Entomopathogenic nematodes and their symbiotic bacteria: microorganism-host interactions : A review

Abstract
Entomopathogenic nematodes (EPNs) are natural enemies of insect pests and acts as promising bio-control agent of the insect pests of many commercial crops.They are having a symbiotic relationships with a bacterium species. *Photorhabdus* and *Xenorhabdus* bacteria live in association with *Heterorhabditis* and *Steinernema* , respectively. Their association is mutualistic and persistent..With the help of the nematode, the bacteria penetrate and proliferate inside the insect hemocoel, causing septicemia, and providing suitable environment for nematode reproduction.The nematode and bacterial interaction utilize a varieties of pathogenic strategies that provide disease condition of the insect host of several insect orders .The importance for research in EPNs and their symbiotic bacteria are increasing and progress has been made in different fields of biology. Some of the EPN species are used as models for various biological studies especially in molecular genetics. This review describes the current state of information of entomopathogenic nematodes and their symbiotically bacteria and the interaction between them and the resultant death of insect. The studies on EPN-bacterial complex-symbiosis will ensure that EPNs will be an effective biopesticides that will help in sustainable agricultural production.

Key words: Entomopathogenic nematodes (EPNs), Symbiotic bacteria, Microorganism-host interaction, Insect pests

1.Introduction

Agricultural production is threatened by several biotic and abiotic factors. Among those insect pests are the major and conspicuous limiting factors of production. Though chemical pesticides are common recommended practices against insect pests, the use of these pesticides has many health hazards. Now-a-days, biological pesticides are of use as an alternative to chemical pesticides. Biopesticides can assist in maintaining a desirable ecological balance. Insect-pathogenic nematodes or Entomopathogenic nematodes (EPNs) are of interest as biopesticides. They are also called as beneficial nematodes (Chang *et al*., 2019). The EPNs have a wide range of insect hosts in different orders, but are safe for plants, animals and humans.

Nematodes are non-segmented, multicellular worms under the phylum Nematoda. They occupy diverse environmental niches.The beneficial nematode, Steinernematidae and Heterorhabditidae are the important families under the order Rhabditida. Steinernematidae have two genera ; *Steinernema* Travassos, 1927 (comprises over 95 species), and *Neosteinernema* Nguyen and Smart, 1994 (one species *Neosteinernema longicurvicauda*),whereas Heterorhabditidae is is having one genus *Heterorhabditis* Poinar, 1976 with 16 species (Hunt *et al*., 2016; Koppenhofer *et al*., 2020).

The infective juveniles (IJs) of EPNs are found in and become obligate parasites of insects (Kaya and Gaugler, 1993). Third-stage IJ stage is non-feeding, enclosed within the second-stage cuticle, with closed intestinal orifices. The second-stage cuticle is lost only when the nematodes reach

the insect gut. The pathogenic act begins when the IJ stage come in contact with the cuticle of the insect and penetrates through the spiracles, mouth or anus, and establishes itself in the haemolymph after advancing into the haemocoel (Griffin, 2012). After establishment, the nematode releases its symbiotic bacteria. *Heterorhabditis* carrying *Photorhabdus* whereas *Steinernema* carries *Xenorhabdus* bacterium. The bacteria are released either through regurgitation through mouth or esophageal pumping down through anus (Waterfield *et al*., 2009). After the release of the bacteria,the bacteria multiplies and produces a wide range of virulence factors ensuring rapid insect killing (Vicente-Diez *et al*.,2021). Shapiro-Ilan *et al*., (2017) speculated that insect death occurs within 48-72 hours due to joint effects of bacterial toxins and excretion/secretion products of IJs. The nematodes feed on the bacteria as well as decomposing insect tissues and growing to adult male and females, mate and produce eggs that hatch into a next infectious juveniles. With exhaustion of nutrients, a transient developmental stage occurs that leads to the formation of colonized pre-IJ. Pre-IJ leaves the cadaver and become mature IJs, and seeks a new insect host in soil. The life-cycle of EPN is 5-10 days. However the period depends on surrounding environmental condition, associated bacterial load and the susceptibility of insect host. The interaction between nematode-bacterium is of considerable interest in the production of bio-insecticides. Therefore, the bacterium is a promising model for the study of symbiosis and host-pathogen interactions in order to exploit and manage EPNs as a biological control agent.

