Endosymbiotic microbes from entomopathogenic nematode (EPNs) and their applications as biocontrol agents for agro-environmental sustainability

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Abstract

Background: The biological diversity on planet earth is declining day by day, due to different factors such as excessive applications of pesticides. The utilization of chemical pesticides affected environment as well as microorganisms. The awareness among the peoples towards the hazards by the residual toxicity of chemical pesticides should be developed for agro-environmental sustainability.

Main body: Entomopathogenic nematodes (EPNs) are the bacto-helminth parasites which show classical mutualism with the genera Xenorhabdus and Photorhabdus. The nematodes along with its endosymbiotic bacteria have a bio-control potential which could be used to reduce chemical pesticides. Applications of bioagents have been reported and resulted in considerable reduction in pathogens. Furthermore, these bioagents are biodegradable, eco-friendly and easy to apply for protection of crops against diverse pathogenic organism. The nematode-bacterium complexes are effective against huge range of bacteria, fungi, nematodes and insects that are harmful to the crops. Along with biocontrol potential, the endosymbionts produce diverse secondary metabolic compounds, exoenzymes and toxic compounds that show antibiotic, antymycotic, nematicidal, miticidal and anticancerous properties.

Conclusion: The present review deals with the diversity of endosymbiotic microbes from EPNs and their role in bio-control for the agro-environmental sustainability.

Keywords: Agricultural sustainability, Biocontrol, Diversity, Entomopathogenic nematode, Photorhabdus, Xenorhabdus

Background

Entomopathogenic nematodes (EPNs) are microscopic roundworms that belong to the families Heterorhabditidae and Steinernematidae of phylum Nematoda. EPNs are beneficial nematodes that exhibit a holoparasitic mode of survival (Bhat et al. 2020). The EPNs have been reported to survive in most of environmental conditions except psychrophilic conditions of Antarctica (Hominick 2002). The EPNs from genus Steinernema and Heterorhabditis were considered deadly fatal for a number of agricultural insects (Liu et al. 2020). Globally, 17 species of genus Heterorhabditis and 100 species of genus Steinernema have been reported that are found to be lethal for insect pests (Bhat et al. 2020). These nematodes showed mutualistic associations with endosymbiotic bacterial species that live inside the nematode. A major role has been played by these bacterial endosymbionts in nutritional physiology (Feldhaar 2011). The endosymbionts Xenorhabdus and Photorhabdus reside in symbiotic
association with EPNs Steinernema and Heterorhabditis (Kaya and Gaugler 1993).

In EPNs, the third-stage juvenile (dauer juvenile) resides freely in soil with non-foraging behaviour, and carries endosymbiotic bacteria inside the body which is responsible for causing the pathogenicity in their host. Once the dauer juvenile penetrates into the host body through spiracles or through natural body openings, it releases its symbiotic bacteria inside the haemocoel of the insect. The bacterial cells duplicate and generate severe toxins that have a high insecticidal potential and can assassinate its host in less than 2 days (Adams and Nguyen 2002). The infected host dies soon after infection due to contagion. Within the insect cadaver, these bacteria nourish the EPNs and promote the growth and reproduction of EPNs. As the food availability shortens, the dauer juvenile of EPNs comes out of the cadaver and look for a new host. Applications of EPNs with their bacterial endosymbionts become a prime approach in the biocontrol sector as well as in integrated pest management. Moreover, these EPNs now become model organisms and used widely in the fields such as evolutionary biology, biological control, soil ecology as well as the bacterial symbiotic mutualism (Stock 2015). Emerging dissatisfaction due to the excessive applications of chemicals insecticides for insect pest control has increased, as they showed adverse effects on the environment and human health (Tomar et al. 2022). Thus, it has turned out to be essential to diminish the utilization of these insecticides and replaces those using ecologically safer products in a sustainable agriculture perspective.

In recent times, these beneficial endosymbionts become a treasure trove for insecticidal compounds as well as for different bioactive compounds. The endosymbionts can decrease the chemical insecticides used in insect control and plant protection by stabilizing the environmental changes (Thakur et al. 2020, 2021; Migunova and Sasanelli 2021). Undoubtedly, these endosymbionts may become a favourable substitute in increasing the biological control of numerous phyto-insect pests as well as pathogens. EPN-associated bacterial endosymbionts have a high potentiality for agricultural pest control due to the toxic compounds and proteins produced as secondary metabolites (Thakur et al. 2022a, 2022b). The present review deals with distribution, identification and culturing practices of endosymbionts associated with EPNs. The major emphasis has been laid on the bioactive compounds produced by the endosymbionts.

Main body

Entomopathogenic nematode

Since the seventeenth century or possibly prior to these EPNs were familiar (Nguyen and Smart Jr 2004) although in the nineteenth and twentieth century’s vast studies on EPNs were carried out, and it was reported that EPNs were distributed worldwide. Steiner was the first to expound the EPN as Aplectana kraussei (Steinernema kraussei) from a hymenopterous sawfly (Steiner 1923), followed by Neoplectana glaseri from Popillia japonica (scarabaeid beetle) (Steiner 1929), S. feltiae, S. affiniae, and S. carpocapsae were from Cydia pomonella (Weiser 1955). Mracek (2002) reported that the nematodes as most victorious organisms on earth which have been found nearly all types of habitats. In India, various investigations have been accomplished to find out the new EPNs species that resulted in the isolation of a new H. indica species from Coimbatore, Tamil Nadu (Poinar Jr et al. 1992). EPNs species were previously described as an excellent means of biocontrol for the management of agricultural insect pests. Till now, 11 EPNs species were disclosed in India including Heterorhabditis indica, H. bacteriophora, S. sangi, S. bicornutum, S. abbasi (S. thermophilum), S. siamkayai, S. carpocapsae (S. meghalamyense), S. riobreve, S. glaseri, S. surkhetense, and S. hermaphroditum (S. dharanai) (Lalramnghaki 2017).

Distribution of endosymbionts of EPNs

The nematodes of families Steinernematidae and Heterorhabditidae have gained the interest of humanity towards their promising biocontrol capacity of managing insect population (Singh et al. 2022). Xenorhabdus and Photorhabdus, the Gram-negative bacteria, are endosymbionts of the dauer juvenile (IJ3) phase of EPNs. The IJs can perforate the insect via natural body orifice. It liberated the endosymbionts inside the blood stream of the host upon getting entry inside the host. Inside the host blood stream, the bacterial cells reciprocated. The occurrence of huge bacterial cells in the host midgut resulted in demises of insect after 24–48 h (Kaya and Gaugler 1993). Huge numbers of Xenorhabdus and Photorhabdus have been reported as endosymbionts from Steinernema and Heterorhabditis (Tables 1, 2).

Taxonomy of endosymbionts

Bacterial groups are considered as the most diversified biological group having varied phylogeny of the organisms (Adams et al. 2006). The fossil traces of cyanobacteria were found about 2.9 billion years back, and the presence of nematodes was supposed somewhat earlier in the Cambrian radiation (Noffke et al. 2003). In the mid-Paleozoic era, it has been considered that the pri-mogenitor of family Heterorhabditidae and Steinernematidae started to reconnoitre their symbiotic interactions with the members of Enterobacteriaceae (Poinar Jr 1993). Achromobacter nematophilus was the first endosymbiont found in mutualistic association with Neaplectana...
Table 1 Global distribution of endosymbionts *Xenorhabdus*

