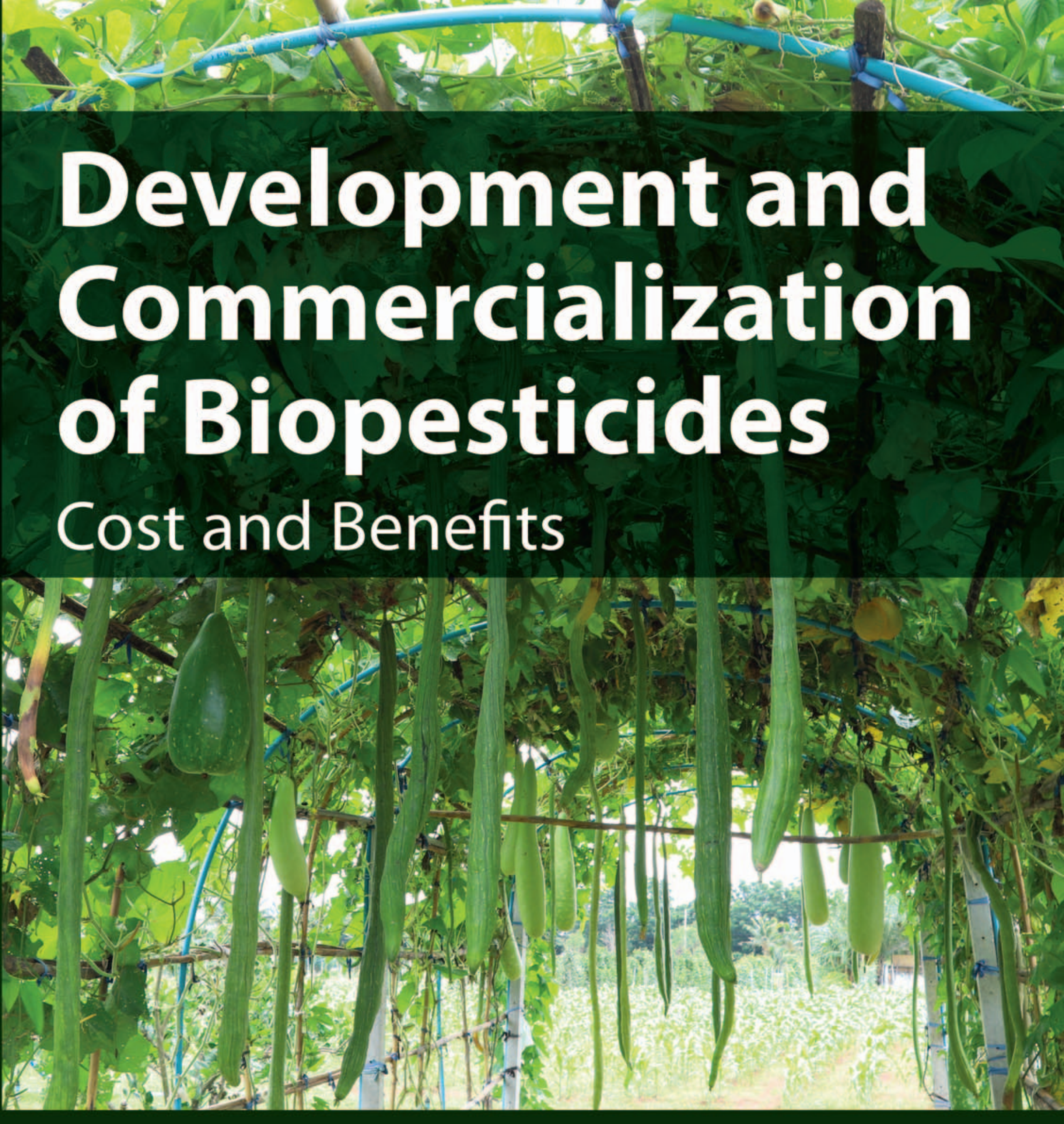

Development and Commercialization of Biopesticides

Cost and Benefits

Edited by
Opender Koul

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CHAPTER

5

Xenorhabdus- and *Photorhabdus*-based products: status and future perspective in agriculture

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New toolbox for pest and disease management

Every sector of the agriculture-related industry looks for strategies to get away from using hazardous chemicals, and the consumer demands eco-friendly products. Growers who can deliver an utterly chemical-free product with sustainable quality will have an edge in the market. Therefore, biological pest control is reasonably acceptable, but the constraint is to obtain well-understood and best products to be effective on a field scale. Thus, the demand for a new toolbox for pest and disease management is inevitable.

