Review Article

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

Abstract

Entomopathogenic nematodes (EPNs) *Steinernema* and *Heterorhabditis* are obligate parasites of insects of many commercial crops and they can provide effective bio-control of the insect pests.They are having a symbiotic relationships with a bacterium species. *Photorhabdus* and *Xenorhabdus* bacteria live in association with *Heterorhabditis* and *Steinernema* ,respectively. In the nematode bacteria association , nematode and bacteria engage in specific, persistent, and mutualistic relationships.With the help of the nematode, the bacteria penetrate and proliferate inside the insect hemocoel, and kill the insect host and digest the host tissues, thereby providing suitable nutrient conditions for nematode growth and development.The EPNs and bacterial complexes are effective over a wide range of insect orders and both the EPN and bacteria utilize a variety of pathogenic strategies. The importance for research in EPNs and their symbiotic bacteria are increasing and progress has been made in the fields of biology, ecology, biodiversity, symbiosis and molecular genetics.The simplicity, tractability, and conserved genes of many EPN species supported their use as models for diverse biological studies.This review describes the current state of knowledge of entomopathogenic nematodes and their symbiotically associated bacteria and the interaction between them and the resultant death of insect. The knowledge gained from this studies should ensure that EPNs will be an effective biopesticides.

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

1.Introduction

Agricultural production is threatened by several biotic and abiotic stresses. Among biotic stresses insect pests are one of the major and conspicuous limiting factors of production. Though chemical pesticides are common recommended practices for the control of insect pests, the use of these pesticides has raised many environmental and biological concerns. Insect-pathogenic nematodes (EPNs) are of interest as biological pesticides to replace chemical pesticides that can assist in maintaining a desirable ecological balance. The EPNs have a wide range of insect hosts which are killed rapidly, but are safe for non-targeted organisms such as plants, animals and humans, they are called as beneficial nematodes (Chang *et al*.,2019).

Nematodes are microscopic, multicellular, and non-segmented worms under the phylum Nematoda. They occupy diverse environmental niches. Steinernematidae and Heterorhabditidae are the important families under the order Rhabditida and are widely used as control agents of insects. There are two genera in Steinernematidae, *Steinernema* Travassos, 1927 (comprises over 95 species), and *Neosteinernema* Nguyen and Smart, 1994(one species *Neosteinernema longicurvicauda*). Heterorhabditidae is solely represented by the genus *Heterorhabditis* Poinar, 1976 with 16 species (Hunt *et al*., 2016; Koppenhofer *et al*., 2020).

The infective juveniles (IJs) of EPNs are soil-adapted and obligate parasites of Insects (Kaya and Gaugler, 1993). IJ stage is non-feeding third-stage larvae enclosed within the second-stage cuticle, with closed intestinal orifices and is lost only when the nematodes reach the insect gut. The pathogenic act begins when the IJ stage attaches to the cuticle of the insect, penetrates through the natural openings, such as the spiracles, mouth or anus, and establishes itself in the hemolymph after advancing into the body cavity (Griffin, 2012). Once established, the nematode releases its mutualistic bacteria into the host hemolymph. Nematode-bacterium associations include those between entomopathogenic nematodes *Heterorhabditis* and *Steinernema* and the *Photorhabdus* and *Xenorhabdus* respectively. The bacteria are released either through regurgitation or esophageal pumping down through the intestine and out of the anus (Waterfield *et al*., 2009). After the bacteria are released, the bacterium produces a wide variety of virulence factors ensuring rapid insect killing (Vicente-Diez *et al*.,2021). Death of the insect occurs within 48-72 hours after infection due to joint effects of bacterial multiplication and excretion/secretion products of IJs (Shapiro-Ilan *et al*., 2017). The nematodes begin to feed on the bacteria and the decomposing insect carcass. During nematode development, male and females mate and produce eggs that hatch into a subsequent generation of infectious juveniles. A high density of nematodes and depletion of nutrients trigger the formation of pre-IJ, a transient developmental stage that leads to the formation of colonized IJs. Immature IJs leave the cadaver, become mature IJs, and seek a new insect host. The life-cycle of EPN is 5-10 days, depending on temperature, bacterial symbiont, and ability to suppress the immunity of the insect host. However, the pathogenicity of EPN species and their symbiotic bacteria is governed by several factors such as host recognition, acceptance and infection behavior. The interaction between nematode-bacterium is of considerable interest in the production of bio-insecticides. The mutualistic relationship between the nematode and bacterium is known to be followed: the nematode is dependent upon the bacterium for quickly killing its insect host, creating a suitable environment for its development by producing antibiotics that suppress competing microorganisms, transforming the host tissues into a food source, and serving as a food resource. The bacterium is dependent upon the nematode for protection from the external environment, penetration into the host's hemocoel, and inhibition of the host's antibacterial proteins. Therefore, the bacterium is a promising model for the study of symbiosis and host-pathogen interactions and information of the nematode biology is important for improving our ability to exploit and manage EPNs as a biological control agent.

