. ISAAA, Ithaca

James C (2012) Global status of commercialized biotech/GM Crops: 2012. ISAAA Briefs No. 44. ISAAA, Ithaca

Jeong HU, Mun HY, Oh HK (2010) Evaluation of insecticidal activity of a bacterial strain *Serratia*, sp EML-SE1 against diamondback moth. J Microbiol 48:541–545

Joshi MC, Sharma A, Kant S, Birah A, Gupta GP, Khan SR, Bhatnagar R, Banerjee N (2008) An insecticidal GroEL protein with chitin binding activity from *Xenorhabdus nematophila*. J Biol Chem 283:28287–28296

Kaur S (2000) Molecular approaches towards development of novel *Bacillus thuringiensis* biopesticides. World J Microbiol Biotechnol 16:781–793

Khan AG (2005) Role of soil microbes in the rhizospheres of plants growing on trace metal contaminated soils in phytoremediation. J Trace Elem Med Biol 18:355–364

Khandelwal P, Banerjee-Bhatnagar N (2003) Insecticidal activity associated with the outer membrane vesicles of *Xenorhabdus nematophilus*. Appl Environ Microbiol 69:2032–2037

Khandelwal P, Choudhury D, Birah A, Reddy MK, Gupta GP, Banerjee N (2004) Insecticidal pilin subunit from the insect pathogen *Xenorhabdus nematophila*. J Bacteriol 186:6465–6476

Klein MG (1990) Efficacy against soil-inhabiting insect pests. In: Gaugler R, Kaya HK (eds) Entomopathogenic nematodes in biological control. CRC, Boca Raton, pp 195–231

Konig W, Faltin Y, Scheffer J, Schoffler H, Braun V (1987) Role of cell-bound hemolysin as a pathogenicity factor for *Serratia* infections. Infect Immun 55:2554–2561

Kumari P, Kant S, Zaman S, Mahapatro GK, Banerjee N, Sarin NB (2014) A novel insecticidal GroEL protein from *Xenorhabdus nematophila* confers insect resistance in tobacco. Transgenic Res 23:99–107

Lacey LA, Georgis R (2012) Entomopathogenic nematodes for control of insect pests above and below ground with comments on commercial production. J Nematol 44:218–225

Lacey LA, Kaya HK (2007) Field manual of techniques in invertebrate pathology, 2nd edn. Springer, Dorcrecht

Lacey LA, Frutos R, Kaya HK, Vail P (2001) Insect pathogens as biological control agents: do they have a future? Biol Control 21:230–248. doi:10.1006/bcon.2001.0938

Lamelas A, Gosalbes MJ, Manzano-Marín A (2011) *Serratia symbiotica* from the aphid *Cinara cedri*: A missing link from facultative to obligate insect endosymbiont. PLoS Genet 7:1–11

Landsberg MJ, Jones SA, Busby JN, Rothnagel R, Busby JN, Marshall SDG, Simpson RM, Lott JS, Hankamer B, Hurst MRH (2011) 3D structure of the *Yersinia entomophaga* toxin complex and implications for insecticidal activity. Proc Natl Acad Sci USA 108:20544–20549

Lee SC, Stoilova-McPhie S, Baxter L, Fülöp V, Henderson J, Rodger A, Roper DI, Scott DJ, Smith CJ, Morgan JA (2007) Structural characterization of the insecticidal toxin Xpt A1 reveals a 1.15 MDa tetramer with a cage like structure. J Mol Biol 366:1558–1568

Lengyel K, Lang E, Fodor A, Szállás E, Schumann P, Stackebrandt E (2005) Description of four novel species of *Xenorhabdus*, family Enterobacteriaceae: *Xenorhabdus budapestensis* sp. nov., *Xenorhabdus ehlersii* sp. nov., *Xenorhabdus innexi* sp. nov. and *Xenorhabdus szentirmaii* sp. nov. Syst Appl Microbiol 28:115–122