Current agricultural context: food losses in fields and postharvest supply chain

Within the framework of the 2030 Agenda of the United Nations, one of the noteworthy Sustainable development goals (SDGs) is ensuring an adequate food supply chain for a growing population while conserving natural resources. Farmers' losses represent between 60% and 80% (depending on different estimation methodologies) of total value chain losses (Delgado et al., 2017; Sagar et al., 2018). The leading causes of preharvest reduction in crop yields on the farm include infestation by pests and diseases (Jacometti et al., 2010), which might be exacerbated by the drought increased by global warming. Fungal diseases are also responsible for postharvest food decay (Delgado et al., 2017). The damage caused by insects and mites ranges from 8% to 23% in the United States, inflicting a loss between 5% and 15% from a total of $US 200 billion in revenue each year (Balog et al., 2017). The effects on nonindustrialized countries are even worse due to both the direct losses (pests and climate) and the indirect drivers, such as market prices or the quality of public

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services (including, for example, road infrastructure or other social services) (FAO, 2019). In addition, invasive pests represent new threats and challenges as global trade expands and climatic conditions shift. Recent estimates suggest that the crop yield losses caused by invasive pests will increase to 25% in the European Union (EU) by 2080 (Balog et al., 2017).

The end of chemical synthetic pesticides management

Industrialized agriculture model production has used plants protection products (PPPs), chiefly chemical synthetic pesticides, to protect crops from several pests and diseases and maintain the highest standards of food production rates (Therond et al., 2017). Indeed, "feeding the world" is a common rationale for the overuse of agrochemicals and insurance-based pest management approaches in crop protection. However, because of their known ability to cause much adverse health and environmental effects, it is more evident than ever that society needs the implementation of a new agricultural concept based on a more sustainable and ecological approach (Damalas, 2009; Nicolopoulou-Stamati et al., 2016). This urgent need has produced many innovative ideas, including agricultural reforms based on agroecological principles (Wezel et al., 2020) and the redesign of the food supply chain, reducing inefficient practices (Krishnan et al., 2020). Scientific research, integrated pest management (IPM) technology, and farmer education to halt or drastically reduce our overreliance on systemic agrochemicals globally are crucial steps to achieving the SDGs (Chai et al., 2021; Pecenka et al., 2021; Veres et al., 2020).

Options to reduce food losses while protecting the environment

During the last decade, the trends set by Agroecology have expanded from the field, farm, and agroecosystem scale to encompass the whole food system. Nowadays, there is a consensus about the necessity of new agriculture techniques based on principles of Agroecology to end the environmental degradation and loss of biodiversity (Therond et al., 2017). Within principles of Agroecology, Wezel et al. (2020) establish that future agriculture has to improve the care of soil health and biodiversity and reduce or eliminate dependency on purchased inputs. In recent years, agroecological approaches have also gained prominence in scientific, agricultural, and political discourse. Many countries worldwide are adopting regulations to implement these ecologically-friendly and sustainable approaches. For example, the EU has been working around the Integrated Pest Management policy step by step and is increasing the measures inside the European Green Deal (Barzman et al., 2015; Pecenka et al., 2021). The IPM is a central organizing principle to guide pesticide use based on "a use-as-needed approach": optimizing pesticide inputs, preventing overuse via practices such as scouting with applications dictated by a range of parameters, including economic thresholds, heat unit accumulations, and historical data (Barzman et al., 2015; Pecenka et al., 2021). Legislation is also increasingly restrictive with agrochemicals in the cropping systems. In the same line, for example, EU Directive 2009/128/EC "Sustainable use of plant protection products" marks that in Sustainable Agriculture, preventive or indirect control measures are the first choice, with direct control only applied when the rest of the measures are not adequate, and implementing actions that are selective to the target organism (EU, 2009).

Similarly, the Regulation (EU) 2015/408 has proposed a chemical substitution list currently used in industrialized agriculture, motivating the development of new physical, chemical, and biological control methods as alternatives to pesticides (EU, 2015). Beyond social lobby and scientific reports arguing against the use of synthetic pesticides, on-farm pest and diseases elimination programs are today often based on a combination of nonchemical and chemical control methods (Damalas, 2009) and, in many cases, are still managed with inertia toward "intelligent pesticide management" (Nicholls, 2010). Despite the sector's wishes, the complete elimination of agrochemicals is still not achievable.