2. Entomopathogenic nematodes (EPNs)

2.1. EPNs mode of reproduction

For a steinernematid to reproduce there must be both the male and female present in the cadaver. On the other hand, most of the IJs develop into self-fertile hermaphrodites in case of Heterorhabditids and *S. hermaphroditum*, in the first generation and facilitating out-crossing in subsequent generations. Mating behaviors differ in the two genera, with *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 symbiont transmission. This observable fact has evolved to optimize female's reproductive success in response to food demanding conditions, as a form of parental behavior, and that the bacteria later adapted its mode of transmission to the nematode's reproductive biology. It is also observed that great reproductive potential of EPNs dampens the potential negative effects of their natural enemies.

2.2. EPNs mode of infection

The infection behavior of IJs is influenced by several factors like host status, the species, and the status of the IJ (male or female, status of infectivity, age, presence of other nematodes) and duration of activity (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 bacteria are

essential to the EPN. They kill the insect host in a short period, providing a suitable environment for the EPN to reproduce and produce antibiotics and secondary metabolites that prevent any development of other microorganisms and convert the host tissue into food. In exchange, the EPN provide protection and access to the host hemolymph. 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 a vital role against insect hosts.

3. Symbiotic bacteria

The bacteria play the major role in killing the host and virulence of EPNs (Boemare *et al*., 1996; Lu *et al*., 2017). The life cycle of all EPN symbiotic bacteria is similar and can be divided into three phases: phoretic in the nematode host, pathogenic in the insect body, and saprophytic in the insect cadaver. This bacterium is lethal to most soil dwelling insects but is completely safe for a large variety of plant and animal species.

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 genetic and physiologic approaches to each other (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 quiescent state, protected within their nematode body. Differences are present in the location of symbiont bacteria, the nematode host range, and symbiotic strategies for parasite success, and arrays of 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. In *Heterorhabditis*, *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.The ventricular portion of the intestine of the steinernematid IJ is specifically modified for storage of symbiotic bacteria (*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, although any one bacterial species may be associated with more than one nematode species (Akhurst and Boemare 1990). Whereas the symbiotic *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 and *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*, *S. jeffreyense, S. saccharii*	Ferreira *et al*., 2013
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 *X. thuongxuanensis*	*S. sangi*	Kailliez *et al*., 2010 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 *X. ehlersii*	*S. longicaudatum*	Ubaub and Stock ,2018 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 and *Heterorhabditis* species

Bacterium	Nematode	
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. 11 eorgiana, 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,*	Fischer-Le Saux *et al*., 1999;

	H. zealandica	Machado *et al*., 2018
P. luminescens subsp.*kayaii* *P. luminescens* subsp. *thracensis*	*H. bacteriophora*	Hazir *et al*., 2004 Tailliez *et al*., 2010 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.

6. References

Adeolu, M., Alnajar, S., Naushad, S., Gupta, RS. 2016. Genome-based phylogeny and taxonomy of the ‘Enterobacteriales’: Proposal for Enterobacterales ord. nov. divided into the families Enterobacteriaceae, Erwiniaceae fam.nov., Pectobacteriaceae fam.nov., Yersiniaceae fam.nov., Hafniaceae fam.nov., Morganellaceae fam.nov., and Budviciaceae fam. Nov. International Journal of Systematic and Evolutionary Microbiology 66, 5575-5599

Akhurst, R., Boemare, NF. 1990.Biology and taxonomy of *Xenorhabdus* In: Entomopathogenic nematodes in biological control. R.Gaugler and HK Kaya (Eds.). Boca Raton, CRC Press.pp.75-87.