Li M, Wu G, Liu C, Chen Y, Qiu L, Pang Y (2009) Expression and activity of a probable toxin from *Photorhabdus luminescens*. Mol Biol Rep 36:785–790. doi:10.1007/s11033-008-9246-z

Liu D, Burton S, Glancy T (2003) Insect resistance conferred by 283-kDa *Photorhabdus luminescens* protein TcdA in *Arabidopsis thaliana*. Nat Biotechnol 21:1307–1313

Liu W, Ye W, Wang Z, Wang X, Tian S, Cao H, Lian J (2006) *Photorhabdus luminescens* toxin-induced permeability change in *Manduca sexta* and *Tenebrio molitor* midgut brush border membrane and in unilamellar phospholipid vesicle. Environ Microbiol 8:858–870

Marra MC, Piggott NE, Goodwin BK (2010) The anticipated value of SmartStax™ for US corn growers. AgBio Forum 13:1–12

Mohan S, Sabir N (2005) Biosafety concerns on the use of Photorhabdus luminescens as biopesticide: experimental evidence of mortality in egg parasitoid *Trichogramma* spp. Curr Sci 89:1268–1272

Moran NA (2002) Microbial minimalism: genome reduction in bacterial pathogens. Cell 108: 583–586

Morgan JA, Sergeant M, Ellis D, Ousley M, Jarrett P (2001) Sequence analysis of insecticidal genes from *Xenorhabdus nematophilus* PMFI296. Appl Environ Microbiol 67:2062–2069

Naranjo SE (2005) Long-term assessment of the effects of transgenic Bt cotton on the function of the natural enemy community. Environ Entomol 34:1211–1223

Neale MC (1997) Bio-pesticides – harmonisation of registration requirements within EU directive 91–414. An industry view. Bull Eur Mediterranean Plant Protect Organ 27:89–93

Nuñez-Valdez ME, Calderón MA, Aranda E, Hernández L, Ramírez-Gama RM, Lina L, Rodríguez-Segura Z, Gutiérrez M, Villalobos FJ (2008) Identification of a putative Mexican strain of *Serratia entomophila* pathogenic against root-damaging larvae of Scarabaeidae (Coleoptera). Appl Environ Microbiol 74:802–810

Ochman H, Lawrence JG, Groisman EA (2000) Lateral gene transfer and the nature of bacterial innovation. Nature 405:299–304

Ogier JC, Calteau A, Forst S, Blair HG, Roche D, Rouy Z, Suen G, Zumbihl R, Givaudan A, Tailliez P, Médigue C, Gaudriault S (2010) Units of plasticity in bacterial genomes: new insight from the comparative genomics of two bacteria interacting with invertebrates, *Photorhabdus* and *Xenorhabdus*. BMC Genomics 11:568

Ohno S (1970) Evolution by gene duplication. Springer, New York, p 160

Parkhill J, Wren BW, Thomson NR (2001) Genome sequence of *Yersinia pestis*, the causative agent of plague. Nature 413:523–527

Paul VJ, Frautschy S, Fenical W, Nealson KH (1981) Antibiotics in microbial ecology: isolation and structure assignment of several new antibacterial compounds from the insect-symbiotic bacteria *Xenorhabdus* spp. J Chem Ecol 7:589–597

Pechy-Tarr M, Bruck DJ, Maurhofer M, Fischer E, Vogne C, Henkels MD, Donahuer M, Grunder J, Loper JE, Keel C (2008) Molecular analysis of a novel gene cluster encoding an insect toxin in plant-associated strains of *Pseudomonas fluorescens*. Environ Microbiol 10: 2368–2386

Pereira EJ, Lang BA, Storer NP, Siegfried BD (2008) Selection for Cry1F resistance in the European corn borer and cross-resistance to other Cry toxins. Entomol Exp Appl 126:115–121

Perlak F, Oppenhuizen M, Gustafson K, Voth R, Sivasupramaniam S, Heering D, Carey B, Ihrig RA, Roberts JK (2001) Development and commercial use of Bollgard cotton in the USA-early promises versus today's reality. Plant J 27:489–502

