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Bio-efficacy of *Photorhabdus* Insecticidal Toxin for Insect Pest Control in Agriculture

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A B S T R A C T

The use of chemical fertilizers and chemical insecticides in agricultural field by the farmers to get increased yield, is a routine practice. Adverse effects of chemical pesticides on non-target organisms, food safety, and development of insect resistance are well known. It has led the researchers to focus on the development of alternative eco-friendly measures. Biological control is aimed to reduce population of ecologically and agriculturally harmful invasive species. The chief bioinsecticides available are *Bacillus thuringiensis*, insect parasitic nematodes, and entomopathogenic bacteria. Bacteria of the genus *Xenorhabdus* and *Photorhabdus* are considered to be symbiotically associated with the genera *Steinernema* and *Heterorhabditis*, respectively. They are not isolated outside this relationship and play important role for killing the insects. *Photorhabdus* has received commercial attention as a new biocontrol agent because of high pathogenicity against a wide variety of insects. Two of the four insecticidal toxins are known to possess oral toxicity to *Lepidopteran* insects. *Photorhabdus* with its peculiar characteristics can to be used as novel biopesticide against insect pest.

Key words: Entomopathogenic bacteria, Biopesticide, *Photorhabdus, Bacillus thuringiensis,* Insect parasitic nematodes, Insecticidal toxin

It is a well-known fact that the Chemical fertilizers and insecticides allow growers to maximize their crop yield. However, at the same time it is also a fact that there are adverse effects of chemical pesticides on non-target organisms and food safety. This has necessitated the researchers to consider the development of alternative eco-friendly measures. Under these circumstances, alternative method to chemical insecticides can be proposed by use of biopesticides. Biological control is aimed to reduce population of ecologically and agriculturally harmful invasive species. The biopesticides are crop protection agents composed of living micro-organisms or natural products. Use of biopesticides is nontoxic to environment, has efficiency to kill the insect pest, and reliable way to control the insect pest.

The biopesticides are classified into microbial pesticides, botanical pesticides, and zooid pesticides. Microbes like bacteria, fungi, viruses, nematodes, and protists are known insect pathogens, and are replacing chemical insecticide for agricultural insect-pest management (Kushwah *et al*. 2017). The chief bioinsecticides available are *Bacillus thuringiensis*, insect parasitic nematodes, and entomopathogenic bacteria (Chattopadhyay *et al.* 2004). The bacterial species inhabit bodies of insects establishing different levels of mutualistic relationships, only a limited number of them behave as insect pathogens (Luca 2015). These entomopathogenic bacteria can be used as a biological control of insect pest. One of the entomopathogenic bacteria, *Bacillus thuringiensis* widely used for control of insect pest. It produces delta endotoxins which are proteinaceous insecticidal toxins and readily biodegradable, and thus have a short half-life inside the insect mid gut (Chattopadhyay *et al*. 2004). The two entomopathogenic nematode genus,

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Steinernema and *Heterorhabditis* are used as natural insecticides. They possess extreme virulence for insects at a non specific broad host range and are safe for mammals. *Xenorhabdus* and *Photorhabdu luminescens* are symbiotically associated with the *Steinernema* and *Heterorhabditis*, respectively, play important role for killing the insects.

Potential role of Photorhabdus luminescens as a biopesticide

The genera *Photorhabdus* and *Xenorhabdus* are entomopathogenic members recognized as endosymbionts of insecticidal nematodes. The first one of the above are typically related to entomopathogenic nematodes within the genus *Heterorhabditis,* while the second one are related to *Steinernema species.* The pathogenic action occurs when the nematodes have actively entered the insect body and delivered symbiotic bacteria within the insect hemocoel. Here the bacteria proliferate producing various antimicrobial compounds to contrast the growth of other microorganisms. They also release different enzymes that contribute to the degradation processes within the hemocoel, thus creating a perfect environment which encourages the development of the nematode population (Luca 2015).