| Endosymbiotic bacteria     | Nematode species | Host organism            | Country         | References                      |
|----------------------------|------------------|--------------------------|-----------------|---------------------------------|
| *Xenorhabdus szentirmaii*  | Steinernema rarum| *Galleria mellonella*    | Argentina       | Lengyel et al. (2005)           |
| *Xenorhabdus miraniensis*  | Steinernema spp. | *G. mellonella*          | Australia       | Tailiez et al. (2006)           |
| *Xenorhabdus magdalemensis*| Steinernema australis | *G. mellonella*     | Chile           | Tailiez et al. (2012)           |
| *Xenorhabdus ehlersii*     | Steinernema longicaudum | *G. mellonella*   | China           | Lengyel et al. (2005)           |
| *Xenorhabdus ishibashii*   | Steinernema aciari | *G. mellonella*          | China           | Kuvata et al. (2013)            |
| *Xenorhabdus budapestensis*| Steinernema cerataphorum | *G. mellonella*   | China           | Yang et al. (2012a, b)          |
| X. szentirmaii             | Steinernema costaricense | *G. mellonella*     | Costa Rica      | Lengyel et al. (2005)           |
| *Xenorhabdus poinarii*     | Steinernema cubanum | *G. mellonella*         | Cuba            | Fischer-Le Saux et al. (1999)   |
| *Xenorhabdus bovienii*     | Steinernema poinari | *G. mellonella*         | Czech Republic  | Sajnaga et al. (2018)           |
| *Xenorhabdus nematophila*  | Steinernema carpocapsae | *Cydia pomonella*     | Czechoslovakia  | Martens and Goodrich-Blair (2005)|
| X. szentirmaii             | Steinernema feltiae | *Feltia segetum (Agrotis segetum)* | Denmark       | Ehlers et al. (1997)           |
| *Xenorhabdus indica*       | Steinernema yirigalemense | *G. mellonella*     | Ethiopia        | Tamiru et al. (2012)           |
| *Xenorhabdus kokoei*       | Steinernema boemarei | *G. mellonella*         | France          | Tailiez et al. (2006)           |
| X. poinarii                | Steinernema khangou | *G. mellonella*         | Florida         | Banya and DiGennaro (2021)      |
| *Xenorhabdus doucetiae*    | Steinernema diapreesi | *Diaprepes abbreviates* | Florida         | Tailiez et al. (2006)           |
| X. bovienii                | Steinernema silvaticum | *G. mellonella*         | Germany         | Akhurst and Boemare (1988)      |
| *Xenorhabdus andreanii*    | Steinernema krausei | *Cepaleia abietis*       | Germany         | Akhurst (1982b)                 |
| X. bovienii                | Steinernema tibialis | *G. mellonella*         | Georgia         | Gorgadze et al. (2015)          |
| *X. griffiniae*            | Steinernema hermaphroditum | *G. mellonella*     | Indonesia       | Tailiez et al. (2006)           |
| X. kudzoi                  | Steinernema vulcanicum | *G. mellonella*         | Italy           | Clusi et al. (2011)             |
| X. bovienii                | Steinernema ichneumae | *G. mellonella*         | Italy           | Tarasco et al. (2011)           |
| *Xenorhabdus japonicus*    | Steinernema Kushida | *G. mellonella*         | Japan           | Nishimura et al. (1994)         |
| X. bovienii                | Steinernema litorale | *G. mellonella*         | Japan           | Ozdemir et al. (2020)           |
| *Xenorhabdus sp.*          | Steinernema monticolum | *A. segetum, A. ipsilon, arapediasia tertetrella* | Korea          | Kang et al. (2003)              |
| X. hominickii              | Steinernema karii  | *G. mellonella*         | Kenya           | Tailiez et al. (2006)           |
| X. stockiae                | Steinernema surkhentse | *G. mellonella*         | Nepal           | Bhat et al. (2017)              |
| X. romanii                 | Steinernema puertoricense | *G. mellonella*     | Puerto Rico     | Tailiez et al. (2006)           |
| X. kudzoi                  | Steinernema arenanum | –                       | Russia          | Tailiez et al. (2006)           |
| X. khoisaee                | Steinernema britecheimi | *G. mellonella*     | South Africa    | Cimen et al. (2016a)            |
| X. khoisaee                | Steinernema fabii | *G. mellonella*         | South Africa    | Abate et al. (2018)             |
| X. indica related endosymbiots | Steinernema biddulphi | *G. mellonella*     | South Africa    | Cimen et al. (2016b)            |
| X. bovienii                | Steinernema citrea | *G. mellonella, Terebrinio molitor* | South Africa    | Stokwe et al. (2011)           |
| X. khoisaee                | Steinernema jeffreyense | *G. mellonella*     | South Africa    | Dreyer (2018)                   |
| X. khoisaee                | Steinernema khoisaeeae | *G. mellonella*         | South Africa    | Ferreira et al. (2013)          |
| *Xenorhabdus griffiniae*   | Steinernema lilchii | *G. mellonella*         | South Africa    | Dreyer (2018)                   |
| *Xenorhabdus khoisaeeae*   | Steinernema sacchari | *Eldana saccharina, G. mellonella* | South Africa    | Dreyer (2018)                   |
| *Xenorhabdus budapestensis*| Steinernema bicornutum | *G. mellonella*     | Serbia          | Lengyel et al. (2005)           |
| X. indica                  | Steinernema abasii | *G. mellonella*         | Sultanate of Oman | Tsai et al. (2008)             |
| *Xenorhabdus stockiae*     | Steinernema siamkayai | *G. mellonella*         | Thailand        | Ardpairin et al. (2020)         |
| X. poinarii                | Steinernema glaserei | *Popillia japonica*     | USA             | Akhurst (1983b)                 |
| *Xenorhabdus cabanillasii* | Steinernema niobrave | *Helioverpa zea*        | USA             | Tailiez et al. (2006)           |
| *Xenorhabdus koppenhoferi* | Steinernema scarabaei | *Anomala orientalis*    | USA             | Tailiez et al. (2006)           |
| X. bovienii                | Steinernema intermedium | *G. mellonella*     | USA             | Akhurst (1983b)                 |
| *Xenorhabdus mauleonii*    | Steinernema spp. | *G. mellonella*         | USA             | Tailiez et al. (2006)           |
| *Xenorhabdus innexi*       | Steinernema scapterisci | *Scaptericus vicinus* | USA             | Kim et al. (2017)               |
| *Xenorhabdus vietnamensis* | Steinernema sangi | *G. mellonella*         | Vietnam         | Kämpfer et al. (2017)           |
| *Xenorhabdus thuongxuanensis* | Steinernema sangi | *G. mellonella*         | Vietnam         | Kämpfer et al. (2017)           |
| *Xenorhabdus eapokensis*   | Steinernema eapokense | *G. mellonella*         | Vietnam         | Kämpfer et al. (2017)           |
orhabditis (Boemare et al. 1993). The earlier classification of Heter- 
alistic association with the nematodes of genera having mutu-
Xenorhabdus among the other genera was X. luminescens (Akhurst 1983a). A noticeable separation of the nematophilus genus includes bacterial symbionts and X. luminescens X. This novel emergence of the novel genera Xenorhabdus did not suit with any of the previously approved genera that results in the characteristic features of A. nematophila did not suit with any of the previously approved genera that results in the emergence of the novel genera Xenorhabdus. This novel genus includes bacterial symbionts X. luminescens and X. nematophilus (Akhurst 1983a). A noticeable separation of the bacterial classification (Stackebrandt 2006). New species of endosymbionts were discovered by following the 98.7% relatedness concept of 16S rRNA gene, still an initial move towards identification along with the threshold of 70% in DNA-DNA hybridization and the threshold of 80% in DNA-DNA hybridization for efficiently balancing negligible criterion in the bacterial taxonomy approach (Goris et al. 2007). The gene

### Table 2: Global distribution of endosymbionts Photorhabdus

| Endosymbiotic bacteria | Nematode species | Host organism | Country | References |
|------------------------|------------------|---------------|---------|------------|
| Photorhabdus luminescens subsp. luminescens | Heterorhabditis bacteriophora | Heliothis punctigera | Australia | Machado et al. (2018) |
| Photorhabdus luminescens subsp. laurandii | H. bacteriophora | H. punctigera | Australia | Tailiez et al. (2010) |
| Photorhabdus luminescens subsp. kaiyai | H. bacteriophora | H. punctigera | Australia | Tailiez et al. (2010) |
| Photorhabdus luminescens subsp. thracensis | H. bacteriophora | H. punctigera | Australia | Machado et al. (2018) |
| Photorhabdus khanii | H. bacteriophora | H. punctigera | Australia | Tailiez et al. (2010) |
| Photorhabdus carribbeanensis | H. bacteriophora | H. punctigera | Australia | Machado et al. (2018) |
| Photorhabdus asymbiotica | Heterorhabditis gerrardi | Tenebrio molitor | Australia | Akhurst et al. (2004) |
| Photorhabdus australis subsp. australis | H. gerrardi | T. molitor | Australia | Plichta et al. (2009) |
| Photorhabdus luminescens subsp. thailandensis | H. gerrardi | T. molitor | Australia | Machado et al. (2021) |
| Photorhabdus australis | H. gerrardi | T. molitor | Australia | Machado et al. (2018) |
| Photorhabdus subsp. guanajuatensis | Heterorhabditis atacamensis | G. mellonella | Chile | Machado et al. (2019) |
| Photorhabdus bodei | Heterorhabditis bechniana | G. mellonella | China | Machado et al. (2018) |
| Photorhabdus luminescens subsp. floridensis | Heterorhabditis floridensis | G. mellonella | Florida | Blackburn et al. (2016) |
| Photorhabdus luminescens subsp. akhurstii | Heterorhabditis georgiana | G. mellonella | Georgia | Machado et al. (2018) |
| Photorhabdus stackebrandii | H. georgiana | G. mellonella | Georgia | Machado et al. (2018) |
| Photorhabdus kleini | H. georgiana | G. mellonella | Georgia | Machado et al. (2018) |
| Photorhabdus luminescens subsp. akhurstii; | H. indicus | Scirpophaga excerptalis | India | Machado et al. (2021) |
| Photorhabdus aegyptia | Heterorhabditis indicus | S. excerptalis | India | Machado et al. (2021) |
| Photorhabdus asymbiotica | H. indicus | S. excerptalis | India | Machado et al. (2021) |
| Photorhabdus temperata | Heterorhabditis downesi | G. mellonella | Ireland | Machado et al. (2018) |
| Photorhabdus cinerea | H. downesi | G. mellonella | Ireland | Machado et al. (2018) |
| Photorhabdus luminescens subsp. mexicana | Heterorhabditis mexicana | G. mellonella | Mexico | Machado et al. (2019) |
| Photorhabdus luminescens subsp. sonorense | Heterorhabditis sonorense | G. mellonella | Mexico | Orozco et al. (2013) |
| Photorhabdus heterorhabditis | Heterorhabditis zealandica | Heteronychus arator | New Zealand | Ferreira et al. (2014) |
| Photorhabdus luminescens subsp. laurandii | Heterorhabditis safricana | G. mellonella | South Africa | Geldenhuys et al. (2016) |
| Photorhabdus luminescens subsp. noenieputensis | Heterorhabditis noenieputensis | G. mellonella | South Africa | Ferreira et al. (2013) |
| Photorhabdus hainanensis | Undescribed spp. | G. mellonella | South Africa | Tailiez et al. (2010) |
| Photorhabdus namnanaensis | H. baujardi | G. mellonella | Thailand | Glaeser et al. (2017) |
| Photorhabdus temperate | Heterorhabditis megidis | Papilia japonica | USA | Toth and Lakatos (2008) |
| Photorhabdus cinerea | Heterorhabditis megidis | P. japonica | USA | Machado et al. (2018) |
| Photorhabdus tasmanensis | Heterorhabditis marelatus | G. mellonella | USA | Machado et al. (2018) |
| Photorhabdus luminescens | Heterorhabditis baujardi | G. mellonella | Vietnam | Glaeser et al. (2017) |
sequencing technique 16 R-gene for classification was regarded as inappropriate because of little differences along with lateral gene transfer (LGT) (Tailliez et al. 2010). The multilocus sequence analysis (MLSA) technique has been extensively utilized for the recognition of many novel species of bacteria (Liu et al. 2017). The enhanced sequencing methodologies permit the utilization of whole genetic sequence for the identification and classification (Lee et al. 2016). A remarkable potential of genome taxonomy has been shown in the high-resolution classification of genera Photorhabdus (Machado et al. 2018).

**Life history of endosymbionts**

The EPNs are the organisms that cause diseases in insects. In the nematode life cycle specialized infective juvenile (IJ) is the only form that lives outside the host insect (Poinar Jr et al. 1979). Inside the intestinal tract of infective juvenile these symbiotic bacteria populate. The other juvenile stages IJ1 and IJ2 cannot live without their host and do not carry symbiotic bacteria in their intestinal tract. The endosymbionts (Photorhabdus and Xenorhabdus) of EPNs exhibit almost similar life cycles. The infective juvenile works as a carrier in transmitting the bacterial endosymbionts (Sicard et al. 2004). Upon entering to the insect body, nematode moves to the haemocoel cavity of the insect and released its endosymbionts. These endosymbiotic bacteria have the ability to escape from the insect immune system and are responsible for causing pathogenicity in the insects by releasing a variety of insecticidal toxins (Koppenhöfer et al. 2007).

In addition to pathogenicity against insects, these bacteria defend the insect cadaver from invading microorganisms (pathogens, competitors, and predators) by producing antimicrobial compounds and also contribute to nematode reproduction (Gulcu et al. 2012). Inside host insect nematodes undergo about 2–3 rounds of reproduction before the nutrients depleted after that infective juvenile (IJ1) form way out from the insect corpse and seek for a new host (Grewal and Georgis 1999).