Perry RD, Fetherston JD (1997) *Yersinia pestis* – etiologic agent of plague. Clin Microbiol Rev 14: 35–66

Poinar GO Jr (1979) Nematodes for the biological control of insects. CRC, Boca Raton, p 227

Poinar GO (1993) Origins and phylogenetic relationships of the entomophilic rhabditids, *Heterorhabditis* and *Steinernema*. Fund Appl Nematol 16:333–338

Radake SG, Undirwade RS (1981) Seasonal abundance and insecticidal control of shoot and fruit borer, *Earias* spp. on okra, *Abelmoschus esculentus* (L.). Indian J Entomol 43:283–287

Rae R, Riebesell M, Dinkelacker I (2008) Isolation of naturally associated bacteria of necromenic *Pristionchus nematodes* and fitness consequences. J Exp Biol 211:1927–1936

Reineke A, Karlovsky P, Zebitz CPW (1999) Amplified fragment length polymorphism analysis of different geographic populations of the gypsy moth, *Lymantria dispar* (Lepidoptera: Lymantriidae). Bull Entomol Res 89:79–88

BCC Research (2010) Biopesticides: the global market-BCC Research Report, CHM029C, February 2010

Ribeiro C, Vignes M, Brehelin M (2003) *Xenorhabdus nematophila* (Enterobacteriaceae) secretes a cation selective calcium-independent porin which causes vacuolation of the rough endoplasmic reticulum and cell lysis. J Biol Chem 278:3030–3039

Ruffner B, Péchy-Tarr M, Ryffel F, Hoegger P, Obrist C, Rindlisbacher A, Keel C, Maurhofer M (2013) Oral insecticidal activity of plant-associated *Pseudomonads*. Environ Microbiol 15: 751–763. doi:10.1111/j.1462-2920.2012.02884.x

Ruiu L, Satta A, Floris I (2013) Emerging entomopathogenic bacteria for insect pest management. Bull Insectol 66:181–186

Sanahuja G, Banakar R, Twyman RM, Capell T, Christou P (2011) *Bacillus thuringiensis*: a century of research, development and commercial applications. Plant Biotechnol J 9:283–300

Sanchis V (2011) From microbial sprays to insect-resistant transgenic plants: history of the biospesticide *Bacillus thuringiensis*. A review. Agron Sustain Dev 31:217–231. doi:10.1051/agro/2010027

Saour G (2014) Sterile insect technique and F1 sterility in the European grapevine moth, *Lobesia botrana*. J Insect Sci 14:8

Sayyed AH, Moores G, Crickmore N, Wright DJ (2008) Cross-resistance between a *Bacillus thuringiensis* Cry toxin and non-Bt insecticides in the diamondback moth. Pest Manag Sci 64:813–819

Schnepf E, Crickmore N, Van Rie J, Lereclus D, Baum J, Feitelson J, Zeigler DR, Dean DH (1998) *Bacillus thuringiensis* and its pesticidal crystal proteins. Microbiol Mol Biol Rev 62:775–806

Sergeant M, Jarrett P, Ousely M, Morgan AW (2003) Interactions of insecticidal toxin gene products from Xenorhabdus nematophila PMF1296. Appl Environ Microbiol 69:3344–3349

Sergeant M, Baxter L, Jarret P (2006) Identification, typing, and insecticidal activity of *xenorhabdus* isolates from entomopathogenic nematodes in United Kingdom soil and characterization of the xpt toxin loci. Appl Environ Microbiol 72:5895–5907

Shahid AA, Rao AQ, Bakhsh A, Husnain T (2012) Entomopathogenic fungi as biological controllers: new insights into their virulence and pathogenicity. Arch Biol Sci Belgrade 64:21–42

Sheets JJ, Hey TD, Fencil KJ, BurtonS NW, Lang AE, Benz R, Aktories K (2011) Insecticidal toxin complex proteins from *Xenorhabdus nematophilus*: structure and pore formation. J Biol Chem 286:22742–22749