Akhurst, RJ., Boemare, NE. 1988. A numerical taxonomic study of the genus *Xenorhabdus* (Enterobacteriaceae) and proposed elevation of the subspecies of *X. nematophilus* to species. Journal of General Microbiology 134(7), 1835-1845

Akhurs,t RJ., Boemare, NE., Janssen, PH., Peel, MM., Alfredson, DA., Beard CE. 2004.Taaxonomy of Australian clinical isolates of the genus *Photorhabdus* and proposal of *Photorhabdus asymbiotica* subsp. *asymbiotica* subsp.nov. and *P.asymbiotica* subsp. *australis* subsp.nov. International Journal of Systematic and Evolutionary Microbiology 54, 1301-1310.

Akhurst, RJ.1983.Taxonomic study of *Xenorhabdus*, a genus of bacteria symbiotically associated with insect pathogenic nematodes. International Journal of Systematic Bacteriology 33,38-45.

Alatorre-Rosas, R., Kaya, HK. 1990. Interspecific competition between entomopathogenic nematodes in the genera *Heterorhabditis* and *Steinernema* for an insect host in sand. Journal of Invertebrate Pathology 55(2), 179-188.

An, R., Grewal, PS. 2011. *Photorhabdus luminescens* subsp. *kleinii* subsp.,nov. (Enterobacteriales: Enterobacteriaceae).Current Microbiology 62,539-543.

Bager, R., Roghanian, M., Gerdes, K., Clarke, DJ. 2016. Alarmone (p) ppGpp regulates the transition from pathogenicity to mutualism in *Photorhabdus* luminescens. Molecular Microbiology 100,735-747.

Bhat, AH., Chaubey, AK., Puza, V. 2019. The first report of *Xenorhabdus indica* from *Steinernema pakistanense*: co-phylogenetic study suggests co-speciation between *X.indica* and its steinernematid nematodes. Journal of Helminthology 93(1), 81-90.

Bhat, AH., Sharma, L., Chaubey, AK. 2020. Characterisation of *Steinernema surkhetense* and its symbiont *Xenorhabdus stockiae* and a note on its geographical distribution. Egyptian Academic Journal of Biological Sciences 13(1),105-122.

Bird, AF., Akhurst, RJ.1983.The nature of the intestinal vesicle in nematodes of the family Steinernematidae. International Journal of Parasitology 13,599-606.

Boemare, NE. , Boyer-Giglio, MH. , Thaler, JO. , Akhurst, RJ. , Brehelin, M. 1992. Lysogeny and bacteriocinogeny in *Xenorhabdus nematophilus* and other *Xenorhabdus* spp. Applied and Environmental Microbiology 58, 3032- 3037

Boemare, NE., Laumond, C., Mauleon, H.1996. The entomopathogenic nematode bacterium complex: Biology, life cycle and vertebrate safety. Biocontrol Science and Technology 6, 333-346.

Boemare, NE., Akhurst, RJ., Mourant, RG. 1993. DNA relatedness between *Xenorhabdus* spp. Enterobacteriaceae), symbiotic bacteria of entomopathogenic nematodes, and a proposal to transfer *Xenorhabdus luminescens* to a new genus, *Photorhabdus* gen. nov. International Journal of Systematic Bacteriology 43, 249-255.

Brivio, MF., Mastore, M. 2020. When appearance misleads: The role of the entomopathogen surface in the relationship with its host. Insects 11, 387. DOI: 10.3390/insects11060387.

Brivio, MF., Toscano, A., De Pasquale, SM., De Lerma Barbaro, A., Giovannardi, S., Finzi, G, 2018. Surface protein components from entomopathogenicnematodes and their symbiotic bacteria: effects on immune responses of the greater wax moth, *Galleria mellonella* (Lepidoptera: Pyralidae). Pest Managent Science 74, 2089-2099. doi: 10.1002/ps.4905

Burnell, AM., Stock, P.2000. *Heterorhabditis, Steinernema* and their bacterial symbionts: lethal pathogens of insects. Nematology 2, 31-42.

Caldas, C., Cherqui, A., Pereira, A., Simoes, N. 2002. Purification and characterization of an extracellular protease from *Xenorhabdus nematophila* involved in insect immune-suppression. Applied and Environmental Microbiology 68, 1297-1304.