Photorhabdus is a bioluminescent, Gram-negative bacteria member of the family Enterobacteriaceae. The bacterium is motile by means of peritrichous flagella, facultative anaerobic, having both a respiratory and a fermentative metabolism and requires optimum growth temperature usually ∼28°C (Bergey's Manual of Systematics of Archaea and Bacteria). The natural habitat for *Photorhabdus* is the intestinal lumen of entomopathogenic nematodes of the genus *Heterorhabditis* and insects infected by these nematodes. It has a pathogenic interaction with a wide range of insects, whilst also maintaining a mutualistic interaction with nematodes from the family *Heterorhabditis* (Waterfield *et al*. 2009). *Photorhabdus* species appears in two forms, primary and secondary variants, which differ in morphological and physiological characters. While the primary variants occur in infective stage nematodes, secondary variants occur after sustained growth. Primary variants produce extracellular protease, extracellular lipase, intracellular protein crystals CipA and CipB, antibiotics, and exhibit bioluminescence. Secondary variants lack enzymatic activity (protease & lipase), antibiotic activity, and have lesser bioluminescence. They further also differ in colony morphology, pigmentation, dye adsorption, metabolism and ability to support nematode growth and reproduction.

The *Photorhabdus* bacteria symbiotically associated with *Heterorhabditis*, have only been identified from 6 out of the 14 recognized *Heterorhabditis* species, *H. indica, H. bacteriophora, H. megidis, H. zealandica, H. downesi*, and *H. marelatus* (Ruisheng *et al*. 2011). Currently, three species of *Photorhabdus*, *P. asymbiotica*, *Photorhabdus luminescens*, and *P. temperata*, have been described with the recognition of five *P. luminescens* subspecies, three *P. temperata* subspecies, and two *P. asymbiotica* subspecies. Poinar *et al.* (1980) studied on *P. luminescens* Hb strain associated with *H. bacteriophora*, found that the symbionts cannot exist in the environment in the absence of their nematode associates after 24 hours. Morgan (1997) shown that both *X. nematophilus* and *Photorhabdus luminescens* have restricted survival in terms of their colony forming ability in soil, water and also reported the decline of cells to below detection limits within 7-day release. Brunel *et al.* (1997) showed that restriction analysis of PCR-amplified 16S rDNA is a highly effective technique for distinguishing between the bacterial symbionts of entomopathogenic nematodes.

A variety of bacterial virulence factors are involved in the interaction with the susceptible host. Different *Photorhabdus* and *Xenorhabdus* species produces an insecticidal toxin complex (Tc) which has high potential for pest management. Generally, the Tcs are high-molecular weight and multi-subunit proteins that include three components, A, B and C which are orally active against different insects. While the mode of action is not completely understood, all these components are needed to achieve full toxicity (Luca 2015). Another example of insecticidal proteins produced by these bacterial species is represented by the Photorhabdus insect related (Pir) proteins, produced by *P. luminescens*, which shows similarity to *B thuringiensis* (Bt) delta-endotoxins and are proposed to be mimics of the juvenile hormone esterases (JHEs) interfering with insect development regulation (Luca 2015). In case of *B thuringiensis*, the δ-endotoxins are produced as protoxins and form a crystalline inclusion which is solubilized during passage through the insect gut. The high pH condition lies in the midgut and which leads to the action of proteases to solubilize the protein and produce a smaller active toxin (Bravo *et al*. 2007).

The endotoxins of *B thuringiensis* have been used as sprayable microbial insecticides for nearly 3 decades with limited success in the commercial marketplace. The genetically variant pests emerged which are not susceptible to the BT delta endotoxins. These resistant pests possess a mechanism to break down these toxins. BT resistance was first discovered in *P. interpunctella* in 1985. Various other insect species have developed resistance to BT toxin in the laboratory, including *Ostrinia nubilalis*, *Heliothis virescens*, *Pectonophora gossypiella, Culex quinquefasciatus*, *Aedes aegypti, Trichloroplusia ni, L decemlimeata*, *Spodoptera exigua*, *Spodoptera littoralis*, and *Chryosomela scripta* (Chattopadhyay *et al*. 2004). *P. xylostella* was found to have become resistant to biological control by the Bt toxin (*Bacillus thuringiensis*) in the field. The genes encoding the toxin complexes (Tcs or Xpt) described in *Xenorhabdus* and *Photorhabdus* does not have any similarity to the Bt δ-endotoxins. It implies that these bacteria have the potential to develop as insecticidal agents. The effective, reliable use of these proteins to control insect pests in the field remained vague until advances in plant transformation technology allowed the stable introduction of these genes in a variety of crop species. Although the numerous problems are encountered with the applications of synthetic insecticides. Due to the serious health hazards linked with the synthetic insecticides, biopesticide product such as toxin offers a good

alternative for controlling the pest population affecting agricultural crops. This literature review is a comprehensive summary of previous research on the role of *Photorhabdus luminescens* to control the insect pests and deliberates their potential role in agriculture.