Sicard M, Le Brun N, Pages S, Godelle B, Boemare N, Moulia C (2003) Effect of native *Xenorhabdus* on the fitness of their *Steinernema* hosts: contrasting types of interactions. Parasitol Res 91:520–524

Sicard M, Ferdy JB, Pages S, LeBrun N, Godelle B, Boemare N, Moulia C (2004) When mutualists are pathogens: an experimental study of the symbioses between *Steinernema* (entomopathogenic nematodes) and *Xenorhabdus* (bacteria). J Evol Biol 17:985–993

Sikorowski PP, Lawrence AM, Inglis GD (2001) Effects of *Serratia marcescens* on rearing of the tobacco budworm (Lepidoptera: Noctuidae). Am Entomol 47:51–60

Silva CP, Waterfield NR, Daborn PJ, Dean P, Chilver T, Au CP, Sharma S, Potter U, Reynolds SE, ffrench-Constant RH (2002) Bacterial infection of a model insect: *Photorhabdus luminescens* and *Manduca sexta*. Cell Microbiol 4:329–339

Somvanshi VS, Lang E, Ganguly S, Swiderski J, Saxena AK, Stackebrandt E (2006) A novel species of *Xenorhabdus*, family Enterobacteriaceae: *Xenorhabdus indica* sp. nov, symbiotically associated with entomopathogenic nematode *Steinernema thermophilum*. Syst Appl Microbiol 29:519–525

Spinner JL, Jarrett CO, LaRock DL (2012) Yersinia pestis insecticidal-like toxin complex (Tc) family proteins: characterization of expression, subcellular localization, and potential role in infection of the flea vector. BMC Microbiol 12:1–14

Srinivasan A, Giri A, Gupta V (2006) Structural and functional diversities in Lepidopteran serine proteases. Cell Mol Biol Lett 11:132–154

Steinhaus EA (1941) A study of bacteria associated with thirty species of insects. J Bacteriol 42:757–790

Steinhaus EA (1951) Possible use of *Bacillus thuringiensis* Berliner as an aid in the biological control of the alfalfa caterpillar. Hilgardia 20:350–381

Sulakvelidze A (2000) *Yersinia* other than *Y. enterocolitica, Y. pseudotuberculosis,* and *Y. pestis*, the ignored species. Microbes Infect 2:497–513

Swihart KG, Welch RA (1990) Cytotoxic activity of the Proteus hemolysin HpmA. Infect Immun 58:1861–1869

Tailliez P, Pagès S, Ginibre N, Boemare N (2006) New insight into diversity in the genus *Xenorhabdus*, including the description of ten novel species. Int J Syst Evol Microbiol 56: 2805–2818

Talekar NS, Shelton AM (1993) Biology, ecology, and management of the Diamondback moth. Annu Rev Entomol 38:275–301

Vallet-Gely I, Opota O, Boniface A, Novikov A, Lemaitre B (2010) A secondary metabolite acting as a signalling molecule controls *Pseudomonas entomophila* virulence. Cell Microbiol 12: 1666–1679

Vermunt AMW, Koopmanschap AB, VLak JM, de Kort CAD (1997) Cloning and sequence analysis of cDNA encoding a putative juvenile hormone esterase from the Colorado potato beetle. Insect Biochem and Mol Biol 27:919–928

Vigneux F, Zumbihl R, Jubelin G, Ribeiro C, Poncet J, Baghdiguian S, Givaudan A, Brehélin M (2007) The xaxAB genes encoding a new apoptotic toxin from the insect pathogen *Xenorhabdus nematophila* are present in plant and human pathogens. J Biol Chem 282: 9571–9580

Vodovar N, Vinals M, Liehl P, Basset A, Degrouard J, Spellman P, Boccard F, Lemaitre B (2005) Drosophila host defense after oral infection by an entomopathogenic *Pseudomonas* species. Proc Natl Acad Sci USA 102:11414–11419