*Photorhabdus* bacteria are the endosymbionts of *Heterorhabditis* that make a colony behind the basal bulb in the anterior portion of the intestine. They were also reported scattered in the remaining portion of the intestine (Ciche et al. 2003). The *Xenorhabdus* are endosymbionts of *Steinernema* that colonize in the specific bilobed vesicle of intestine (Martens et al. 2003). The life cycle exhibited by these bacteria is quite unique and interesting as they lived in symbiotic association with nematode and are pathogenic to harmful insects. The endosymbiotic complexes of *Steinernema* show a very high resemblance with *Heterorhabditis* and have a little variability in their life cycle that leads to the independent classification of the two genera. Endosymbiont *Photorhabdus* are emanated via the anterior region, *i.e.* mouth of nematode (Ciche et al. 2008), whereas *Xenorhabdus* is liberated from the posterior region, via anus, of the nematode (Sicard et al. 2004). Host immune suppressing proteins were released by *Steinernema* that might support their endosymbionts release (Simoes and Rosa 1998) but in *Heterorhabditis*, this process is still mysterious (Forst and Clarke 2002). Brillard et al. (2002) reported that both genera show haemotoxic behaviour.

The endosymbionts of both genera release certain protein toxins as well as exoenzymes that are responsible for causing septicaemia in host insects that leads to insect death (Forst and Clarke 2002). In the late infectious state, the proteinaceous toxins produced by bacteria damaged the midgut of the insect mainly the epithelium lining (Silva et al. 2002) (Figs. 1, 2). *Xenorhabdus* and *Photorhabdus* exhibit colony pleomorphism, *i.e.* phase variation phenomenon in which coexistence of two different variants was observed in single bacterial species. Differences in trait numbers were observed in these variants like release of antibiotics, proteins, pigment substances, lipases, and bioluminescence (Turlin et al. 2006). The endosymbiotic bacteria show two phases in their life cycle that are morphologically distinct from each other. In *Xenorhabdus* spp., the cells under phase II are somewhat larger, mobile with crystalline inclusion bodies and liberate secondary metabolic substances such as lipases, proteases, and some other bioactive components. Phase II cells are much smaller than phase I, immobile and can easily be recognized by staining with dyes (triphenyltetrazolium chloride and bromothymol blue). The cells in phase I condition when exposed to nutritional media having the fusion of two dyes triphenyltetrazolium chloride and bromothymol blue, they shows clearly distinct dark blue bacterial colonies with red core. But an exemption was also noticed that phase I bacterial cells do not absorb dye (bromothymol blue). It has stated that in two phases of their life cycle, the phase I bacterial cells were normally found in association with reproducing nematodes, while the phase II bacteria were found in the nematode infected cadaver (Turlin et al. 2006).

**Culturing practices and cost effectiveness of endosymbionts**

The endosymbionts *Xenorhabdus* and *Photorhabdus* can be easily isolated from the dead larval cadaver of *Galleria mellonella* L. infected with IJs of EPNs after 24 h. The infected cadavers should be sanitized with merthiolate (0–1% for 120–180 min) and cleaned multiple times with distilled water. After that transferred to the yeast-soy-based (YS) broth and changed into suspension with the help of tissue homogenizer.
The suspension should be spread over the nutrient agar (NA) containing bromothymol blue and triphenyltetrazolium chloride. The endosymbionts can be isolated using hanging drop system method. The dead cadavers should be soaked into absolute ethanol for surface cleaning and allowed to dehydrate in sterile Petri dish. With the help of a sterilized forceps, dead cadaver should be dissected and a drop of haemolymph dragged out using a sanitized loop. The loop containing haemolymph streaked over the NBT agar and should be placed at room temperature for growth (Poinar and Thomas 1966). An alternative approach for endosymbionts isolation was by directly crushing the IJs. The IJs (50-100I) have sterilized with thiomersal (1% for 2 h) and centrifuged. After centrifugation the pellet containing IJs should be washed several times and placed onto nutrient broth (NB) luria–bertani (LB), tryptic soy broth (TSB). The suspension should be spread over the NBT agar media or luria–bertani media and cultivated at room temperature.

**Role of endosymbionts as biocontrol agents**

Endosymbionts metabolism produces a wide range of secondary metabolites for potential applications in different sectors. In EPNs infected insects, these endosymbionts defend the host cadaver from the invading microbiome by the secretion of a variety of compounds possessing antibiotic activities. These metabolites are also used as biocontrol negotiators for the management of viruses, fungi, nematodes, and insects (Lulamba et al. 2021). The bioactive components produced by *Xenorhabdus* and *Photorhabdus* play an essential role in the biological transformation of host insects. Production of different kinds of antibiotics, proteases, adhesions, lipases, and haemolysins were observed from endosymbionts. Even a single *Xenorhabdus* strain produces multiple metabolites that act against variety of pathogenic organisms. Webster et al. (2002) recorded the productions of several antimicrobials such as β-lactam carbapenem, isopropylstilbenes from *Photorhabdus* species. The discovery of such antimicrobial complexes acquires
major attention of agronomic and pharma industries (Hazir et al. 2016) (Tables 3, 4). Numerous bactericidal, fungicidal, entomopathogenic as well as nematode killing activities have been reported from endosymbionts (Muangpat et al. 2020). All the metabolic complexes that showed different activities against insects, parasites, fungi, and viruses were possessed a particular gene that encoded for particular traits (da Silva et al. 2020). Various strains of *Xenorhabdus* produces a variety of secondary metabolites that show a wide range of bioactivity, antibacterial activities, antifungal activities, nematicidal activities, insecticidal activities as well as several cytotoxic properties (Brachmann and Bode 2013) (Tables 3, 4).

**Insecticidal activity**

Insecticidal activities are the insect killing or insect controlling mechanisms, showed by *Photorhabdus* and *Xenorhabdus*. Nematodes penetrate the host body through naturalistic orifices and liberate the bacteria through defecation that releases compounds that suppress the insect immunity (Webster et al. 2002). They have been found to infect *Cydia pomonella*, *Delia radicum* *Diaprepes abbreviates*, *Thrips* spp., *Ottorhynchus sulcatus*, *Phyllopertha horticola* as well as the larvae of several other insect orders including dipterans and lepidopterans (Georgis et al. 2006). Several *X. nematophila* strains produce UnA protein that inhibits the accumulation of haemocytes which results in the formation of cover or sheath around the bacteria and nematode (Ribeiro et al. 2003). Dunphy and Webster (1991) reported that these protein and lipopolysaccharides in *G. mellonella* stop the cluster formation of haemocytes that hinder phenoloxidase activation which is a major tool of insect immune system (Forst et al. 1997). Eicosanoids responsible for the cellular immunity in insect’s also suppressed by *X. nematophila* strains. They hinder the action of phospholipase A2 (PL.A2) upon which the production of eicosanoids depends (Kim et al. 2018). Without eicosanoids, insects were died due to acute bloodstream infection or septicemia. An insecticidal protein (57 kDa) produced by *X. budapestensis* D43 trigger the phenoloxidase cascade in *G. mellonella* and resulted in extreme immune responses such as productions of a high amount of quinones (Yang et al. 2012a, b). This extreme production of quinine is lethal for the insect larvae. Even
### Table 3  Bioactive complexes produced by *Xenorhabdus*

| Bacterial Spp             | Bioactive complexes/secondary metabolite                      | Biological asset                                                                 | References                               |
|---------------------------|----------------------------------------------------------------|----------------------------------------------------------------------------------|------------------------------------------|
| *X. beddingii*            | R-type bacteriocins                                             | Bactericidal                                                                      | Boemare et al. (1992)                   |
| *X. bovienii*             | Xenocyloins                                                      | Insecticidal                                                                      | Proschak et al. (2014)                  |
|                           | Amicoumacin                                                     | Antibacterial, insecticidal, antifungal, anti-cancer, and anti-inflammatory        | Park et al. (2016)                      |
|                           | Indoles                                                         | Antibiotic                                                                        | Li et al. (1995a)                       |
|                           | Dithiolopyrrolones                                              | Antibiotic                                                                        | Li et al. (1995a)                       |
| *X. budapestensis*        | Bicornitun                                                      | Antibacterial and antifungal                                                      | Tobias et al. (2017)                    |
|                           | GP-19                                                           | Antibacterial and antifungal                                                      | Xiao et al. (2012)                      |
|                           | EP-20                                                           | Antifungal                                                                        |                                         |
|                           | Fabclavine                                                      | Antibacterial, antifungal, antiprotozoal and cytotoxic                            | Wenski et al. (2020)                    |
| *X. cabanillasii*         | Nemaucin                                                        | Antibacterial and antifungal                                                      | Gualtieri et al. (2012)                 |
|                           | Rhabdopeptide                                                   | Antiprotozoal, insecticidal and cytotoxic                                          | Reimer et al. (2013)                    |
|                           | Cabanillasin                                                    | Antifungal                                                                         | Houard et al. (2013)                    |
| *X. doucetiae*            | Xenoamicin                                                      | Antiprotozoal                                                                     | Bode et al. (2017)                      |
|                           | Xenorhabdin                                                     | Antibacterial                                                                      |                                         |
|                           | Xenocoumacin                                                    | Antibacterial, antifungal and antilucent                                            |                                         |
| *X. indica*               | Taxilllaids                                                     | Antiprotozoal and cytotoxic                                                       | Kronenwerth et al. (2014)               |
| *X. kozodoii*             | Xenocoumacin                                                    | Antibacterial, antifungal and antilucent                                            | Tobias et al. (2017)                    |
| *Xenorhabdus* Spp         | Xenobactin                                                      | Antibacterial and antiprotozoal                                                    | Grundmann et al. (2013)                 |
| *X. khoisanae* strain SB10| PAX lipopeptides                                                | Antimicrobial                                                                      | Booysean et al. (2021)                  |
|                           | Xenocoumacin                                                    | Antimicrobial                                                                      | Booysean et al. (2021)                  |
| *X. innexi*               | Rhabdopeptides                                                  | Antiprotozoal, insecticidal and cytotoxic                                          | Hacker et al. (2018)                    |
| *X. mauleonii*            | Xenoamicin                                                      | Antiprotozoal                                                                      | Tobias et al. (2017)                    |
|                           | and xenocoumacin                                                | Antibacterial, antifungal and antilucent                                            |                                         |
|                           | xenorhabdin                                                     | Antibacterial                                                                      |                                         |
| *X. nematophila*          | Pristinamycin                                                   | Antibacterial                                                                      | Brachmann et al. (2012)                 |
|                           | Xenorhabdins                                                    | Antibacterial                                                                      | Qin et al. (2013)                       |
|                           | Xenonides                                                       | Antibacterial and antifungal                                                      |                                         |
|                           | PAX peptides                                                    | Antibacterial and antifungal                                                      | Haiz et al. (2016)                      |
|                           | Nematofin                                                       | Antibacterial and antifungal                                                      | Cai et al. (2017)                       |
|                           | Xenoacin                                                        | Antibacterial                                                                      | Rathore (2013)                          |
|                           | Xenorhabdinc (R-type bacteriocins)                              | Antibacterial                                                                      | Eugenia Nuñez-Valdez et al. (2019)      |
|                           | Xenocoumacins                                                   | Antibacterial, antifungal and antilucent                                            | Guo et al. (2017)                       |
|                           | Xenortides                                                      | Antiprotozoal and cytotoxic                                                       | Esmai et al. (2018)                     |
|                           | Rhabdopeptides                                                  | Antiprotozoal, insecticidal and cytotoxic                                          | Zhao et al. (2018)                      |
|                           | Xenematides                                                     | Antibacterial and insecticidal                                                     | Crawford et al. (2012)                  |
|                           | Rhabducin                                                       | Insecticidal                                                                        | Crawford et al. (2012)                  |
|                           | Benzylidene-acetone                                             | Antibacterial, immuno-suppressant and insecticidal                                 | Ji et al. (2004)                        |
| *X. szentirmai*           | Fabclavines                                                     | Antibacterial, antifungal, antiprotozoal and cytotoxic                            | Wenski et al. (2020)                    |
|                           | Szentiamide                                                     | Antibacterial, antifungal and cytotoxicity                                        | Nollmann et al. (2015)                  |
|                           | Xenofuranones A and B                                           | Insecticidal                                                                       | Dongare et al. (2021)                   |
|                           | Xenocoumacin                                                    | Antimicrobial                                                                      | Dreyer et al. (2019)                    |
|                           | PAX lipopeptides                                                | Antimicrobial                                                                      | Dreyer et al. (2019)                    |
programmed cell death in insect haemocytes was also caused by several species of *Xenorhabdus* such as *X. nematophila*, *X. beddingii*, *X. japonica*, and *X. kozodoii* (Cho and Kim 2004).