Cariss, SJ., Constantinidou, C., Patel, MD., Takebayashi, Y., Hobman, JL., Penn, CW., Avison MB. 2010. YieJ (CbrC) mediates CreBC-dependent colicin E2 tolerance in *Escherichia coli*. Journal of Bacteriology 192(13),3329-3336.

Chaston, JM., Suen, MG., Tucker, SL., A W *et al*..2011. The entomopathogenic bacterial endosymbionts *Xenorhabdus* and *Photorhabdus* : Convergent lifestyles from divergent genomes.PLoS One 6.e27909.10.1371/journal.pone.0027909.

Chang, DZ., Serra, L., Lu, D., Mortazavi, A., Dillman, AR. 2019. A core set of venom proteins is released by entomopathogenic nematodes in the genus *Steinernema*. PLoS Pathogens 15, e1007626. doi: 10.1371/journal.ppat.1007626

Ciche, TA., Kim, KS., Kaufmann-Daszczuk, B., Nguye,n KC., Hall, DH.2008. Cell invasion and matricide during *Photorhabdus luminescens* transmission by *Heterorhabditis bacteriophora* nematodes. Applied and Environmental Microbiology 74, 2275-2287

Clarke, DJ. 2017. The regulation of secondary metabolism in *Photorhabdus*. In: The molecular biology of *Photorhabdus* bacteria. Current Topics in Microbiology and Immunology.Vol.402.Ffrench-Constant R(Ed.).Springer, Cham.pp.81-102.

Derzelle, S., Duchaud, E., Kunst, F., Danchin, A., Bertin, P. 2002. Identification, characterization, and regulation of a cluster of genes involved in carbapenem biosynthesis in *Photorhabdus luminescens*. Applied and Environmental Microbiology. 68(8), 3780-3789. DOI:10.1128/AEM. 68.8.3780-3789.2002.

Dreyer, J., Malan, AP., Dicks, MT. 2017. First report of a symbiotic relationship between *Xenorhabdus griffiniae* and an unknown *Steinernema* from South Africa. Archives of Microbiology 200,349-353.

Eckstein, S., Dominelli, N., Brachmann, A., Heermann, R. 2019. Phenotypic heterogeneity of the insect pathogen *Photorhabdus luminescens*: insights into the fate of secondary cells. Applied and Environmental Microbiology 85(22). https://doi.org/10.1128/AEM.01910-19

Eleftherianos, I., Ffrench-Constant, R., Clarke, DJ., Dowling, AJ. 2010. Dissecting the immune response to the entomopathogen *Photorhabdus*.Trends in Microbiology 18(12), 552-560.

Ferreira, T., Reenen, C., Pages, S., Tailliez, P., Malan, AP., Dicks, LM. 2013. *Photorhabdus luminescens* subsp. *noenieputensis* subsp.nov., a symbiotic bacterium associated with a novel *Heterorhabditis* species related to *Heterorhabditis indica*. International Journal of Systematic and Evolutionary Microbiology 63(5), 1853-1858.

Ferreira, T., Van, CA., Tailliez, P., Pages, S., Malan, A., Dicks, LMT.,2014. First report of the symbiotic bacterium *Xenorhabdus indica* associated with the entomopathogenic nematode *Steinernema yirgalemense*. Journal of Helminthology 1-5. DOI: 10.1017/S0022149X14000583.

Ferreira, T., Reenen, C., Endo, A., Tailliez, P., Pages, S., Sproer, C., Malan, AP., Dicks, LMT. 2014. *Photorhabdus heterorhabditis* sp. nov., a symbiont of the entomopathogenic

nematode *Heterorhabditis zealandica*. International Journal of Systematic and Evolutionary Microbiology 64,1540-1545.

Fischer-Le Saux, M., Viallard, V., Brunel, B., Normand, P., Boemare, NE. 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. International Journal of Systematic Bacteriology 49(4),1645-56. doi: 10.1099/00207713-49-4-1645.

Fujimoto, A., Lewis, EE., Cobanoglu, G., Kaya, HK. 2007. Dispersal , infectivity and sex ratio of early or late emerging infective juveniles of the entomopathogenic nematode *Steinernema carpocapsae*. Journal of Nematology 39, 333-337.

Gatsogiannis, C., Lang, A., Meusch, D., Pfaumann, V., Hofnagel, O., Benz, R., Raunser, S.2013. A syringe-like injection mechanism in *Photorhabdus luminescens* toxins.Nature 495, 520-523.