Interaction of Photorhabdus with insect tissue

Photorhabdus is unique bacteria has contrasting, but obligate, interactions with two different animal hosts. *Photorhabdus* bacteria are symbiotically associated with the entomopathogenic nematodes *Heterorhabditis*, representing an emerging model system for studies on invertebrate microbe symbiosis (Ruisheng *et al*. 2011). The bacteria use the nematode as a habitat to persist in the soil and as a vector for transmission from insect to insect, while the nematode uses the bacteria to provide access to a rich nutrient source that permits high levels of nematode reproduction. The similarity in lifestyles appears to be a consequence of convergent evolution as there is substantial evidence that *Xenorhabdus* and *Photorhabdus* have evolved to this complex lifestyle independently (Clarke 2014).

The *Photorhabdus* bacteria play important role for nematode during growth and development within insect and colonize within the Infective Juvenile (IJ). The IJ is a free living, soil-dwelling, infectious stage of the nematode. The IJ nematode penetrates the insect body, usually through natural body openings (mouth, anus, and spiracles) or areas of thin cuticle. Upon entering an insect host, the nematodes release the bacteria by regurgitation directly into the insect hemocoel (Clark 2008). The destiny of the bacteria in the haemocoel varies with the insect species, the physiological state of the insect, the bacterial species, and its physiological state (Morgan *et al*. 1997). The nematodes and bacteria work together to overcome the immune response of their insect host, thus allowing the bacteria to proliferate. *Photorhabdus* uses lipopolysaccharide (LPS) modification to resist the action of the host-derived antimicrobial peptide production (Abdel-Razek *et al*. 2003). Dunphy and Webster (1991) reported that LPS is a component of the outer membrane of the bacterial symbiont cells and has been shown to damage the hemocytes (invertebrate immune system cell) and inhibit activation of the humoral immune system. The bacteria grow exponentially in the insect reaching cell densities up to 10^9cfu/insect within 48 hours. This rapid growth is facilitated by the secretion of toxins and other molecules that damage host tissues and reduce the effectiveness of the normally highly potent insect innate immune system (Clarke 2008). Death of the insect is normally concomitant with the entry of the bacteria into the post-exponential phase of growth and, at this stage; all of the internal organs and tissues of the insect will have been converted into bacteria-associated biomass. Developing nematodes nourishes on mixture of bacteria and bio converted host tissue, enabling them produce one to three generation until the food resources within the cadaver are depleted (Ferreira et al. 2014). When the dauers escape the insect cadaver to search for new prey, they carry the symbiont in their gut, ensuring the vertical transmission of the mutualistic association. Insect death also signals the end of the pathogenic period, and the start of the mutualistic period, of the *Photorhabdus* life cycle. Although the nematode hosts are the natural vectors of their propagation in the insects, in nutritional terms *Photorhabdus* might be considered entomophilic rather than nematophilic microorganisms.

Effect of bacterial insecticidal toxin on different insect hosts (Table 1)

Boemare and Akhurst (1988) reported that *Photorhabdus* and *Xenorhabdus* spp are highly pathogenic to a variety of insect larvae. *Photorhabdus* is highly virulent to model insect larvae such as *Galleria mellonella* (the greater wax moth) and *Manduca sexta.* Tiarin *et al*. (2014) conducted in vivo pathogenicity assay with isolated *Photorhabdus* strains SF41T and SF783 on *Galleria mellonella*, after 16 hrs, the mortality of larvae injected with strains SF41T and SF783 was higher than 80%, while larvae in the control group (injected with *E. coli* DH5a) survived. Razek (2003) reported that in laboratory pathogenicity bioassays, *X. ehlersii* was the most potential as a pathogen to kill *Ae. aegypti* larvae within 48 hrs. Forst *et al.* reported that 20 cells of *X. nematophilus* killed as much as 90% of the larvae of *Manduca sexta* (Fukruksa *et al*. 2017). Bowen *et al.* (1998) studied the *Xenorhabdus* toxin and encode its complexes. Identified Tc toxins form a large protein complex consisting of about 10 polypeptides ranging from 30 to 200kDa, toxic to insects either by ingestion or injection to *Manduca sexta*. Brown *et al.* (2004) purified A24 toxin from *Xenorhabdus nematophila* which is active against *Galleria* and *Helicoverpa*.