Another cytotoxic protein CyA produced from *X. nematophila* can destroy the insects within one or two days (Khush et al. 2002). It was reported that toxin complexes of *X. nematophilus* include three insecticidal proteins  xptA2,  xptB1, and  xptC1 that united to form a tetramer (∼1120 kDa) that attached to the outer membranes of insects and form pores in the membrane and have very high insecticidal activity against lepidopteran insects (Sheets et al. 2011). PMFI296 genes of *X. nematophila* produce insect killing proteins such as  xptA1,  xptA2,  xptB1 and  xptC1. These proteins are highly virulent against insects and affect the haemocytic activity of insects (Reimer et al. 2009). Srf ABC toxin derived from the fosmid clones of *X. stockiae* HN_xs01 strain also has insect killing properties. Srf ABC toxin brings G2/M at halt and causes necrobiosis or cell death in CF-203 cells (midgut cells) of *H. armigera* larvae (Yang et al. 2019). Chitinase protein (76-kDa) from *X. nematophila* strain ATCC 19061 exhibit endochitinase activity, β-N-acetylglucosaminidase and chitobiosidase activities were found highly lethal against *H. armigera* (Mahmood et al. 2020).

Four major categories of toxin complexes are recognized such as: the *Photorhabdus* insect-related (Pir) proteins, toxin complexes (Tcs), make caterpillars’ floppy (Mcf) toxins and *Photorhabdus* virulence cassettes (PVC) from *Photorhabdus*. Pir-AB toxic proteins found from the

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**Table 4** Bioactive complexes produced by *Photorhabdus*

| Bacterial Spp         | Bioactive complexes/secondary metabolite               | Biological asset | References          |
|-----------------------|----------------------------------------------------------|------------------|---------------------|
| *Photorhabdus luminescens* | Anthraquinone                                             | Insecticidal     | Zhou et al. (2019)  |
|                       | 3,5-Dihydroxy-4-isopropylstilbene                        |                  |                     |
|                       | 1,3-Dihydroxy-2-(isopropyl)-5-(2-phenylethenyl)benzene   |                  |                     |
|                       | 1,6-Dihydroxy-4-methoxy-9,10-anthraquinone               | Antibiotic       | Richardson et al. (1988) |
|                       | 1,8-Dihydroxy-3-methoxy-9,10-anthraquinone               | Antibiotic       | Hu et al. (1998)    |
|                       | 1-Hydroxy-2,6,8-trimethoxy-9,10-anthraquinone            |                  |                     |
| Carbenapem            | Anthraquinone                                             | Antibiotic       | Derzelle et al. (2002) |
| GameXpeptides         | Anantraquinone                                             | Antibiotic       | Brachmann et al. (2012) |
| Indigoidine           | Anthraquinone                                             | Antibiotic       | Hu et al. (1999)    |
| Indole                | Anthraquinone                                             | Antibiotic       |                     |
| Luminizone A          | Anthraquinone                                             | Antibiotic       |                     |
| Phurealpids           | Anthraquinone                                             | Antibiotic       |                     |
| Photobactin           | Anthraquinone                                             | Antibiotic       |                     |
| Pyrone                | Anthraquinone                                             | Antibiotic       |                     |
| Stilbene and its derivatives | Anthraquinone (1,3-dimethoxy-8-hydroxy-9,10-anthraquinone and 3-methoxychrysazine) | Insecticidal | Yang et al. (2019) |
| Trans-cinnamic acid   | Anthraquinone                                             | Insecticidal     |                     |
| Rhabduscin            | Anthraquinone                                             | Insecticidal     |                     |
| *Photorhabdus temperata* | Anthraquinone (1,3-dimethoxy-8-hydroxy-9,10-anthraquinone and 3-methoxychrysazine) | Insecticidal |                     |
|                       | Benzaldehyde                                              | Antimycotic      |                     |
|                       | Stillbene                                                 | Insecticidal     |                     |
| *Photorhabdus asymbiotica* | Glidobactin/Cepafungin I                            | Insecticidal     | Theodore et al. (2012) |
| *Photorhabdus spp.*   | Galtox                                                    | Insecticidal     | Ahuja et al. (2021) |
|                       |                                                          |                  |                     |
P. luminescens TT01 genome possess insecticidal properties. The toxic proteins were effective against insect pests (Duchaud et al. 2003) as they acts as binary toxin (Yang et al. 2019) and shows resemblance with δ-endotoxins of Bacillus thuringiensis. Toxin complexes include numerous subunits having high molecular weight that shows insecticidal properties (ffrench-Constant 2007). Genomic study of P. luminescens W14 elucidate the availability of tc loci and cytolytic RTX-like toxins that shows resemblance with the toxins of Erwinia chrysanthemi, Vibrio cholera, Erwinia tarda and Serratia marcescens (ffrench-Constant et al. 2000). The four complexes encoded are tca, tcb, tcc, and tcd present on different locus and producing variety of compounds (Sheets and Aktories 2016). Oral toxicity by tca and tcd toxic complexes was reported in Manduca sexta, and these complexes were regarded as latent substitute of transgenic Bt formation (Bowen et al. 1998).

Even the cell-free filtrate of Photorhabdus spp. also exhibits insect killing properties. The genome of Photorhabdus laumondii (TT01 strain) encodes for a wide range of metabolic compounds including lipases, toxins, adhesins, proteases, and haemolysins and variety of antibiotic substances (Zamora-Lagos et al. 2018). Crude extract of P. luminescence laumondii (TT01strain) reported to cause toxicity in Bemisia tabaci (Shrestha and Lee 2012) and crude extract of P. luminescense sonorensis was effective against Helicoverpa zea. Many secondary metabolites produced by Photorhabdus also pathogenic to insects. These metabolites are anthraquinone derivatives, stilbene derivatives and genistene that are highly virulent against insects (Chalabaev et al. 2008). Anthraquinone derivatives 3-methoxychrysazine and 1,3-dimethoxy-8-hydroxy-9,10-anthraquinone, extracted from P. temperata effective against various mosquito spp. (Ahn et al. 2013). Baur et al. (1998) also describe that these anthraquinone acts as an obstacle for ants and birds. Stilbenes derived from Photorhabdus hamper the activity of phenoloxidase by interfering and interrupting the insect (Manduca sexta) immune system. This phenoloxidase is the major component of insect immune system and is responsible for melanisation (Eleftherianos and Revenis 2011). TccC3 (adenosine diphosphate (ADP)–ribosyltransferases) and TccC5 (DP-ribosylated Rho guanosine triphosphatase) toxic proteins produced by P. luminescens hinder the mechanism of phagocytosis in insect cells and intracellular actin polymerization (Lang et al. 2010). P. luminescens, also produces tyrosine based compounds, rhabduscin that suppress the insect immune system (Crawford et al. 2012). Photorhabdus temperata M1021 releases benzaldehyde, a lethal compound that acts upon the insect immune system and kill their insect hosts by seizing their immune responses in G. mellonella. Additionally, benzaldehyde also reduces the phenoloxidase activities and melanisation (Ullah et al. 2014). Cell-free filtrate and suspensions culture of Xenorhabdus spp. were found highly effective against Hopilia philanthus (scarabaeid beetles) (Ansari et al. 2003), Otioryhynchus sulcatus (vine weevil), Spodoptera exigua (beet armyworm), Schistocerca gregaria (desert locust) (Mahr et al. 2008), Tribolium castaneum (Red flour beetle) (Shrestha and Kim 2010), Thrips tabaci (onion thrips), Frankiiniella occidentalis (western flower thrips) (Gerritsen et al. 2005), Plutella xylostella (diamond back moth) (Mahr et al. 2008) and G. mellonella (greater wax moth) (Mahr et al. 2004) under the laboratory bioassay study. X. nematophila and P. luminescens were also used against Luciaphorus pernicious and are highly lethal (Bussaman et al. 2012). Xenorhabdus and Photorhabdus strains possess tpx40 gene that produce ubiquitous insect killing proteins and is effective against the insect larvae belonging to order Diptera and Lepidoptera. They affect the midgut and cause injury in the fat bodies (Brown et al. 2006). Even Xenorhabdus stockiae, X. indica, P. luminescence subsp. hainanensis and P. luminescence subsp. akhurstii also cause mortality in the mosquito larvae (Aedes albopictus and Aedes aegypti) (da Silva et al. 2020).