2. Entomopathogenic nematodes (EPNs)

2.1. EPNs mode of reproduction

In case of *Steinernema* sp. both the male and female are required for whereas, most of the IJs develop into self-fertile hermaphrodites in case of Heterorhabditis sp. and *S. hermaphroditum* in the first generation. But,subsequent generations facilitate out-crossing. Mating behaviors also differ in the two genera - *Steinernema* males coiling around the female at the vulva, whereas *Heterorhabditis* males align parallel to the female or hermaphrodite. Steinernematid infective juveniles produced via *endotokia matricida* emerge mainly from natural openings of the maternal cadavers, while *heterorhabditids* developing infectives emerge mostly transcuticularly. In *Heterorhabditis*, juveniles developing within the mother become exclusively IJs, while eggs laid into the cadaver may continue development to adult. Ciche *et al*., (2008) suggest that intra-uterine development is an adaptation for bacterial transmission. It is a form of parental behavior

in order to optimize female's reproductive success in response to food demanding conditions. The bacteria later adapted its mode of transmission to the nematode's reproductive biology. It is also observed that great reproductive potential of EPNs reduces the negative effects of their natural enemies.

2.2. EPNs mode of infection

The infection behavior of IJs is influenced by several factors like the species, the status of the IJ (male or female, age, presence of other nematodes),duration of activity and host status (Fujimoto *et al*., 2007). Generally, an insect host is infected by tens to hundreds of individual IJ (group infection). As crowding increases, the reproductive output per invading nematode is reduced (Ryder and Griffin, 2002) and at very high densities no IJs are emerged from the cadaver (Koppenhofer *et al*., 1995). IJs can also differentiate between already infected hosts and non-infected host. A preference to invade already infected hosts is strongest during the initial stages of the infection but eventually turns to repulsion. The bacteria can induce to changes in volatile emissions in infected hosts, which results in the attraction of non-infected hosts. Normally *Heterorhabditis* and *Steinernema* cannot coexist within a host, though they can co-infect. Mutualistic bacteria are the factors for the incompatibility of the heterorhabditids and steinernematid species (Alatorrre-Rosas and Kaya, 1990; Koppenhofer *et al*., 1996). For the nematode, the host insect is a source of food and also a mating assignation. The nematode is dependent upon the bacterium for killing its insect host, producing antibiotics that suppress competing microorganisms, transforming the host tissues into a food source and creating a suitable environment for its development and reproduction. Similarly, the bacterium is dependent upon the nematode for defense from the external environment, penetration into the host's haemocoel, access to the host hemolymph ,and inhibition of the host's antibacterial proteins. The nematode may produce a toxin or a toxic substance that would destroy the inducible enzymatic defense response of the insect. Thus the nematode-bacterium complex plays an essential role against insect hosts.

3. Symbiotic bacteria

The bacteria play the major role in virulence of EPNs (Boemare *et al*., 1996; Lu *et al*., 2017). This bacterium is lethal to most soil dwelling insects but is completely safe non-target organisms.The life cycle of symbiotic bacteria having three phases,viz., phoretic in the nematode host, pathogenic in the insect body, and saprophytic in the insect cadaver.