Glaeser, S., Tobias, N., Thanwisai, A., Chantratita, N., Bode, HB. 2017. *Photorhabdus luminescens* subsp. *namnaonensis* subsp.nov., isolated from *Heterorhabditis baujardi* nematodes in Nam Nao district of central Thailand. International Journal of Systematic and Evolutionary Microbiology 67(4), DOI: 10.1099/IJSEM.0.001761.

Goodrich-Blair, H. 2007. They've got a ticket to ride: *Xenorhabdus nematophila* - *Steinernema carpocapsae* symbiosis . Current Opinion in Microbiology 10, 225-230.

Griffin, CT. 2012. Perspectives on the behavior of entomopathogenic nematodes from dispersal to reproduction: traits contributing to nematode fitness and biocontrol efficacy. Journal of Nematology 44(2),177-84.

Han, R., Ehlers, R. 2001. Effect of *Photorhabdus luminescens* phase variants on the in vivo and in vitro development and reproduction of the entomopathogenic nematodes *Heterorhabditis bacteriophora* and *Steinernema carpocapsae*. FEMS Microbiology Ecology 35(3), 239-247. doi: 10.1111/j.1574-6941.2001.tb00809.x.PMID: 11311434

Hazir, S., Stackebrandt, E., Lang, E., Schumann, P., Ehlers, RU., Keskin, N. 2004. Two new subspecies of *Photorhabdus luminescens*, isolated from *Heterorhabditis bacteriophora* (Nematoda: Heterorhabditidae): *Photorhabdus luminescens* subsp. kayaii subsp. nov. and

Photorhabdus luminescens subsp. thracensis subsp. nov. Systematic and Applied Microbiology 27(1),36-42. doi: 10.1078/0723-2020-00255.

Hunt, DJ., Nguyen, KB., Spiridonov, SE. 2016. Steinernematidae: Species descriptions. Adavances in entomopathogenic nematode taxonomy and phylogeny. Brill. p.111.

Imai, Yu., Meyer, KJ., Linishi, A., Favre-Godal, Q., Green, R., Manuse, S. 2019. A new antibiotic selectively kills gram-negative pathogens. Nature 576(7787),459

Jones, RS., Fenton, A., Speed, MP. 2016. 'Parasite-induced aposematism' protects entomopathogenic nematode parasites against invertebrate enemies. Behavioral Ecology 27, 645-651.

Kampfer, P., Tobias, N., Long, PK., Bode, HB., Glaeser, S. 2017. *Xenorhabdus thuongxuanensis* sp.nov.and *Xenorhabdus eapokensis* sp.nov., isolated from *Steinernema* species. International Journal of Systematic and Evolutionary Microbiology 67(5), DOI: 10.1099/ijsem.0.001770.

Kaya, HK., Gaugler, R .1993. Entomopathogenic nematodes. Annual Review of Entomology 38(1), 181-206.

Koppenhofer, AM., Shapiro, DI., Hiltpold, I.2020.Entomopathogenic nematodes in sustainable food production. Frontiers in Sustainable Food systems. 4,1-14. doi: 10.3389/fsufs.2020.00125

Koppenhofer, AM., Fuzy, AM., Kaya, HK. 1996. Coexistence of two Steinernematid nematode species (Rhabditida: Steinernematidae) in the presence of two host species. Applied Soil Ecology 4,221-230.

Koppenhofer, AM., Kaya HK., Shanmugam S., Wood GL.1995. Inter-specific competition between steinernematid nematodes within an insect host. Journal of Invertebrate Pathology 66, 99-103.

Koppenhofer, HS., Gaugler, R. 2009. Entomopathogenic nematode and bacteria mutualism. In: White J, Torres M (eds) Defensive mutualism in microbial symbiosis. CRC Press, Boca Raton, Florida, pp 99-116

Kuwata, R., Qiu, L.,Wang, W., Harada, Y., Yoshida, M., Kondo, E., Yoshiga, T. 2013. *Xenorhabdus ishibashii* sp.nov., isolated from the entomopathogenic nematode *Steinernema aciari*. International Journal of Systematic and Evolutionary Microbiology 63(5),1690-1695.

Lengyel, K., Lang, E., Fodor, A., Szallas, 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. Systematic and Applied Microbiology 28, 115-122.