Daborn *et al.* (2002) studied single *Photorhabdus* gene, Mcf encodes a novel BH3 containing pro-apoptotic toxin and reported that it destroys mid gut of insect by stopping osmoregulation of columnar epithelial cell. Dong *et al.* (2003) prepared transgenic toxin A of *Photorhabdus* in *Arabidopsis thaliana* and studied its efficacy for control of feeding insect. Eun Kyung Jang *et al.* (2011) isolated and characterized the heat stable insecticidal toxin from *Photorhabdus temparate*. The supernatants extracted from the culture retained up to 95% of insecticidal activity after heat treatment for 30 min at 80°C. Sheets *et al.* (2011) purified a native toxin complex from *X. nemtophilus*. The toxin complex consists of three different proteins, XptA2, XptB1, and XptC1, enclosed to solubilize insect brush border membranes and facilitate pore formation in black lipid membranes. The Pir AB toxins from *P. asymbiotica* exhibited potential for reduction in the survival of the larvae of *Ae. aegypti* and *Ae. albopictus* larvae. An oral dose of *X. nematophila* and P. luminescens cell suspension is lethal to Aedes larvae even within the absence of the entomopathogenic nematode.

Recently, a new family of oral insecticidal toxins produced by *Xenorhabdus* and *Photorhabdus* bacteria has been identified. An insecticidal toxic protein from *P. luminescens* strain W-14 contains high molecular weight complexes which include toxin A and toxin B. These toxin complexes have been well-characterized both at a biochemical level and by identifying their genes. Rajagopal

et al. (2002) purified the toxin complex from *Photorhabdus luminescens akhurstii*, is active against larvae of *S. litura* and *G. mellonella* at a concentration of 0.4 to 0.5 µg upon delivery into haemocoel. Li *et al.* (2009) cloned and expressed the subsp. *Akhurstii YNd185*. Pir killed *G mellonella* at LD50 30ng/larvae via hemocoel injection. It did not significantly increase mortality of *S litura* orally but the treatment did inhibit growth of the insects. Zinc metalloproteases are common in pathogenic bacteria and are often attributed roles in virulence (Chang *et al.* 2013). Several species of insect pathogens, including *Pseudomonas aeruginosa*, *Proteus mirabilis* and *Serratia marcescens* also produces metalloproteases. *Photorhabdus* delivers these metalloproteases are thought to induce death in infected insects by specifically hydrolyzing hemolymph proteins and causing damage to the hemocytes. Proteases trigger a massive programmed cell death of the midgut epithelium in the infected *Manduca sexta*. Prt A activity can be detectable 24 hours after artificial bacterial infection of an insect, suggesting that the protease may play a key role in degrading insect tissues. Chang (2013) also shown that the *Photorhabdus luminescens* strain 0805-P5G protease has a direct toxic effect on two insects viz. *G. mellonella* and *P. xylostella* (Dunphy 1995).

Table 1 Effect of insecticidal toxin secreted by *Photorhabdus* on insect

Bacteria	Toxins secreted	Mode of action	Insect
Photorhabdus Daborn *et al.* (2002)	Single gene, Mcf encodes a novel BH3 containing pro-apoptotic toxin	Destroys mid gut of insect by stopping osmoregulation of columnar epithelial cell	*Manduca sexta*
Photorhabdus Dong *et al.* (2003)	Transgenic toxin A	Feeding insect	*Manduca sexta* and Southern Corn rootworm
Photorhabdus temparate Eun *et al.* (2011)	Heat stable insecticidal toxin	95% of insecticidal activity after heat treatment for 30 min at 80°C	*Galleria mellonella*
Photorhabdus subsp. Akhurstii YNd185; Li *et al.* (2009)	-	-	*Galleria mellonella*
Photorhabdus and *Xenorhabdus* isolates; Mona *et al*. (2009)	Bacterial cell	-	*Galleria mellonella*
Photorhabdus and *Xenorhabdus* isolates (Dilipkumar *et al.* 2017)	Organic fraction of ethyl acetate bacterial crude extracts of the symbiotic bacteria	Antibacterial activity on selected pathogenic bacteria	*Escherichia coli, Staphylococcus aureus, Salmonella typhi* and *Klebsiella pneumoniae*
Photorhabdus luminescens sp. akhurstii strain IARISGMG3 Jyoti *et al.* (2017)	Injected Bacterial cells	Injectable toxicity	*Galleria mellonella*
Photorhabdus subsp. Akhurstii Rajgopal *et al*. (2002)	Purified 1000KDa	Mortality of *S. litura* within 24 hours	*Spodoptera* spp and *Galleria mellonella*
Photorhabdus luminescens Sharad *et al.* (2003)	Formulation of bacteria	-	*Plutella* and *Pieris* spp