Photorhabdus and *Xenorhabdus* are placed within the family Morganellaceae (Enterobacteriales) (Adeolu *et al*., 2016). Comparisons of 16S rDNA sequences show that *Photorhabdus* and *Xenorhabdus* genera are phylogenetically similar, but different in genetic and physiologic approaches (Chaston *et al*., 2011; Sajnaga and Kazimierczak, 2020). The symbiotic bacteria of both genera are motile, gram negative, rod-shaped and are in a nearly dormant state. Differences are present in both the genera in the location of symbiont bacteria, the nematode host range, and symbiotic strategies for parasite success, and released antibiotics and insecticidal toxins. Various surface structures such as pili/fimbriae, flagella, and the outer membrane vesicles (OMVs) present in the bacteria help adherence to gut epithelial cells. *Photorhabdus* primarily colonizes the anterior region of the intestine just posterior to the basal bulb and is also located throughout the remainder of the intestine in *Heterorhabditis*. The ventricular portion of the intestine of the steinernematid IJ is specifically modified for storage of *Xenorhabdus* and is called an intestinal vesicle (Bird and Akhurst,1983). *S.carpocapsae* intestinal vesicle contains a sub-cellular structure with which *X. nematophila* associates during initial stage of colonization, followed by bacterial division and filling of the vesicle. Most *Photorhabdus* spp. are luminescent and catalase positive, whereas *Xenorhabdus* spp. have no luminescence and are catalase negative. Parasitic success positively correlates with the number of bacteria carried per IJ. An IJ carries between 0 and 2000 cells of its symbiont bacterium (Murfin *et al*., 2012).Currently, 19 species of *Photorhabdus* and 26 species of *Xenorhabdus* have been described (Koppenhofer *et al*., 2020)(Table 1 and Table 2).

The mutualistic bacteria have evolved many strategies to invade the host, to overcome its immune responses, to infect and to kill the host insect. Each *Steinernema* species has a specific natural association with only one bacterial species *Xenorhabdus*, although any one bacterial species may be associated with more than one nematode species (Akhurst and Boemare 1990). *Heterorhaditis-Photorhabdus* relationships are more flexible: many species, both bacteria and nematodes, are able to engage in symbiotic associations with multiple species of symbiotic partners (Koppenhofer and Gaugler, 2009).The association between the bacterium and nematodeis essentially monoxenic, but other bacterial species have been isolated from the infective juvenile from various steinernematid and heterorhabditid species. A bacterial gene serves to retain the specificity between the bacterium and nematode. A few *X. nematophila* cells initiate the colonization of an infective juvenile and that these grow inside the lumen of the

intestine in a reproducible polyphasic pattern during colonization. The bacteria alone, are lethal to most insects, with LD_{50} values as low as 1 cell per insect. This quick kill may reduce the necessity to evolve a high degree of adaptation to a specific host as many nematode-bacterial complexes, most notably *S. carpocapsae* - *X. nematophila* and *H. bacteriophora* - *P.luminescens*, have host ranges that encompass hundreds of insect species. On the other hand, *S. scapterisci-X. innexi*, show a much narrower degree of host specificity. Nematode mutualistic bacteria are released after 30 mins of establishment in the case of *Heterorhabditis*, and 4-6 h for *Steinernema* .The host insect granted the release in a variable time frame depending on the species. Symptoms of infection are highly characteristic, heterorhabditid nematodes turn the host cadaver red, purple, orange, yellow, brown or sometimes green, whereas steinernematid nematodes turn the insect cadaver tan, ochre, gray or dark gray (Eckstein *et al*.,2019).The color difference is due to the pigment released by the symbiotic bacteria in the cadaver. *Xenorhabdus* and *Photorhabdus* produce phenotypic variant cell types. Both the phases have distinguished physiological, biochemical, and behavioral features. They differ in exoenzyme production, antibiotic production, outer membrane proteins, and cell surface structures (fimbriae and flagellae), symbiotic capabilities with nematode partners. The primary variant is associated with the gut of infective juvenile nematodes, whereas the secondary spontaneously arises when the bacteria are in culture or in the insect cadaver during the late stages of nematode reproduction. The transformation is believed to adapt to the external environment. The primary variant are smaller, 3-4µm in length, oval or circular shape, and induce more secretary toxins, enzymes and antibiotics, the cells are hemolytic and highly motile; they adsorb certain dyes and produce crystalline inclusions, whereas secondary variant cells are larger, 6-7 µm in length, and do not produce enzymes or antibiotics or flat colonies. In *Photorhabdus*, bioluminescence traits are greatly reduced or lost in the secondary cells. The *Photorhabdus* primary variants are associated with the production of stilbene antibiotic, antraquinone pigment and florescence that have a direct role in pathogenicity against insects and can support both nematode growth and development (Eckstein *et al*., 2019; Yuksel *et al*., 2022). Redox stress influences the formation of the *Photorhabdus* phenotypic secondary variants (Clarke, 2017). Therefore, in liquid culture method of mass culturing technique, the bacteria must be kept in their primary phenotypic variant (Lulamba *et al*., 2021).