Li, J., Chen, G., Webster, JM.1997. Nematophin, a novel antimicrobial substance produced by *Xenorhabdus nematophilus* (Enterobactereaceae). Canadian Journal of Microbiology 43(8),770-773.

Lu, D., Macchietto, M., Chang, D., Barros, MM., Baldwin, J., Mortazavi, A., Dillman, AR. 2017.Activated entomopathogenic nematode infective juveniles release lethal venom proteins. PLoS Pathogens 13(4),e1006302. doi:10.1371/ journal.ppat.1006302.

Lulamba, TE., Green, E, Serepa-Dlamini, MH. 2021. Genome assembly and annotation of *Photorhabdus heterorhabditis* strain ETL reveals genetic features involved in pathogenicity with its associated entomopathogenic nematode and anti-host effectors with biocontrol potential applications. Gene. 795, 145780. doi: 10.1016/j.gene.2021.145780.

Machado, RAR., Wuthrich, D., Kuhnert, P., Arce, CCM., Thonen, L., Ruiz, C., Zhang, X., Robert, CAM., Karimi, J., Kamali, S., Ma, J., Bruggmann, R., Erb, M. 2018. Whole-genome-based revisit of Photorhabdus phylogeny: proposal for the elevation of most *Photorhabdus* subspecies to the species level and description of one novel species *Photorhabdus bodei* sp. nov., and one novel subspecies *Photorhabdus laumondii* subsp. *clarkei* subsp. nov. International Journal of Systematic and Evolutionary Microbiology 68, 2664-2681. DOI: https : //doi.Org/10.1099/ ijsem.0.002820, PMID: 29877789.

Machado, RAR., Muller, A., Ghazal, SM., Thanwisai, A., Pages, S., Bode, HB., Hussein, MA., Khlil ,KM., Tisa, LS. 2021.*Photorhabdus heterorhabditis* subsp.aluminescens subsp. nov., *Photorhabdus heterorhabditis* sub sp. heterorhabditis subsp.nov., *Photorhabdus australis* subsp.thailandensis subsp.nov., *Photorhabdus australis* subsp.australis subsp.nov., and Photorhabdus aegyptia sp.nov.isolated from Heterorhabditis

entomopathogenic nematodes. International Journal of Systematic and Evolutionary Microbiology 71(1),10.1099/ijsem.0.004610.hal-03143425

Maglangit, F., Yu, Y., Deng, H. 2021. Bacterial pathogens: threat or treat(a review on bioactive natural products from bacterial pathogens. Natural Product Reports 38, 782-821.

Massaoud, MK., Marokhazi, J., Fodor ,A., Venekei, I. 2010. Proteolytic enzyme production by strains of the insect pathogen *Xenorhabdus* and characterization of an early-log-phase-secreted protease as a potential virulence factor. Applied and Environmental Microbiology 76, 6901-6909.

Meligy, AMA. 018. Comparative study of element contents in seven isolates of entomopathogenic nematodes. Egyptian Journal of Biological Pest Control 28,1.DOI 19.1186/s41938-017-0002-3.

Morales-Soto, N., Forst, SA. 2011. The xnp1 P2-like tail synthesis gene cluster encodes xenorhabdicin and is required for interspecies competition. Journal of Bacteriology 193, 3624-3632 . https://doi.org/ 10.1128/JB. 00092-11

Mracek, Z., Nguyen, KB., Tailliez, P., Boemare, N., Chen S. 2006. *Steinernema sichuanense* n.sp (Rhabditida, Steinernematidae), a new species of entomopathogenic nematode from the province of Sichuan, east Tibetan Mts., China. Journal of Invertebrate Pathology 93(3), 157-169.

Murfin, KE., Dillman, AR., Foster, JM., Bulgheresi, Slatko ,BE., Sternberg, PW., Goodrich-Blair, H. 2012. Nematode-Bacterium symbioses-cooperation and conflict revealed in the 'Omics' age. The Biological Bulletin 223(1),85-102.

Ng, WL., Bassler, BL. 2009. Bacterial quorum-sensing network architectures. Annual Review of Genetics 43,197-222.