Specific toolbox: biopesticides

Developing diverse pest control strategies that include safe products and practices inside an ecologically-based framework requires a specific toolbox for farmers, optimizing sustainable production, environmental quality, and human health. In recent decades, biopesticides are one of these tools promoted in pest management as possible alternatives to synthetic pesticides. Their definition by US Environmental Protection Agency is the following: (1) naturally occurring substances that control pests (biochemical pesticides), (2) microorganisms that control pests (microbial pesticides), and (3) pesticidal substances produced by plants that contain added genetic material (plant-incorporated protectants, PIPs) (Seiber et al., 2014). The PPP include synthetic PPPs and biopesticides in the European Union. All PPPs contain at least one active substance/ingredient and often contain components such as safeners, coformulants, adjuvants, and synergists (Scheepmaker et al., 2019). The European meaning of biopesticides is products derived from a biological origin and distinguishes only two categories: biological control products (homologous of US biochemical pesticides) and microbial biological control agents (homologous of US microbial pesticides), but do not recognize PIPs as biopesticides. Indeed, the regulatory European framework legislation on genetically modified food and feed is the strictest worldwide (EU, 2003). Currently, biopesticides comprise a small share (~5%) of the total crop protection market globally, valued at $3 billion in 2015 (Damalas and Koutroubas, 2018). Biopesticides use steadily increases 10% every year (Kumar and Singh, 2015). More than 200 products had been sold in the US market, compared to only 60 comparable products in the EU due to its lengthy, expensive, and cumbersome approval procedures of biopesticides in comparison with the rest of the World (Balog et al., 2017; Damalas and Koutroubas, 2018; Scheepmaker et al., 2019).

Biopesticides can be used in organic and conventional farming and enhance agoecological systems. Compounds derived from natural sources (plant, animal, bacterial, algae, or fungal origins) have the potential to be used for food safety and crop protection due to their antimicrobial properties against a broad range of pathogens and pests (Gyawali and Ibrahim, 2014). About 90% of the microbial biopesticides derive from the single bacterium *Bacillus thuringiensis* (*Bt*) and several *B. thuringiensis* subspecies (Lacey et al., 2015). These bacterial species produce insecticidal proteins during the sporulation phase as parasporal crystals (also called δ-endotoxins), predominantly comprising one or more proteins (Cry and Cyt toxins), disrupting the insect gut when ingested. These toxins, completely biodegradable, are highly specific to their target insects but innocuous to humans, vertebrates, and plants (Bravo et al., 2007). Therefore, *Bt* has become a viable alternative for controlling insect pests in agriculture. The live microbe form is an effective microbial pesticide. Purified toxins from this strain are the most widely used biochemical pesticide, and the DNA encoding the *Bt* toxin makes a powerful PIP. Currently, approximately 75% of all biopesticide use consists of *Bt*-based products. This microorganism dominates the current biopesticide landscape, but emerging approaches are poised to capture additional market share, thanks mainly to emerging resistance to *Bt*-based biopesticide products (Heckel, 2021).

Future biopesticides have to explore beyond *Bt* and go deeper in search of new natural sources of new entomopathogenic biotools. The development, use, and future directions of insect-specific viruses, bacteria, fungi, and nematodes as components of integrated pest management strategies for controlling arthropod pests of crops are fundamental for ensuring global food supply (Lacey et al., 2015). Another possibility is enhancing *Bt* by synergism with other biomolecules or beneficial microorganisms (Jung and Kim, 2006, 2007; Park et al., 2016). This chapter provides an overview of the beneficial functions of the entomopathogenic bacteria, *Xenorhabdus* and *Photorhabdus*, explaining the current knowledge of their biology to understand the many valuable properties that these bacteria can give in the pest/diseases management for crop protection (Fig. 5.1).

Biology of *Xenorhabdus* and *Photorhabdus* soil microorganisms: potential biofactory for novel compounds

The soil microbiome drives numerous vital/beneficial ecosystem functions and services, such as primary production, carbon sequestration, and nutrient mineralization (Thakur and Geisen, 2019). The structure and function of the soil microbiome depend on the interactive effects among associated-plant inputs, trophic regulations, and abiotic variables present in the soil. Several studies have focused on the soil microbiome—plant association for a wide range of microorganisms and plant species. Many beneficial free-living microorganisms are part of the functional biodiversity in the rhizosphere. Beneficial microorganisms can protect plants from pests or enhance their diseases resistance (e.g., induced systemic resistance, ISR) and abiotic stress tolerance. Entomopathogenic bacteria are beneficial microorganisms widespread in nature and include mainly members of the genera *Bacillus*, *Paenibacillus*,