Vodovar N, Vallenet D, Cruveiller S, Rouy Z, Barbe V, Acosta C, Cattolico L, Jubin C, Lajus A, Segurens B, Vacherie B, Wincker P, Weissenbach J, Lemaitre B, Médigue C, Boccard F (2006) Complete genome sequence of the entomopathogenic and metabolically versatile soil bacterium *Pseudomonas entomophila*. Nat Biotechnol 24:673–679

Wang JX, Li LY (1987) Entomogenous nematode research in China. Rev Nematol 10:483–489

Waterfield N, Dowling A, Sharma S (2001) Oral toxicity of *Photorhabdus luminescens* W14 toxin complexes in *Escherichia coli*. Appl Environ Microbiol 67:5017–5024

Waterfield NR, Daborn PJ, ffrench-Constant RH (2002) Genomic islands in *Photorhabdus*. Trends Microbiol 10:541–545

Waterfield NR, Daborn PJ, Dowling AJ, Yang G, Hares M, ffrench-Constant RH (2003) The insecticidal toxin makes caterpillars floppy 2 (Mcf2) shows similarity to HrmA, an avirulence protein from a plant pathogen. FEMS Microbiol Lett 229:265–270

Waterfield N, Hares M, Yang G, Dowling A, ffrench-Constant R (2005a) Potentiation and cellular phenotypes of the insecticidal Toxin complexes of *Photorhabdus* bacteria. Cell Microbiol 7:373-382

Waterfield N, Kamita SG, Hammock BD, ffrench-Constant R (2005b) The *Photorhabdus* Pir toxins are similar to a developmentally regulated insect protein but show no juvenile hormone esterase activity. FEMS Microbiol Lett 245:47–52

Waterfield NR, Sanchez-Contreras M, Eleftherianos I, Dowling I, Yang G, Wilkinson P, Parkhill J, Thomson N, Reynolds SE, Bode HB, Dorus S, ffrench-Constant R (2008) Rapid virulence annotation (RVA): identification of virulence factors using a bacterial genome library and multiple invertebrate hosts. Proc Natl Acad Sci USA 105:15967–15972

Waterfield NR, Ciche T, Clarke D (2009) Photorhabdus and a host of hosts. Annu Rev Microbiol 63:557–574

Whitehouse MEA, Wilson LJ, Fitt GP (2005) A comparison of arthropod communities in transgenic Bt and conventional cotton in Australia. Environ Entomol 34:1224–1241

Wilkinson P, Waterfield NR, Crossman L, Corton C, Sanchez-Contreras M, Vlisidou l, Barron A, Bignell A, Clark L, Ormond D, Mayho M, Bason N, Smith F, Simmonds M, Churcher C, Harris D, Thompson NR, Quail M, Parkhill J, ffrench-Constant RH (2009) Comparative genomics of the emerging human Pathogen Photorhabdus asymbiotica with the insect pathogen *Photorhabdus luminescens*. BMC Genomics 10:302–324

Woese CR (1987) Bacterial evolution. Microbiol Rev 51(221):271

Wolters M, Boyle EC, Lardong K (2013) Cytotoxic necrotizing factor-Y boosts *Yersinia* effector translocation by activating Rac. J Biol Chem 288:23543–23553

Wu K, Lu Y, Feng H, Jiang Y, Zhao J (2008) Suppression of Cotton Bollworm in multiple crops in china in areas with Bt toxin-containing cotton. Science 321:1676–1678

Yang G, Dowling AJ, Gerike U, ffrench-Constant RH, Waterfield NR (2006) *Photorhabdus* virulence cassettes confer injectable insecticidal activity against the wax moth. J Bacteriol 188:2254–2261

Young SD, Townsend RJ, Swaminathan J, O'Callaghan M (2010) *Serratia entomophila*-coated seed to improve ryegrass establishment in the presence of grass grubs. N Z Plant Protect 63: 229–234