Nollmann, FI., Heinrich, AK., Brachmann, AO., Morisseau, C., Mukherjee, K., Casanova-Torres, AM., Strobl, F., Kleinhans, D., Kinski, S., Schultz, K., Beeton, ML., Kaiser, M., Chu, YY., Ke, LP., Thanwisai, A., Bozhuyuk, KAJ., Chantratita, N., Gçtz, F., Waterfield, NR., Vilcinskas, A., Stelzer, EHK., Goodrich-Blair, H., Hammock, BD., Bode, HB. 2015. A *Photorhabdus* natural product inhibits insect juvenile hormone Epoxide Hydrolase. ChemBioChem 16,766 - 771.

Ogier, JC., Duvic, B., Lanois, A., Givaudan, A., Gaudriault, S.2016. A new member of the growing family of contact-dependent growth inhibition systems in *Xenorhabdus doucetiae*. PLoS ONE 11(12),e0167443 doi: 10.1371/journal. pone. 0167443.

Orozco, RA., Hill, T., Stock, SP. 2013. Characterization and phylogenetic relationships of *Photorhabdus* sub sp. *sonorensis* (y-proteobacteria: enterobacteriacae), the bacterial symbiont of the entomopathogenic nematode *Heterorhabditis sonorensis* (Nematoda: Heterorhabditidae). Current Microbiology 66(1),30-39.

Pantel, L., Florin, T., Dobosz, M., Mankin, A., Polikanov, YS. 2018. Odilorhabdins, antibacterial agents that cause miscoding by binding at a new ribosomal site. Molecular Cell 70(1), 83-94.

Park, Y., Kang, S., Sadekuzzaman, M., Kim, H., Jung, JK., Kim, Y.2017. Identification and bacterial characteristics of *Xenorhabdus hominickii* ANU101 from an entomopathogenic nematode,*Steinernema monticolum*. Journal of Invertebrate Pathology 144,74-87.

Poinar, GO Jr., Thomas, GM.1965. A new bacterium, *Achromobacter nematophilus* sp.nov.(Achromobacteriaceae: Eubacteriales) associated with a nematode. International Bulletin of Bacteriological Nomenclature and Taxonomy 15, 249-252.

Ryder, JJ ., Griffin, CT. 2002. Density dependent fecundity and infective juvenile production in the entomopathogenic nematode *Heterorhabditis megidis*. Parasitology 125,83-92.

Sajnaga, E., Kazimierczak, W. 2020. Evolution and taxonomy of nematode-associated entomopathogenic bacteria of the genera *Xenorhabdus* and *Photorhabdus* : an overview. Symbiosis 80, 1-13

Sajnaga, E., Kazimierczak, W., Skowronek, M., Lis, M., Skrzypek, T., Wasko, A. 2018. *Steinernema poinari* (Nematoda: Steinernematidae): a new symbiotic host of entomopathogenic bacteria *Xenorhabdus bovienii*. Archives of Microbiology 200(9),1307-1316.

Shapiro-Ilan, DI., Hazir, S., Glazer, I. 2017. Basic and applied research: Entomopathogenic nematodes. In: Lacey LA (ed) Microbial agents for control of insect pests: from discovery to commercial development and use. Academic Press, San Diego, pp 91-105.

Sheets, J., Aktories, K.2017.Insecticidal toxin complexes from Photorhabdus luminescens. In: The molecular biology of *Photorhabdus* bacteria.In: Current Topics in Microbiology and Immunology.Vol.402. Ffrench-Constant (Ed.).Springer, Cham.pp.3-24.

Stock, P. 2015. Diversity, biology and evolutionary relationships. In: Nematode Pathogenesis of Insects and Other Pests. R Campos-Herrera (Ed.).Springer ,Cham.3-28.

Tailliez, P., Laroui, C., Ginibre, N., Paule, A., Pages, S., Boemare, N. 2010. Phylogeny of *Photorhabdus* and *Xenorhabdus* based on universally conserved protein-coding sequences and implications for the taxonomy of these two genera. Proposal of new taxa: *X.vietnamensis* sp. Nov., *P.luminescens* subsp. *caribbeanensis* subsp.nov., *P.luminescens* subsp. *hainanensis* subsp. nov., *P. temparata* subsp. *khanii* subsp.nov., *P.temperata* subsp. *tasmaniensis* subsp. *thracensis* as *P.temperata* subsp. *thracensis* comb.nov. International Journal of Systematic and Evolutionary Microbiology 60(8), 1921-1937.