Antibacterial activity
Endosymbionts Xenorhabdus spp. and Photorhabdus spp. exhibit antibacterial properties. The endosymbionts directly interfere in the development of the target bacteria by multiple targets systems such as biosynthesis of bacterial protein and cell-wall, DNA replication and repair system, via membrane destruction, and by metabolic pathway that ultimately inhibit the growth of the target bacteria. Leucine responsive protein (lpr) produced from X. nematophila showed bactericidal activities towards Bacillus subtilis and Micrococcus luteus (Cowles et al. 2007). Paul et al. (1981) reported that Xenorhabdus spp. secreted several metabolites having antibacterial properties. Xenocoumacin (xcnKL strain), ngrA-derived compound from Xenorhabdus spp. has widely adapted as antimicrobial and antibacterial properties (Singh et al. 2021). Xenocoumacin II and nematophin isolated from X. nematophilus possess modest antibacterial activities (Lang et al. 2008). Amicoumacin and xenocoumacin derived from Xenorhabdus showed a strong inhibition towards Staphylococcus aureus (Reimer et al. 2009). Antibiotic complexes were also reported from the genus Photorbahdus. Anthraquinone and trans-stilbenes were the antibacterial complexes discovered from P. temperata and P. luminescens (Boemare and Akhurst 2006). A monoterpenoid compound trans-4-phenyl-3-buten-2-one (benzylideneacetone) isolated from X. nematophila...
show their potential effect against phytopathogen *Agrobacterium vitis*, *Pseudomonas syringae*, *Pectobacterium carotovorum*, *Ralstonia solanacearum*, and *P. carotovorum* (Ji et al. 2004). A lysine-rich cyclolipopetide (PAX-peptide-antimicrobial-Xenorhabdus) from *X. nematophila* shows modest activity against several bacteria (Gualtieri et al. 2009). Several other bioactive compounds such as indole derivatives (Sundar and Chang 1993), benzylidenacetone (Ji et al. 2004), ribosomal-encoded bacteriocins (Singh and Banerjee 2008), PAX peptides (Fuchs et al. 2011), xenocoumacins (Reimer and Bode 2014), and depsipeptides (Kronenwerth et al. 2014) were purified from *Xenorhabdus* spp.

*Photorhabdus* spp. released an array of secondary metabolites with a broad range of antibiotic properties that hinder the decaying of insect cadaver (Stock et al. 2017). Antibacterial capacity of *P. luminescens* was also revealed in the previous studies. Poinar Jr et al. (1980) demonstrated that *P. luminescens* hinder the development of the *B. subtilis* and *Bacillus cereus*. They were reported effective against phytopathogen *Erwinia carotovora* (Akhurst 1982a). Paul et al. (1981) reported two compounds, complex V (3.5-dihydroxy-4-isopropyl-trans-stilbene) and complex VI (3.5-dihydroxy-4-ethyl-trans-stilbene) derived from *P. luminescens* showing antibiotic actions. Antibiotic effect of *Photorhabdus* spp. against *B. subtilis* was also reported by Chen et al. (1996) 1,2-isopropyl-5-(3-phenyl-oxiranyl)-benzene-1,3-diol (syn. 2-Isopropyl-5-(3-phenyl-2-oxiranyl)-1,3-benzenediol), a new antibacterial complex was also recorded from *P. luminescens* that exhibit potent bactericidal activities (Hu et al. 2006). A carbanep complex (1-carbanep-2-em-3-carboxylic acid) secreted by *Photorhabdus* represent insecticidal potential against various bacterial species including *Klebsiella pneumonia*, *Enterobacter cloacae*, and *Escherichia coli* (Derzelle et al. 2002).

Anthraquinone by products 1,8-dihydroxy-9,10-anthraquinone and 3,8-dihydroxy-9,10-anthraquinone derived from Type II polypeptide synthase enzymes of *Photorhabdus* shows antibacterial activities (Challinor and Bode 2015). Bactericidal effect of *Photorhabdus* luminescens subsp. *akhurstii* (bSBR36.2_TH) was reported against *E. coli*, *Bacillus subtilis*, *S. aureus* RN4220, and *S. pyogenes* (Derzelle et al. 2002). *P. luminescens* produces photobactin (2-2, 3-dihydroxyphenyl)-5-methyl-4, 5-dihydro-oxazole-4-carboxylic acid [4-(2,3-dihydrobenzoylamo)n-butyl]-amide) a catecholate siderophore that shows antibiosis against insects (Ciche et al. 2003). Bactericidal activities of *Photorhabdus* spp. were effective against Gram negative bacteria *Erwinia amylovora* responsible for causing fire blight in rosaceae (Hevesi et al. 2004) and manage two more bacterial spp. *Xanthomonas* and *Pseudomonas* in plants (Uma et al. 2010). Numerous biological activities including bactericidal activities were observed from isopropylstilbene compound produced by *Photorhabdus* spp. against *S. aureus* and *E. coli* (Shi et al. 2017). Muangpat et al. (2017) emphasized the bactericidal properties of *Photorhabdus* spp. The growths of about 10 drug-resistant bacterial strains together with *S. aureus* strain PB57, PB36 and ATCC20475 prohibited by the extract (ethyl acetate) of *P. temperata*. Bacterial spp. *P. luminescens* subsp. *akhurstii* was recorded to effectively suppress the *S. aureus* strain PB36 (Muangpat et al. 2020).

**Antifungal activity**

The metabolites produced by endosymbionts have been reported to inhibit the growth of the multiple fungi. Huge numbers of research studies were carried out and being continued to measure the efficacy of endosymbionts against fungal pathogens. *X. nematophila* produces cyclolipopetide having lysin rich residue is highly effective against fungal pathogens including plant as well as animal fungal pathogens (Gualtieri et al. 2009). Chen et al. (1994) evaluated the inhibitory effect of *X. nematophilus* X. bovienii and *P. luminescens* against 32 fungal species and they found effectiveness against all fungal species. Even the growth of 7 major phytopathogenic fungi: *Trichoderma pseudokgingi*, *Botrytis cinerea*, *Mucor piriformis*, *Ceratozysis ulmi*, *Pythium coloratum*, *Ceratozysis dryocotidis*, and *Pythium ultimatum* were completely suppressed by these endosymbionts. Webster et al. (1995) reported the fungicidal activities of endosymbionts *Xenorhabdus* and *Photorhabdus*. The metabolic complexes from *Xenorhabdus* and *Photorhabdus* were isolated and it is found that these compounds have defensive and fungus eliminating properties. High antimycotic effect was recorded in *Sclerotinia sclerotiorum* when cell free filtrate of *X. szentirmaii* was applied (Chacon-Orozco et al. 2020). It was reported that inside insect cadaver antimycotic substances were produced by *Photorhabdus* spp. that protect the cadaver and prevent the growth from invading fungal pathogen (Chen et al. 1994). Crude extract of *Photorhabdus* spp. was assessed against fungal phytopathogens such as *Phomopsis* sp., *Fusi cladosporiun efficium*, *Glomerella cingulata*, *Phytophthora cactorum*, and *Monilinia fructicola*. Modest effect of endosymbionts against fungal phytopathogens was observed (Shapiro-Ilan et al. 2009). Strong fungicidal effect of *Photorhabdus* spp. was observed towards *Monilipophora roreri* (San-Blas et al. 2012). Even several specified secondary metabolic complexes have been applied to assess their antimycotic peoerties. Li et al. (1995b) applied 3,5-dihydroxy-4-isopropylstilbene on...
fungi Aspergillus fumigatus, A. flavus, Cryptococcus neoformans, Botrytis cinerea, and Candida tropicalis. Photorhabdus temperata SN259 strain produces seven metabolic complexes among which two stilbene complexes such as 3-hydroxy-2-isopropyl-5-phenethyl phenyl carbamate and 2-isopropyl-5-(E)-2-phenoxyethenyl) benzene-1,3-diol (syn. 3,5-dihydroxy-4-isopropylstilbene) were applied to investigate its impact upon E. oxysporum, Rhizoctonia solani, Pythium aphanidermatum, and Exserohilum turcicum. A highly strong inhibitory effect has been observed against P. aphanidermatum (Shi et al. 2012). Transcinnamic acid isolated from P. luminescens prevents the growth of the Fuscidium effusum (Bock et al. 2014). P. temperata M102 derived metabolite benzaldehyde was assayed against three phytopathogens Phytophthora capsici, Corynespora cassiciola, and R. solani. Benzaldehyde shows high inhibitory effect against the fungal phytopathogen. Even Photorhabdus spp. has been reported to hinder the growth of about 32 fungal species (Ullah et al. 2014). Trans-cinnamic acid (TCA) isolated from Photorhabdus sp. hinder the growth of Colletotrichum acutatum, Colletotrichum gloeosporioides and Colletotrichum fragariae (Chen et al. 1996). TCA also inhibits the growth of E. effusum (Shapiro-Ilan et al. 2014). The efficiency of cell free filtrate from P. luminescens strain VS, P. temperata and P. luminescens strain K122 was assessed against various phytopathogenic fungal species such as: Armillaria tabescens, Fuscidium carpophilum, E. effusum, Glomerella cingulata and Monilinia fructicola. It was found that these supernatants completely suppress the growth of the phytopathogenic fungi (Hazir et al. 2016). Under an in vitro inhibition test, fungi Alternaria alternate and Fusarium oxysporum sp. asparagus were treated with the crude extract of P. luminescens sp. sonorenensis, that show mild effect against these fungi although the growth was retarded (Orozco et al. 2016). Secondary metabolic complexes produce by P. akhurstii exhibit fungicidal activities against Colletotrichum gloeosporioides (Tu et al. 2022).

Nematicidal activity

The cell free substrate of Photorhabdus spp. not only exhibit insecticidal, antibiotic and antimycotic activities but also possesses nematicidal properties. They have been reported to kill or manage various species of plant parasitic nematodes. Several strains of Photorhabdus spp. were evaluated against Meloidogyne incognita and Bursaphelenchus xylophilus. They were highly lethal against second-stage juveniles of M. incognita and adults as well as fourth-stage juveniles of Bursaphelenchus xylophilus (Hu et al. 1999). Crude extract of P. luminescens sonorenensis CH35 strain was applied against three nematode species namely: M. incognita, Caenorhabditis elegans and S. carpocapsae. It was found that this supernatant was highly effective against M. incognita (J2), while it exhibited very low nematicidal activities against C. elegans and S. carpocapsae (Orozco et al. 2016). The metabolic substances stilbene (3,5-Dihydroxy-4-isopropylstilbene) and indole produced through P. luminescens strain MD were tested against A. rhizogenoides rhytium, Bursaphelenchus spp., C. elegans and M. incognita. High nematode killing abilities were exhibited by these derivatives against fourth-stage juvenile and adult forms of three tested species; however, no nematicidal effect of these metabolites has been observed against M. incognita (J2) (Hu et al. 1999). Cell-free supernatant of Xenorhabdus spp. is highly toxic against M. incognita and showed inhibitory effects (Grewal et al. 1999). Lewis et al. (2001) worked on the interactions of S. feltiae and X. bovienii with M. incognita and found that tomato plant infected with M. incognita when treated with same rate of S. feltiae, affect egg production with lesser galls in their roots. X. bovienii also shows inhibitory effect against M. incognita.