The virulence of *Photorhabdus* is clearly genetically complex and genes, activities, and functions appear to be redundant. Therefore, it has been exceedingly difficult to identify specific genes that are required for virulence using traditional transposon-based genetic screens. Nevertheless, mutants in the production and/or assembly of the O-antigen of LPS has identified on several occasions in independent studies highlight the importance of this structural molecule during pathogenicity (French-Constant *et al*. 2003).

Use of Photorhabdus bacteria in pest management

Biological control agents, like bacterial entomopathogens, are recognized as lower risk substances and various benefits are related to their use than conventional chemical pesticides. Their mode of action is complex than conventional chemicals (Luca 2015). They target a variety of action sites which prevents the development of resistant pest. *Photorhabdus* has received commercial attention as a new biocontrol agent because of high pathogenicity towards a wide variety of insects. *Photorhabdus* bacteria produce an array of high molecular weight toxins having both oral and injectable activities against insect (French-Constant *et al*. 2003). *Photorhabdus* genome consists of 23 biosynthetic gene clusters out of which near about 6.5% of the genome encodes for secondary metabolites. Several studies showed that the bacterium can be used as an insecticide; as foliar application or in the form of alginate beads (Razek *et al.* 2003, Rajagopal *et al.* 2006). The effectiveness of bacteria as insecticide is often associated with a proper application in the field.

Important points to be considered while using the bacterial insecticide in the field

1. The bacterial insecticidal toxins should with stand with the environment until ingested by target insect.
2. The biopesticide should have maximized shelf life, enhanced efficacy along with property to disperse and adhere on the foliage.

A variety of adjuvants and additives for microbial formulations have been developed by the industry. These include dispersants, surfactants, wetters, spreaders, drift control agents, pH buffers, antifoam agents, carriers, phagostimulants and attractants (Sharad *et al*. 2003). The solid and liquid formulations are available on the basis of application and environment conditions. Dusts, granules, briquettes and wettable powders come under solid formulation. The liquid formulation contains concentrates of suspension, emulsions, and encapsulations. Advanced technologies aiming at increasing residual effects comprise micro encapsulations and micro granules. During the past years, the number of microbial products available, have grown significantly and many efforts are made to increase the awareness and to promote the adoption of biopesticides in integrated pest management programs (Luca 2015).

Besides optimizing the efficacy, modern pest management strategies tend to reduce the impact on the environment and on non-target organisms. Although the products contain entomopathogenic bacteria can be used for pest management in organic farming, their use in rotation or combination with chemicals is strongly encouraged to achieve full efficacy and eco-sustainability.

CONCLUSION

Photorhabdus luminescens kills insect hosts within 24 hours after release from the nematodes into the insect hemolymph. The toxic component secreted by *Photorhabdus* is a high molecular weight complex of four proteins encoded by the genes tca, tcb, tcc, and tcd. The cipA and cipB genes in the *Photorhabdus* genome code for two inclusion proteins, including CipA and CipB whose sizes are 11648Da and 11308Da, respectively. These genes have been cloned and studied and now being utilized for development of transgenic insect resistant plants. The toxin secreted by *Photorhabdus* has wide host range. Two of the four insecticidal toxins are known to possess oral toxicity to *Lepidopteran* insects. Protein complexes encoded by the genes tca and tcd have shown oral toxicity to *Manduca sexta*. The present review explores *Photorhabdus* as a novel biopesticide against insect pest.

Upcoming prospects

Currently we are investigating the characteristics of the bacterial isolates obtained from entomopathogenic nematode. Till now we have isolated fifteen bacterial isolates and completed their characterization and molecular identification. The study of bacterial toxin purification and its effect on insect pest is in progress.

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