Tailliez, P., Pages, S., Edgington, S., Tymo, LM., Buddie, AG.2012. Description of Xenorhabdus magdalenensis sp.nov., the symbiotic bacterium associated with Steinernema austral. International Journal of Systematic and Evolutionary Microbiology 62, 1761-1765.

Tailliez, P., Pages, S., Ginibre, N., Boemare, N. 2006. New insight into diversity in the genus *Xenorhabdus*, including the description of ten novel species. International Journal of Systematic and Evolutionary Microbiology 56, 2805-2818.

Thomas, GM., Poinar, GO Jr.1979. *Xenorhabdus* gen. nov., a genus of entomopathogenic nematophilic bacteria of the family Enterobacteriaceae. International Journal of Systematic Bacteriology 29,352-360.

Tobias, NJ., Heinrich, AK., Eresmann, H., Wright, PR., Neubacher, N., Backofen, R. Bode ,HB.2017. *Photorhabdus*–nematode symbiosis is dependent on hfq-mediated regulation of secondary metabolites. Environmental Microbiology19, 119-129.

Toth, T., Lakatos,T. 2008. *Photorhabdus temperata* subsp. *cinerea* subsp.nov., isolated from *Heterorhabditis* nematodes. International Journal of Systematic and Evolutionary Microbiology 58,2579-2581.

Toubarro, D., Lucena, M., Nascimento, G., Costa, G., Montiel, R., Varela, A. 2009. An apoptosis–inducing serine protease secreted by the entomopathogenic nematode *Steinernema carpocapsae*. International Journal for Parasitology 39(12), 1319-30.

Ubaub, L., Stock, P. 2018.First report of *Steinernema longicaudum* and its bacterial symbionts, *Xenorhabdus* species, in pummelo orchards of Davao region, Philippines.Philipp.Scient 53, 66-77.

Vicente-Díez, I., Blanco-Perez, R., González-Trujillo, MDM., Pou, A., Campos-Herrera, R. 2021. Insecticidal effect of entomopathogenic nematodes and the cell-free supernatant from their symbiotic bacteria against *Philaenus spumarius* (Hemiptera: Aphrophoridae) Nymphs. Insects 12(5),448

Viljakainen, L. 2015. Evolutionary genetics of insect innate immunity. Briefings in Functional Genomics 14(6), 407-412.

Vlisidou, I., Hapeshi, A., Healey, J., Smart, K., Yang ,G., Waterfield, NR. 2019. The *Photorhabdus asymbiotica* virulence cassettes deliver protein effectors directly into target eukaryotic cells. eLife .8,e46259.DOI: https://doi.org/10.7554/eLife.46259.

Wang, Y., Gaugler, R. 1999. *Steinernema glaseri* surface coat protein suppresses the immune response of *Popillia japonica* (Coleoptera: Scarabaeidae) larvae. Biological Control 14(1),45-50.

Waterfield, N., Ciche, T., Clarke, D. 2009. *Photorhabdus* and a host of hosts. Annual Review of Microbiology 63,557-574.

Wenski, SL., Cimen, H., Berghaus, N., Fuchs, SW., Hazir, S., Bode, HB . 2020. Fabclavine diversity in *Xenorhabdus* bacteria. Beilstein Journal of Organic Chemistry 16(1), 956-965

Xi, X., Lu, X., Zhang, X., Bi, Y., Li, X., Yu, Z. 2019. Two novel cyclic depsipeptides Xenematides F and G from the entomopathogenic bacterium *Xenorhabdus budapestensis*. The Journal of Antibiotics 72(10),DOI: 10.1038/s41429-019-0203-y.

Yamanaka, S., Hagiwara, A., Nishimura, Y., Tanabe, H., Ishibashi, N.,1992. Biochemical and physiological characteristics of *Xenorhabdus* species, symbiotically associated with entomopathogenic nematodes including *Steinernema kushidai* and their pathogenicity against *Spodoptera litura*(Lepidoptera: Noctuidae).Archives of Microbiology 158, 387-393.

Yuksel, E., Imren, M., Ozdemir, E., Bozbuga, R., Canhilal, R. 2022. Insecticidal effect of entomopathogenic nematodes and the cell-free supernatants from their symbiotic bacteria against different larval instars of *Agrotis segetum* (Denis & Schiffermuller) Lepidoptera: Noctuidae). Egyptian Journal of Biological Pest Control 32, 54. https://doi.org/10.1186/s41938-022-00555-1.