These bacteria are known to produce many virulence factors, antibiotic and exo-enzyme compounds (Salazar-Gutierrez *et al*., 2017; Eckstein and Heermann, 2019). Insects of some species are killed by exotoxin activity whereas others are more susceptible to the endotoxins or nematode toxins. Both bacterial genera secrete extracellular, cytotoxic proteins known as hemolysins and lytic enzymes and natural products that help in killing, degrading the insect cadaver, ensuring quick death of the host. The *Photorhabdus* bacteria having insecticidal toxins, including toxins complexes (Tc's) like proteins, Makes caterpillars floppy(Mcfl-2) like proteins, *Photorhabdus* insect-related proteins (PirAB), Photorhabdus Virulence Cassettes (PVCs), repeats in toxin (Rtx) like proteins (including cytolytic toxins, metalloproteases and lipases), rhs-like elements, haemolysin/cytolysin like proteins secreted via TPS (two partner secretion) pathway, and other enterotoxins (Gatsogiannis *et al*., 2013).These are useful in the killing of an insect (Sheets and Aktories, 2017). The *Photorhabdus* Mcf1 and Mcf2 (makes caterpillars floppy) toxins result in rapid loss of insect body turgor and death. *Photorhabdus* produces enzymes that cause the insect cadaver to glow with a faint but visible yellow-green luminescence. *P. luminescens* also produce an urea lipid compound, 'phurealipids' to inhibit juvenile hormone epoxide hydrolase (JHEH), a key enzyme in insect, can be utilized to trigger the endocrine machinery of an insect at an inappropriate time (Nollmann *et al*., 2015). Bioconversion of the insect cadaver by exoenzymes produced by the bacteria allows the bacteria to multiply and the nematode to reproduce. During this process, the bacteria produce antibiotics to prevent invasion of the insect cadaver by bacterial or fungal competitors. Finally, elimination of competitors allows the bacteria and the nematode to re-associate specifically before leaving the insect cadaver. One defensive compound produced by *Xenorhabdus* is a highly specific phage tail like anti-competitor toxin known as a bacteriocin (Boemare *et al*., 1992). This molecule inhibits the growth of other closely related by binding to membrane bound receptors and depolarizing the membrane (Morales-Soto and Forst, 2011). Growth of *Xenorhabdus* is accompanied by the production of exo and endotoxins. In *Xenorhabdus*, the xenorhabdolysin (C1) hemolysin is extremely virulent and triggers apoptosis in insect (Burnell and Stock, 2000). The proteolytic enzyme of *Xenorhabdus* strains that was secreted the protease B and the Succ-AAPF-SBzl-hydrolyzingenzyme, appeared from the early logarithmic phase of growth (Massaoud *et al*., 2010). Protease B could also be detected in the hemolymph of *Xenorhabdus* infected *G.mellonella* larvae from 15 h post infection. The N-terminal sequence of protease B showed

similarity to a 55-kDa serralysin type metalloprotease in *X. nematophila*, which had been identified as an orthologue of *Photorhabdus* PrtA peptidase). In *X.nematophila*, PrtA, is involved in the immune-suppression of insect (Caldas *et al*., 2002). Ribosomal-encoded bacteriocins (xenorhabdicins) are found in *X.nematophilus*. *Xenorhabdus* spp. synthesizes secondary metabolites like ammonia, indole and stilbene derivatives. The indole-containing Xenematide from *X.nematophila* exhibits moderate antibacterial and insecticidal activities.During the nematode normal cycle of reproduction, the stilbene might act as a food signal stimulating the recovery of IJs, thus functioning as a link between food availability with both reproduction and development of *Heterorhabditis* (Bager *et al*., 2016).Two novel depsipeptides, xenematides F and G, were isolated from *X.budapestensis* SN84 with high antibacterial activity (Xi *et al*., 2019).