Cell-free filtrate of X. bovienii was applied against Meloidogyne javanica and M. incognita and a moderate effect was observed (Kepenekci et al. 2018). Bi et al. (2018) reported about seven metabolites (Rhabdopeptide I-O or 1–7) from X. budapestensis SN84 that possess nematode killing properties and is found effective against M. incognita (J2). Among all seven isolated metabolites, rhabdopeptide J2 was highly effective. Xenorhabdus spp. along with neem cake and furadan was applied to control the M. incognita infestation in Grapevines. It was reported that all treatment significantly suppresses the nematodes population in grapevines (El-Deen et al. 2019).

Bioformulations produced from endosymbionts

Bioformulations are biological pesticides invented with useful microbiomes including bacteria, viruses, fungi, nematodes, and plant-based extracts as well as semiochemicals, as active component. Generally, bioformulations developments are based upon the bioresource detection, optimization, stabilization, and risk executive energy. The biopesticides have been regarded as best alternate of chemical insecticides in sustainable crop production due to their eco-friendly behaviour (Gašić and Tanović 2013). Nowadays, peoples become aware about the hazards caused by extensive use of synthetic chemical insecticides and moving towards healthy and organic foodstuff. Bioformulation applications easily tackle the insect resistance problems, provide effective protection to the crops and are an important part of integrated pest management (IPM) strategies (Elad et al. 1996). A wide range of biopesticides from entomopathogenic bacteria were available for insect pest management. These
formulations are in the form of dry (granular) and wet products (liquid and wet powder) (Singh et al. 2014). Dust powder, seed dressing powder, water dispersible granules as well as wet powders form with dilution attributes are considered under dry formulations. Emulsions, suspoemulsions, suspension concentrates, capsule suspensions, oil dispersions, and ultra-low-volume formulations were considered under wet products/formulation (Knowles 2005). The endosymbionts *Xenorhabdus* and *Photorhabdus* exhibited insecticidal, antimycotic, bactericidal and nematicidal activities so they can be employed for bioformulation production (Namsena and Bussaman 2020). Namsena et al. (2016) reported three bioformulations of *Xenorhabdus* (*X. stockiae* PB09) in the form of liquid supernatant, cell pellet, wettable powder form and observed high mortality in mites even after storage up to 45 days at 4 °C. *P. luminescens* along with paraffin oil, Tween-20 and sucrose were applied directly as a spray against *Pieris brassicae*, and 100% larval mortality was observed within 24 h of foliar application (Mohan et al. 2003). Sodium alginate capsules were also prepared from *P. luminescens akhurstii* that was reported to cause 100% killing of *S. litura* within 48 h of its infection (Rajagopal et al. 2006). Toxicity in *Prays oleae* was also recorded by *P. temperate*, when ingested directly (Tounsi et al. 2006) (Fig. 3).

**Conclusion and future prospects**

Entomopathogenic nematodes (EPNs), the valuable nematodes, have been reported as the finest biocontrol agents. EPNs are considered as the best substitute of chemical insecticides due to their high potential of infecting the insects hidden even in mysterious places with high multiplication ability as well as their eco-friendly nature. In the field of biological pest management, the application of EPNs along with their bacterial symbionts becomes a popular approach of pest control. The diversified secretion systems of entomopathogenic bacteria were involved in the release secondary metabolite. These secondary metabolites are toxic proteins that possess high insecticidal potential along with high antimicrobial activities. *Xenorhabdus* and *Photorhabdus* strains produced multiple metabolites that act against a variety of organisms including, protozoans, fungi, nematodes

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**Fig. 3** Application strategies for bacterial endosymbionts
insects, and even against cancerous cells. Various kinds of antibiotic were also produced from several species of symbiotic bacteria that showed the importance of these bacterial symbionts in the drug industry. There is a need of more surveys on EPNs to discover more species of endosymbiotic bacteria. The secondary metabolites, rich sources of toxic and bioactive compounds from endosymbiotic bacteria, need to be optimized and explored in the future; along with that there is a necessity of development of some by-product from these bacteria and their metabolites.

Abbreviations
X. nematophila: Xenorhabdus nematophilia; P. luminescens: Photorhabdus luminescens; IJs: Infective juveniles; EPNs: Entomopathogenic nematodes; BCAs: Biocontrol agents; %: Percent.

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References
Abate BA, Slippers B, Wingfield MJ, Malan AP, Hurley BP (2018) Diversity of entomopathogenic nematodes and their symbiotic bacteria in South African plantations and indigenous forests. Nematology 20:355–371
Adams BJ, Nguyen KB (2002) Taxonomy and systematics. In: Gaugler R (ed) Entomopathogenic nematology. CABI Internacional, Wallingford, pp 19:4564–4575
Ahn J Y, Lee J Y, Yang E J, Lee Y J, Koo KB, Song KS, Lee KY (2013) Mosquitocidal activity of anthraxgonines isolated from symbiotic bacteria Photorhabdus of entomopathogenic nematode. J Asia-Pac Entomol 16:317–320
Ahuja A, Kushwah J, Mathur C, Chauhan K, Dutta TK, Sormvanshi VS (2021) Identification of Galtocx, a new protein toxin from Photorhabdus bacteri-symbionts of Heterorhabditis nematodes. Toxicon 194:53–62.
Gerritsen LJ, Georgiev J, Wiegers GL (2005) Oral toxicity of \textit{Photorhabdus} toxins against thrips species. J Invertebr Pathol 88:207–211

Glaser SP, Tobias NJ, Thanwasi A, Chandra P, Bode HB, Kämpfer P (2017) \textit{Photorhabdus luminescens} subspp. nannomaceus subspp. nov., isolated from \textit{Heterorhabditis biquardi} nematodes. Int J Syst Evol Microbiol 67:1046–1051

Gorgadze O, Lorkhipanidze M, Ogier J-C, Taillez P, Burjandadze M (2015) [i] \textit{Steinernema bilisilvisis} sp. n. [//Nematoda: Steinernematidae]—a new species of entomopathogenic Nematode from Georgia. J Agric Sci Technol 5:264–276

Gómez I, Konstantinidou KT, Klappebach JA, Coeney T, Vandamme P, Tieje JM (2007) DNA–DNA hybridization values and their relationship to whole-genome sequence similarities. Int J Syst Evol Microbiol 57:81–91

Grewal P, Georgis R (1999) Entomopathogenic nematodes. In: Hall FR, Menn JJ (eds) Biopesticides: use and delivery. Springer, pp 271–299

Grewal PS, Lewis EE, Grewal P, Sardanelli S (2001) Interactions between the \textit{Xenorhabdus} sp. and \textit{Steinernema} sp. nov. \textit{Xenorhabdus} sp. sp. nov., and \textit{Xenorhabdus} budapestensis sp. nov. \textit{xenorhabdus} sp. nov., and \textit{Xenorhabdus bovienii} sp. nov. \textit{Xenorhabdus} sp. sp. nov. and \textit{Xenorhabdus} bovienii sp. nov., isolated from \textit{Steinernema} species. Int J Syst Evol Microbiol 67:1107–1114

Kang S-J, Han S-C, Yi Y-K, Kim Y-G (2003) Identification of an entomopathogenic nematode, \textit{Steinernema monticolus}, and its novel symbiotic bacterium. In: Proceedings of the Korean Society of Applied Entomology Conference. Korean Soc Appl Entomol., p 83

Kaya HK, Gaugler R (1993) Entomopathogenic nematodes. Ann Rev Entomol 38:181–206

Kepenecki I, Hazir S, Oksal E, Lewis EE (2018) Application methods of \textit{Steinernema feltiae}, \textit{Xenorhabdus bovienii} and \textit{Purpureocillium lilacinum} to control root-knot nematodes in greenhouse tomato systems. Crop Prot 108:31–38

Khush RS, Leulier F, Lemarteau B (2002) Pathogen surveillance—the flies have it. Science 296:273–275

Kim IH, Aryal SK, Aghai DT, Casanova-Torres AM, Hillman K, Kozuch MP, Mans EJ, Mauer TJ, Ogier JC, Ensign JC, Gaudriault S (2017) The insect pathogenic bacterium \textit{Xenorhabdus bovienii} has attenuated virulence in multiple insect model hosts yet encode a potent mosquitocidal toxin. BMC Genomics. 18(1):1–25

Kim Y, Ahmed S, Stanley D, An C (2018) Ecosanoid-mediated immunity in insects. Dev Comp Immunol 83:130–143

Knowles A (2005) New developments in crop protection product formulation. T&F Informa UK Ltd, pp 23–34

Koppenhöfer AM, Grewal PS, Fuzy EM (2007) Differences in penetration routes and establishment rates of four entomopathogenic nematode species into four white grub species. J Invertebr Pathol 94:184–195

Kronenwerth M, Bozhüyük KA, Kahnt AS, Steinilber D, Gaudriault S, Kaiser M, Bode HB (2014) Characterisation of \textit{Taxallia} sp. nov. and \textit{Xenobactin} from \textit{Xenorhabdus indica}. Chem Eur J 20:1747B–17487

Kuwata R, Chu L-H, Wang W, Harada Y, Yoshida M, Kondo E, Yoshiga T (2013) \textit{Xenorhabdus ishibashii} sp. nov., isolated from \textit{Steinernema} acari. Int J Syst Evol Microbiol 63:1600–1695

Lalramnghaki H (2017) Characterization of a new isolate of entomopathogenic nematode, \textit{Steinernema ranisong(i) (Rhabditida, Steinernematidae)}, and its symbiotic bacteria \textit{Xenorhabdus vietnamensis} (\textit{y}-Proteobacteria) from Mizoram, northeastern India. J Parasit Dis 41:1123–1131

Lang G, Kalveigne T, Peters A, Wiese J, Imhoff JF (2008) Linear and cyclic peptides from the entomopathogenic \textit{Bacterium} \textit{Xenorhabdus nematophilus}. J Nat Prod 71:1074–1077

Lang AE et al (2010) \textit{Photorhabdus luminescens} toxins ADP-ribosylate actin and Rha to actin filament clustering. Science 327:1139–1142

Lee PJ, Ahn J-Y, Kim Y-H, Kim SW, Kim J-Y, Park J-S, Lee J (2004) Cloning and heterologous expression of a novel insecticidal gene (\textit{tccc1}) from \textit{Xenorhabdus nematophilus} strain. Biochem Biophys Res Commun 319:1110–1116