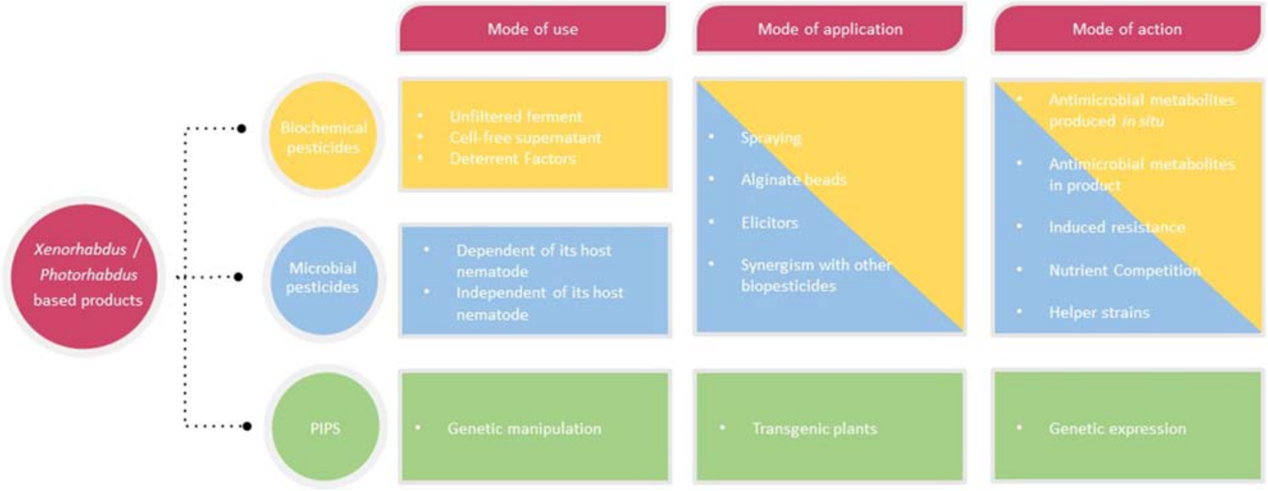

FIGURE 5.1

Illustration of possible scopes of mode use, mode of application, and mode of action of *Xenorhabdus* and *Photorhabdus* bacteria-based products.

Brevibacillus, *Serratia*, *Pseudomonas*, *Xenorhabdus*, or *Photorhabdus* (Glare et al., 2017). Entomopathogenic bacteria can be obligate or facultative arthropod pathogens and display different host ranges and mechanisms of infection. They all have similar abilities to produce a vast diversity of natural antibiotic products to overcome insect immune responses and host-microbiota (Gyawali and Ibrahim, 2014). Insect pathogenic (entomopathogenic) bacteria of the genera *Photorhabdus* (ffrench-Constant et al., 2007; Waterfield et al., 2004) and *Xenorhabdus* (Sergeant et al., 2003), which have been described as secondary metabolite producers, are becoming a potential novel source of biopesticides. Overall, *Xenorhabdus* and *Photorhabdus* are soil Gram-negative γ-Proteobacteria belonging to the family Morganellaceae (Adeolu et al., 2016). They have a mutualistic symbiosis stage with nematodes of the genera *Steinernema* and *Heterorhabditis*, respectively, and an entomopathogenic stage. The classification system of entomopathogenic nematode microsymbionts has recently recognized 26 species of the genus *Xenorhabdus* and 19 species of the genus *Photorhabdus* (Machado et al., 2018; Sajnaga and Kazimierczak, 2020). Despite their similar lifestyle and close phylogenetic origin (Tailliez et al., 2010), *Xenorhabdus* and *Photorhabdus* bacterial species differ significantly in the nematode host range, symbiotic strategies for parasite success, and arrays of released antibiotics and insecticidal toxins (Bode, 2009). The nonfeeding entomopathogenic nematode (EPN) state, free-living infective juvenile (IJ), stays in the soil and carries their associated microbiota (Ogier et al., 2020; Stock, 2015). The nematodes in the genus *Steinernema* carry, among other microorganisms, mutualistic *Xenorhabdus* bacteria in a specialized vesicle named “the receptacle,” placed in the anterior part of the gut. However, *Heterorhabditis* nematodes, which do not have such a specialized structure, use their intestinal lumen to harbor *Photorhabdus* until they find insect larvae to infection (Boemare, 2002). Then, the EPNs release the bacteria into the hemocoel through defecation or regurgitation (Boemare, 2002). Bacteria in the genera *Xenorhabdus* and *Photorhabdus* respond to environmental change by the existence of two phenotypically different forms. This phenotypic switching occurs in a small proportion of cells, so a sizable clonal population is probably never genetically homogeneous. Both bacteria stages synthesize numerous specialized metabolites that have roles in symbiosis and pathogenicity (Neubacher et al., 2020; Tobias et al., 2017). Thus, despite changes in growth conditions that can suddenly occur, some cells always express the phenotype needed for survival (Forst et al., 1997).