Certain *Photorhabdus* species exploit lipopolysaccharide modifications to resist the action of insect antimicrobial peptides (AMPs), while *X.nematophila* interfers with the expression mechanisms of host AMPs (Brivio *et al*., 2018). Protease might play a role both in inactivation of the insects's defense systems and in the digestion of the insect. *Photorhabdus* secreted more than four proteases such as PrtA peptidase, PhpC (Photorhabdus protease C), thermolysin-like enzymes, and other enzymes that may be used in the suppression of the immune responses. Lipases contribute efficiently in the degradation of insect larvae adipose tissue. Lipases have also been proven to have a role in host specificity through bioactivities against selected hosts.

Photorhabdus have a wide-ranging secondary metabolism which produces a wide range of bioactive compounds with different activities, to modulate the interaction between the bacterium and other organisms in the ecosystem (Clarke, 2017). Some are produced to increase the fitness of bacteria by killing off the indigenous insect microbiota (Derzelle *et al*., 2002), others are very potent antagonist of insect AMPs and some others are signals that protect the insect cadaver niche (Jones *et al*., 2016) such as signal molecules to mediate the communication between cell-to-cell and to coordinate the expression of genes (Ng and Bassler, 2009). These secondary metabolites are generally produced through the action of enzymes called polyketide synthase (PKS) and non-ribosomal peptide synthatase (NRPS) (Tobias *et al*., 2017). These bioactive molecules disrupt the insects' metabolic and functional properties, leading to septicemia (Brivio *et al*., 2018). In addition to preserving the insect cadaver until the nematode life cycle is complete, antibiotic production is a key mechanism to assure nematode-bacterial specificity.

Oxin-antitoxin systems are implicated in killing related bacteria and self protection (Cariss *et al*., 2010). *Photorhabdus* bacteria accumulate Cu, Fe, and Co for activation of their metalloenzymes to enhance their virulence potential and reduce mortality (Meligy, 2018).

The release of bacteria by nematodes is clearly a challenge to the host that, once initiated, is difficult to overcome, as each nematode can release 50-200 bacteria directly into the hemolymph (Goodrich-Blair, 2007). Insect innate immunity consists of mechanisms that lead to the production of antimicrobial peptides (AMPs) and reactive oxygen species (ROS), and cellular functions involving phagocytosis, encapsulation and nodulation (Viljakainen, 2015). EPNs and their associated bacteria have evolved with several defense mechanisms to elude and counteract the host insect immune responses (Brivio and Mastore, 2020). The products of these bacteria are capable of damaging hemocytes, and enzymes such as the RTX-like metalloprotease of *Photorhabdus* that can slash hemolymph proteins involved in regulating host immune effector genes (Vlisidou *et al*., 2019). The AMPs from bacteria with novel mode of action, are nematophin from *X. nematophilus* strain BC1 (Li *et al*., 1997), odilorhabdin from *X.nematophila* (Pantel *et al*., 2018), darobactin from *P. khanii* HGB1456 (Imai *et al*., 2019) and photoditritide from *P. 8eorgiana* Meg1 (Maglangit *et al*., 2021). *S.carpocapsae* can produce proteolytic secretions that can interfere with the host immune system .The surface proteins of *S.glaseri* protect from encapsulation by the host immune system of *Popillia japonica* (Wang and Gaugler, 1999). Lytic surface coat proteins, hydrophobic exudates, and lipopolysaccharide-like binding proteins produced by nematodes can all facilitate the parasite's evasion of encapsulation.The proteolytic secretions or proteases secreted by *Steinernema* contribute to virulence appears to be through immune suppression, e.g., inhibiting phenoloxidase activity and/or affecting hemocyte function (Toubarro *et al*., 2009), whereas *Heterorhabditis* do not secrete any proteases and are not virulent in the absence of their bacterial symbionts. *Heterorhabditis* appears to actively modulate the insect immune system by reducing the phagocytic capability of the circulating hemocytes in *Manduca sexta* (Eleftherianos *et al.*, 2010).