Lee D-E, Lee J, Yi M-Y, Myeong J-H, Kim K-H (2013) Uncultured bacterial diversity in a seawater recirculating aquaculture system revealed by 16S rRNA gene ampiclon sequencing. J Microbiol 54:296–304

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

Lewis E, Grewal P, Sardanelli S (2001) Interactions between the \textit{Steinernema feltiae}–\textit{Xenorhabdus bovienii} insect pathogen complex and the root-knot nematode \textit{Meloidogyne incognita}. Biol Control 21:55–62

Li J, Chen G, Webster JM, Czyzewska E (1999a) Antimicrobial metabolites from a bacterial symbiont. J Nat Prod 58:1081–1086

Li J, Chen G, Wu H, Webster JM (1999b) Identification of two pigments and a hydroxystilbene antibiotic from \textit{Photorhabdus luminescens}. J Biol Chem 273:28287–28296

Kämpfer P, Tobias NJ, Ke LR Bode HB, Glaser SP (2017) \textit{Xenorhabdus huangu- anensis} sp. nov. and \textit{Xenorhabdus eapokensis} sp. nov., isolated from \textit{Steinernema} species. Int J Syst Evol Microbiol 67:1107–1114

Kuang S-J, Han S-C, Yi Y-K, Kim Y-G (2003) Identification of an entomopathogenic nematode, \textit{Steinernema monticolus}, and its novel symbiotic bacterium. In: Proceedings of the Korean Society of Applied Entomology Conference. Korean Soc Appl Entomol., p 83
Liu W-T, Chen T-L, Hou RF, Chen C-C, Tu W-C (2020) The invasion and encapsulation of the entomopathogenic nematode, Steinernema abbreviatus, in Aedes albopictus (Diptera Culicidae) Larvae. Insects 11:832

Lulamba TE, Green E, Serepa-Diamini NH (2021) Photorhabdus sp. ET1 antimicrobial properties and characterization of its secondary metabolites by gas chromatography–mass spectrometry. Life 11:787

Machado RA et al (2018) Whole-genome-based revisit of Photorhabdus phylogeny: proposal for the elevation of most Photorhabdus subspecies to the species level and description of one new species Photorhabdus boedi sp. nov., and one new subspecies Photorhabdus laumondii subsp. clarkei sp. nov. Int J Syst Evol Microbiol 68:2664–2681

Machado RA et al (2019) Photorhabdus khanii subsp. guanguautensis subsp. nov., isolated from Heterorhabditis atacamensis, and Photorhabdus luminescens subsp. mexicana subsp. nov., isolated from Heterorhabditis mexicana entomopathogenic nematodes. Int J Syst Evol Microbiol 69:652–661

Machado RA et al (2021) Photorhabdus heterorhabditis subsp. aluminescens subsp. nov. Photorhabdus heterorhabditis subspecies. Heterorhabditis subspec. nov. Photorhabdus asterias subspp. thailandensis subspp. nov. Photorhabdus asterias subspp. aluminascens subspp. nov., and Photorhabdus aegyptia subspp. isolated from Heterorhabditis entomopathogenic nematodes. Int J Syst Evol Microbiol 71:004610

Mahar AT, Lee SS, Lalonide FD, Impelluso T, Newton PO (2004) Biomechanical comparison of stainless steel and titanium nails for fixation of simulated femoral fractures. J Pediatr Orthop 24:638–641

Mahar A, Jan N, Mahar GM, Mahar AQ (2008) Control of insects with entomopathogenic bacterium Xenorhabdus nematophila and its toxic secretions. Int J Agric Biol 10:52–56

Mahnood S, Kumar M, Kuman P, Mahapatro GK, Banerjee N, Sarin NB (2020) Novel insecticidal chitinase from the insect pathogen Xenorhabdus nematophila. Int J Biol Macromol 159:394–401

Martens EC, Goodrich-Blair H (2005) The Xenorhabdus carposcapes intestinal vesicle contains a subcellular structure with which Xenorhabdus nematophila associates during colonization initiation. Cell Microbiol 7:1723–1735

Martens EC, Heungens K, Goodrich-Blair H (2003) Early colonization events in the mutualistic association between Steinernema carposcapes nematodes and Xenorhabdus nematophila bacteria. J Bacteriol 185:3147–3154

Migunova VD, Sasanelli N (2021) Bacteria as biocontrol tool against pythoparasitic nematodes. Plants 10:389

Mohar S, Raman R, Gaur H (2003) Foliar application of entomopathogenic nematodes and Xenorhabdus nematophila from Mae Wong National Park, Thailand. Front Microbiol 8:1142

Muanwpant P et al (2020) Antibacterial activity of Xenorhabdus and Photorhabdus isolated from entomopathogenic nematodes against antibiotic-resistant bacteria. PLoS ONE 15:1–16

Namsema P, Bussaman P (2020) Formulation development of the bacteria associated with entomopathogenic nematode (Xenorhabdus stockiae PB09) for controlling mushroom mite (Luciaphorus perniciosus) Larvae in laboratory and field conditions. Mahasarakham University, pp 1–139

Namsema P, Bussaman P, Rattanasena P (2016) Bioformulation of Xenorhabdus lagalense. Namsena P, Bussaman P (2020) Formulation development of the bacteria Photorhabdus luminescens subspp. sonorensis. J Invertebr Pathol 141:44–52

Ozdemir E, Bayram Ş, Susuruk IA (2020) First Record of the entomopathogenic nematode Steinernema littorale (Filipjev)(Rhabditida: Steinenermatidae) and its symbiotic bacterium from Turkey, and its efficacy against insects. Insects 11:144

Park Y, Kim Y, Stanley D (2004) The bacterium Xenorhabdus nematophila inhibits phospholipases A2 from insect, prokaryote, and vertebrate sources. Naturwissenschaften 91:371–373

Park HB, Perez CE, Perry EK, Crawford JM (2016) Activating and attenuating the amoxicillin antibiotics. Molecules 21:824

Paul VJ, Frautschy S, Fenical W, Neilson KH (1981) Antibiotics in microbial ecol. J Chem Ecol 7:389–397

Plitcha KL, Joyce SA, Clarke D, Waterfield N, Stock SP (2009) Heterorhabditis gerrardii sp. n. (Nematoda: Heterorhabditidae): the hidden host of Photorhabdus asymbiotica (Enterobacteriaceae: y-Proteobacteria). J Helminthol 83(4):309–320

Poinar G Jr (1993) Origins and phylogenetic relationships of the entomophilic rhabditids, Heterorhabditis and Steinernema. Fundam Appl Nematol 16:333–338

Poinar GO Jr, Thomas GM (1965) A new bacterium, Achromobacter nemaphilus sp. nov. (Achromobacteriae: Eubacteriales) associated with a nematode. Int J Syst Evol Microbiol 15:249–252

Poinar GO, Thomas GM (1966) Significance of Achromobacter nemaphilus Poinar and Thomas (Achromobacteriae: Eubacteriales) in the developmental of the nematode, DD-136 (Necoplectrona sp. Steinernematidae). Parasitol 56:385–390

Poinar G Jr, Wassink H, Leegwater-van Der Linden M, van der Geest L (1979) Serratia marcescens as a pathogen of tsetse flies. Acta Trop 36:223–227

Poinar GO Jr, Thomas G, Haygood M, Neilson KH (1980) Growth and luminescence of the symbiotic bacteria associated with the terrestrial nematode Heterorhabditis bacteriophora. Soil Biol Biochem 12:5–10

Poinar G Jr, Karunakar G, David H (1992) Heterorhabditis indicus n. sp. (Rhabditida: Nematoda) from India: separation of Heterorhabditis spp. by infective juveniles. Fundam Appl Nematol 15:467–472

Proschak A et al (2014) Biosynthesis of the insecticidal xenocyclins in Xenorhabdus bovienii. Chem Bio Chem 15:369–372

Qin Z, Huang S, Yu Y, Deng H (2013) Dithiolopyrrolone natural products: isolation, synthesis and biosynthesis. Mar Drugs 11:3970–3997

Rajagopal R, Mohan S, Bhatnagar RK (2006) Direct infection of Pieris brassicae, symbiotic bacteria from entomopathogenic nematode Xenorhabdus nematophila. Int J Bacteriol 2013:1–9

Rathore JS (2013) Expression, purification, and functional characterization of atypic xencin, its immunity protein, and their domains from Xenorhabdus nematophila. Int J Bacteriol 13:1–9

Reimer D, Bode HB (2014) A natural prodrug activation mechanism in the biosynthesis of nonribosomal peptides. Nat Prod Rep 31:154–159

Reimer D, Luxenburger E, Brachmann AQ, Bode HB (2009) A new type of pyrrolidine biosynthesis is involved in the late steps of xenococumacin production in Xenorhabdus nematophila. Chem Bio Chem 10:1997–2001

Reimer D, Cowles KN, Proschak A, Nollmann FI, Dowling AJ, Kaiser M, Constant RF, Goodrich-Blair H, Bode HB (2013) Rhabdopeptides as insect-specific virulence factors from entomopathogenic bacteria. ChemBioChem 14:1991–1997