Table.1. *Xenorhabdus* strains associated with *Steinernema* species

Bacterium	Nematode	References
X. nematophila	*Steinernema carpocapsae*	Poinar and Thomas ,1965
X. poinarii	*S. glaseri,S. cubanum*	Akhurst ,1983
X. japonica	*S. kushidai*	Yamanaka *et al*. ,1992

X.bovienii	*S.intermedium*	Boemare *et al*., 1993
X. indica	*S.abbasi*	Elawad *et al*. ,1997
X.innexi	*S. scapterisci*	Lengyel *et al*., 2005
X.szentirmaii	*S. rarum*	
X.ehlersii	*S.serratum*	
X.bovienii	*S.sichuanense*	Mracek *et al*., 2006
X. khoisanae	*S. khoisanae*,	Ferreira *et al*., 2013
	S. jeffreyense, S. saccharii	
X. kozodoii	*S.arenarium*	Tailliez *et al*. ,2006
X. hominickii	*S. karii, S. monticolum*	
X.griffiniae	*Steinernema sp.*	Tailliez *et al*. ,2006
		Dreyer *et al*., 2017
X. romanii	*S.puertoricense*	Tailliez *et al*., 2006
X. stockiae	*S. siamkayai*	
X.koppenhoeferi	*S. scarabaei*	Tailliez *et al*., 2012
X.magdalenensis	*S. australe*	Tailliez *et al*. ,2006
X. ishibashii	*S. aciari*	Kuwata *et al*. ,2013
X. indica	*S.yirgalemense*	Ferreira *et al*. ,2014b
X. doucetiae	*S. diaprepesi*	Ogier *et al*. ,2016
X. stockiae	*S.surkhetense*	Bhat *et al*. ,2020
X.vietnamensis	*S. sangi*	Kailliez *et al*., 2010
X. thuongxuanensis		Kampfer *et al*., 2017
X. doucetiae	*S. diaprepesi*	Tailliez *et al*., 2006
X. hominickii	*S. monticolum*	Park *et al*. ,2017

X.bovienii	*S. poinari, S. feltiae, S. kraussei, S. affinae, S. intermedium, S. weiseri, S. silvaticum, S. sichuanense, S. nguyeni, S. poinari, S. tbilisiensis, S. jollieti, S. puntauvense, S. oregeonense, S. litorale*	Sajnaga *et al*., 2018 Akhurst and Boemare, 1988
X. indica	*S. pakistanense*	Bhat *et al*., 2019
X. beddingii	*S. longicaudatum*	Ubaub and Stock ,2018
X. ehlersii		Lengyel *et al*., 2005
X. eapokensis	*S. eapokensis*	Kampfer *et al*., 2017
X. budapestensis	*S. bicornutum, S. ceratophorum*	Lengyel *et al*., 2005
X. cabanillasii	*S. riobrave*	Tailliez *et al*., 2006

Table.2. *Photorhabdus* strains associated with *Heterorhabditis* species

Bacterium	Nematode	References
P. luminescens	*H. bacteriophora, H. indica*	Thomas and Poinar, 1979
P. akhurstii	*H. indica*	Fischer-Le Saux *et al*., 1999, Machado *et al*., 2018
P. asymbiotica subsp. australis	*H. gerrardi, H. indica*	Akhurst *et al*., 2004, Machado *et al*., 2018
P. bodei	*H. beicherriana*	Machado *et al*., 2018
P. caribbeanensis	*H. bacteriophora*	Tailliez *et al*., 2010, Machado *et al*., 2018
P. temperate subsp. *cinerea*	*H. downesi, H. megidis, H. bacteriophora*	Toth and Lakatos, 2008; Machado *et al*., 2018
P. heterorhabditis	*H. zealandica*	Ferreira *et al*., 2014a
P. kayaii	*H. bacteriophora*	Hazir *et al*., 2004, Machado *et al*., 2018