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

Richardson WH, Schmidt TM, Neilson K (1988) Identification of an anthraquinone pigment and a hydroxystilbene antibiotic from Xenorhabdus luminescens. Appl Environ Microbiol 54:1602–1605
Sajnaga E, Kazimierzczak W, Skowronek M, Lis M, Skrzypek T, Waśko A (2018) Steinernema poinari (Nematoda: Steinernematidae): a new symbiotic host of entomopathogenic bacteria Xenorhabdus bovienii. Arch Microbiol 200:1307–1316
Sanluis E, Carrillo Z, Parra Y (2012) Effect of Xenorhabdus and Photorhabdus bacteria and their exudates on Monilophthora roreri. Arch Phytopathol Plant Prot 45:1950–1967
Sergeant M, Jarrett P, Ousley M, Morgan JAW (2003) Interactions of insecticidal proteins of Steinernema and Heterorhabditis spp. and their application in agriculture: what we know and what to do next. J Nematol 49:373
Stokwe NF, Malan AP, Knoetze NKB, R and Tiedt L (2011) Steinernema carpocapsae n. sp. (Rhabditida: Steinernematidae), a new entomopathogenic nematode from South Africa. Nematology 13:569–587
Sundar L, Chang F (1993) Antimicrobial activity and biosynthesis of indole antibiosis produced by Xenorhabdus nematophilus. Microbiology 139:3139–3148
Tailliez P, Pages S, Ginibre N, Boermare N (2006) New insight into diversity in the genus Xenorhabdus, including the description of ten novel species. Int J Syst Evol Microbiol 56:2805–2818
Tailliez P, Laroui C, Ginibre N, Paule A, Pages S, Boermare 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. vietnamiensis sp. nov., P. luminenes sp. subsp. caribbeansensis subsp. nov., P. luminenes sp. subsp. hainanensis subsp. nov., P. temperata subsp. khani subsp. nov., P. temperata subsp. tasmaniensis subsp. nov., and the reclassification of P. luminenes subsp. thailandensis as P. temperata subsp. thailandensis comb. nov. Int J Syst Evol Microbiol 60:1921–1937
Thakur N, Kaur S, Tomar P, Thakur S, Yadav AN (2020) Microbial biopesticides: current status and advancement for sustainable agriculture and environment. Int. J. Nematology 14:741–757
Taraesco E, Santiago Alvarez C, Triggiani O, Quesada Moraga E (2011) Laboratory studies on the competition for insect haemocoeel between Beauveria bassiana and Steinernema chihuase recovered in the same ecological niche. Biocontrol Sci Technol 21:693–704
Thakur N, Kaur S, Tomar P, Thakur S, Yadav AN (2020) Microbial biopesticides: current status and advancement for sustainable agriculture and environment. Int. J. Nematology 14:741–757
Singh AK et al. (2022) Loop-mediated isothermal amplification based identification of entomopathogenic nematodes. J Agric Food Chem 65:60–65
Singh G, Ahuja A, Rao U, Somvanshi VS (2021) Loop-mediated isothermal amplification based identification of entomopathogenic nematodes. J Agric Food Chem 65:60–65
Singh H, Singh A, Sarma B, Upadhyay D (2014) Trichoderma viride and live crickets. J Nat Prod 75:2007–2011
Singh AK et al. (2022) Entomopathogenic nematodes: a sustainable option for insect pest management. In: Rakshit A, Meena VS, Abhilash PC, Sarma BK (eds) Biopesticides. Elsevier, pp. 243-82.
Singh AK, King JB, You J, Cichewicz RH (2012) Production of cytotoxic gloabolicins from Photorhabdus asymbiotica in liquid media and live crickets. J Nat Prod 75:2007–2011
Sundar L, Chang F (1993) Antimicrobial activity and biosynthesis of indole antibiosis produced by Xenorhabdus nematophilus. Microbiology 139:3139–3148
Tailliez P, Pages S, Edgington S, Tymo LM, Buddie AG (2012) Description of Xenorhabdus magdalenensis sp. nov., the symbiotic bacterium associated with Steinernema as acaule. Int J Syst Evol Microbiol 62:1761–1765
Tamura T, Waseyenbergbe L, Hailu T, Ehlers R-U, Poglia V, Mrácek Z (2012) Steinernema epipterae n. sp. (Rhabditida: Steinernematidae), a new entomopathogenic nematode from Ethiopia. Nematology 14:741–757
Taraesco E, Santiago Alvarez C, Triggiani O, Quesada Moraga E (2011) Laboratory studies on the competition for insect haemocoeel between Beauveria bassiana and Steinernema chihuase recovered in the same ecological niche. Biocontrol Sci Technol 21:693–704
Thakur N, Kaur S, Tomar P, Thakur S, Yadav AN (2020) Microbial biopesticides: current status and advancement for sustainable agriculture and environment. Int. J. Nematology 14:741–757
Thakur N, Tomar P, Kaur S, Jhamta S, Thakur R, Yadav AN (2021) Entomopathogenic soil microbes for sustainable crop protection. Soil microbiomes for sustainable agriculture. Springer, Cham, pp. 329-571
Thakur N, Tomar P, Kaur S, Kumari P (2022a) Virulence of native entomopathogenic nematode from major lepidopteran insect species of tomato (Solanum lycopersicum L.). J Appl Biotechnol 10(1):1–9
Thakur N, Tomar P, Sharma S, Kaur S, Sharma S, Yadav AN, Hesham AE (2022b) Synergistic effect of entomopathogens against Spodoptera litura (Fabricius) under laboratory and greenhouse conditions. Egypt J Biol Pest Control 32(1):1–11
Theodore CM, King JB, You J, Cichewicz RH (2012) Production of cytotoxic gloabolicins from Photorhabdus asymbiotica in liquid media and live crickets. J Nat Prod 75:2007–2011
Thomas GM, Poinar JRGO (1979) Xenorhabdus gen. nov., a genus of entomopathogenic, nematophilic bacteria of the family Enterobacteriaceae. In: Int J Syst Evol Microbiol 29:352–360
Tobias NJ (2017) Natural product diversity associated with the nematode symbiont Photorhabdus and Xenorhabdus. Nat Microbiol 2:1676–1685
Tomar P, Thakur N, Sharma A (2022) Infectivity of entomopathogenic nematode against the cabbage butterfly (Pieris brassicae L.) in polyhouse and in field condition. Egypt J Biol Pest Control 32(1):1–11
Töth L, Lakatos T (2008) Photorhabdus temperata subsp. cinerea subsp. nov., isolated from Heterorhabditis nematodes. Int J Syst Evol Microbiol 58:2579–2581
Tounsi S, Aoun AE, Blight M, Rebaï A, Jaoua S (2006) Evidence of oral toxicity of Bacillus thuringiensis Cry1Aa and its improve-ment by heterologous expression of Prays oleae and its improve-ment by heterologous expression of Prays oleae. In: New and future developments in microbial biotechnology and bioengineering. Elsevier. pp. 243-82.
Tomar P, Thakur S, Yadav AN, Sharma A (2022b) Synergistic effect of entomopathogens against Spodoptera litura (Fabricius) under laboratory and greenhouse conditions. Egypt J Biol Pest Control 32(1):1–11
Theodore CM, King JB, You J, Cichewicz RH (2012) Production of cytotoxic gloabolicins from Photorhabdus asymbiotica in liquid media and live crickets. J Nat Prod 75:2007–2011
Thomas GM, Poinar JRGO (1979) Xenorhabdus gen. nov., a genus of entomopathogenic, nematophilic bacteria of the family Enterobacteriaceae. In: Int J Syst Evol Microbiol 29:352–360
Tobias NJ (2017) Natural product diversity associated with the nematode symbiont Photorhabdus and Xenorhabdus. Nat Microbiol 2:1676–1685
Tomar P, Thakur N, Sharma A (2022) Infectivity of entomopathogenic nematode against the cabbage butterfly (Pieris brassicae L.) in polyhouse and in field condition. Egypt J Biol Pest Control 32(1):1–11
Töth L, Lakatos T (2008) Photorhabdus temperata subsp. cinerea subsp. nov., isolated from Heterorhabditis nematodes. Int J Syst Evol Microbiol 58:2579–2581
Tounsi S, Aoun AE, Blight M, Rebaï A, Jaoua S (2006) Evidence of oral toxicity of Bacillus thuringiensis Cry1Aa and its improve-ment by heterologous expression of Prays oleae and its improve-ment by heterologous expression of Prays oleae. In: New and future developments in microbial biotechnology and bioengineering. Elsevier. pp. 243-82.
Tomar P, Chiu J, Lin C, Chien C, Hsieh F, Shih M, Yang Y (2022) Evaluation of the antifungal activities of Photorhabdus akhurstii and its secondary
metabolites against phytopathogenic *Colletotrichum gloeosporioides*. J Fungi 8(4):1–13

Turlin E, Pascal G, Rousselle JC, Lenormand P, Ngo S, Danchin A, Derzelle S (2006) Proteome analysis of the phenotypic variation process in *Photorhabdus luminescens*. Proteomics 6:2705–2725

Ullah I, Khan AL, Ali L, Khan AR, Waqas M, Lee U, Shin JH (2014) An insecticidal compound produced by an insect-pathogenic bacterium suppresses host defenses through phenoloxidase inhibition. Molecules 19:20913–20928

Uma G, Prabhuraj A, Patil M (2010) Antibiotic and antibacterial activity of a symbiotic bacterium, *Photorhabdus luminescens*. J Biol Control 24:168–172

Webster JM, Chen G, Hu K, Li J (2002) Bacterial metabolites. In: Gaugler R (ed) Entomopathogenic nematology. CABI Publishing, Wallingford, pp 99–114

Webster J, Chen G, Li J (1995) Novel fungicidal properties of metabolites, culture broth, stilbene derivatives and indole derivatives produced by the bacteria *Xenorhabdus* and *Photorhabdus* spp. Patent no WO9503695

Weiser J (1955) *Neoaplectana carpocapsae* n. sp. (Anguillulata, Steinerne-matinae), novy cizopasník housenek obalece jablčného, *Carpocapsa pomonella* L. Vestn Česk Spol Zool 19:44–52

Wenski SL, Cimen H, Berghaus N, Fuchs SW, Hazir S, Bode HB (2020) Fabclavine diversity in *Xenorhabdus* bacteria. *Beilstein J Org Chem* 16:956–965

Xiao Y, Meng F, Qiu D, Yang X (2012) Two novel antimicrobial peptides purified from the symbiotic bacteria *Xenorhabdus budapestensis NMC*-10. Peptides 35:253–260

Yang J et al (2012a) An insecticidal protein from *Xenorhabdus budapestensis* that results in prophenoloxidase activation in the wax moth, *Gallina mellonella*. J Invertebr Pathol 110:60–67

Yang J, Zeng HM, Lin HF, Yang XF, Liu Z, Guo LH, Yuan JJ, Qiu DW (2012b) An insecticidal protein from *Xenorhabdus budapestensis* that results in prophenoloxidase activation in the wax moth, *Gallina mellonella*. J Invertebr Pathol 110(1):60–67

Yang X et al (2019) Screening a fosmid library of *Xenorhabdus stockiae* HN*__x01 reveals SrfABC toxin that exhibits both cytotoxicity and injectable insecticidal activity. J Invertebr Pathol 167:107247

Zamora-Lagos MA, Eckstein S, Langer A, Gazanis A, Pfeiffer F, Habermann B, Heermann R (2018) Phenotypic and genomic comparison of *Photorhabdus luminescens* subsp. *laumondii* TT01 and a widely used rifampicin-resistant *Photorhabdus luminescens* laboratory strain. BMC Genomics 19:1–17

Zhaoy L, Kaiser M, Bode HB (2018) Rhabdopeptide/xenortide-like peptides from *Xenorhabdus innexi* with terminal amines showing potent antiprotozoal activity. *Org Lett* 20:5116–5120

Zhou Q, et al (2019) Molecular mechanism of polyketide shortening in anthraquinone biosynthesis of *Photorhabdus luminescens*. Chem Sci 10:6341–6349

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