P. khanii	*H. bacteriophora*	Tailliez *et al*., 2010, Machado *et al*., 2018
P. khanii subsp. *guanajuatensis*	*H. atacamensis*	Machado *et al*., 2019
P luminescens subsp. kleinii	*H. georgiana, H. bacteriophora,*	An and Grewal, 2011 Machado *et al*., 2018
P. laumondii subsp. *clarkei*	*H. bacteriophora*	Fischer-Le Saux *et al*., 1999
P. laumondii subsp. *laumondii*	*H. bacteriophora*	Machado *et al*., 2018
P. luminescens subsp. *sonorensis*	*H. sonorensis*	Boemare *et al*., 1993; Orozco *et al*., 2013
P. luminescens subsp. *mexicana*	*H. mexicana*	Machado *et al*. ,2019
P. luminescens subsp. *namnaonensis*	*H. baujardi*	Glaeser *et al*., 2016
P. stackebrandtii	*H. bacteriophora, H. georgiana*	An and Grewal, 2010, Machado *et al*., 2018
P. tasmanensis	*H. zealandica, H. marelatus*	Tailliez *et al*., 2010, Machado *et al*. 2018
P. temperata	*H. megidis, H. downesi, H. zealandica*	Fischer-Le Saux *et al*., 1999; Machado *et al*., 2018
P. luminescens subsp.*kayaii*	*H. bacteriophora*	Hazir *et al*., 2004 Tailliez *et al*., 2010
P. luminescens subsp. *thracensis*		Machado *et al*., 2018
P. khanii subsp.*guanajuatensis*	*H. atacamensis*	Machado *et al*., 2019

P.luminescens subsp. *noenieputensis*	*Heterorhabditis* sp. Strain 158-C		Ferreira *et al*., 2013
P.heterorhabditis subsp.*aluminescens*	*Heterorhabditis sp.*		Machado *et al*., 2021

4. Microorganism-host interactions: Model system

The symbiotic bacteria of entomopathogenic nematodes emerged as a relevant model systems for studying basic mechanisms and dynamics in host-microbe interactions. The bacteria interact with the nematode mid-gut and the insect hemolymph. As the EPNs bacteria are responsible for the mutualism and pathogenic interactions with their hosts- nematodes and insects; their studies have advanced these organisms as a relevant biological model in fields of soil ecology, symbiotic relationships, and evolutionary biology (Chaston *et al*., 2011; Stock, 2015). The interaction between the infective-stage nematode and its bacterial symbiont is based largely on population-level, lethal time point studies on these organisms. Some limitation arises when investigating temporal dynamics of the bacterium within the nematode as there is a difficulty in isolation and maintenance of individual living nematodes and tracking colonizing bacterial cells over time.

Nematodes also play a significant role in the pathogenicity of the nemato-bacterial complex (Han and Ehlers, 2001) as well as the symbiotic bacteria species/strains producing different amounts and types of toxin complexes (Wenski *et al*.,2020). Therefore, strains could be developed that produce the proteins necessary for evasion, or nematodes could be generated that have significantly decreased delays in the ejection timing of their bacterial endosymbionts, as nematodes that can overwhelm the insect immune system before being encapsulated. The simplicity, tractability, and conserved genes of many nematode species have additional advantage as models for host-bacterial interactions. Their genome contain genes related to both nematode symbiont and insect host specificities, resistance to extreme environmental conditions, and control of both phenotypic and phase variation; which are the main factors for improvement of both nematode and the bacteria (Sheets and Aktories, 2017). Genomic analysis of *Photorhabdus* can interpret the relation between pathogenesis and symbiosis, thereby providing vital information for the development of biocontrol agents. Overall, the interaction can then be characterized as a highly complex interplay between the genotypes of the insect, the nematode

and its mutualistic bacteria, which provide a number of potential knowledge for control of insect pests that could be beneficial to agriculture if applied appropriately.

5. Conclusion

This review highlights the symbiotic bacteria and EPN interactions as prospective in mode of action against insects. The commercial use of the bacterial symbiont is related to the utilization of the nematode species with which they are associated. To achieve full efficacy and eco-sustainability, they can be used in combination with other agrochemicals with lower doses. However, the effectiveness of EPNs is often associated with a proper application in the field, to ensure a proper coverage of foliage. This has led to the development of special processing and formulation of bacteria-based bio-insecticides, with the aim of maximizing shelf-life, improving dispersion and adhesion, reducing spray drift, and above all enhancing efficacy. Advanced technologies aiming at increasing residual effects comprise micro-encapsulations and micro-granules. Further research is required on purifying and characterization of toxins or AMPs present in the symbionts and to clarify cloning the genes encoding this toxin. Understanding the mode of action, ecological adaptations, host range, and dynamics of pathogen-arthropod-plant interactions is essential for successfully utilizing entomopathogen-based biopesticides for pest management in agriculture.

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