Along with the insect infection, *Xenorhabdus* and *Photorhabdus* primary variant cells exhibit a reversible change from (1) Mutualistic-form (*M*-form) characterized to be able to support nematode reproduction and colonization initiation in the infective juvenile (IJ) stage nematode to (2) Pathogenic-form (*P*-form) characterized by their virulence properties (Forst et al., 1997; Somvanshi et al., 2012). Cao and Goodrich-Blair (2020) reported that this shift occurs reciprocally depending on levels of *leucine*-responsive regulatory protein (Lrp) in *Xenorhabdus nematophila* within the receptacles of the EPN species *Steinernema carpocapsae*. Thus, high and low levels of Lrp expression imply higher ratios of *M* and *P* forms, respectively. The primary variant cell (*M*-form or *P*-form) occurs between the inside of the EPN and the insect host’s hemocoel. Besides, the species in the genus *Photorhabdus* have a putative free life cycle with a secondary cell variant living in the rhizosphere (Boemare and Akhurst, 1988; Eckstein et al., 2021; Forst et al., 1997). Primary and secondary cell variants are genetically identical, and epigenetic switching is still puzzling (Fig. 5.2). Researching these bacteria's role and function in the rhizosphere might help produce interesting novel compounds.

Once inside the host, the primary bacterial variant in *M*-form changes by a reversible promoter into *P*-form (Forst et al., 1997), which is bigger and faster-growing and starts to produce a wide range of specialized metabolites derived from nonribosomal peptide synthetase (NRPS) or polyketide synthase (PKS) (Tobias et al., 2017). These natural products secreted within the hemocoel overcome the host's immune response targeting different proteases involved in the insect immune response, for example, phospholipase A2 intermediate in cellular and humoral immune response in insects (Ahmed and Kim, 2018; Darsouei and Karimi, 2018; Mollah and Kim, 2020; Shi and Bode, 2018). These specific metabolites induce the host's death within 48−72 h after the EPN penetration. Then, *Xenorhabdus* and *Photorhabdus* natural products cause rapid changes in their biotic and abiotic environments. The cadaver decomposition processes provide nutrients into an environment with a flourishing microbial community, the host microbiota. When the nutrient plume spreads through the soil, it deserves its name: the cadaver decomposition island (CDI). Interspecific microbial communities, mainly opportunistic bacteria and fungi, compete for nutrients and shape a cadaver's microbial community composition, influencing other ecologically or biogeographically essential events, such as immigration or dispersal of community members. Understanding microbial interactions overall, including those in cadaver communities, may address questions at many research scales, including ecological theories about competition and trophic regulations in soil microbiome (Blanco-Pérez et al., 2017; Thakur and Geisen, 2019). In addition, the EPNs need time and the maintenance of proper conditions within the hemocoel to complete their life cycle. Before a new IJ offspring emerges, the nematode-killed host remains in the soil for 7−15 days or longer, at risk of other soil opportunistic microbiome. In this context, *Xenorhabdus* and *Photorhabdus* have developed a defense mechanism through scavenger deterrence based on specific deterrent factors (e.g., volatile organic compounds) (Gulcu et al., 2012; Shi and Bode, 2018; Ulug et al., 2014) to protect de CDI from another opportunistic colonization (Jaffuel et al., 2022; Karthik Raja et al., 2021; Lulamba et al., 2021). The deep knowledge about the wide range of metabolite production in *Xenorhabdus* and *Photorhabdus* species is crucial for metabolomics engineering and allows researchers to get attention to natural product with commercial relevance. Some of these compounds attracted substantial interest for biosanitary (Booysen and Dicks, 2020; Da Silva et al., 2020; ffrench-Constant et al., 2007) and agricultural purposes (Eroglu et al., 2019; Vicente-Díez et al., 2021b). Since the death of the host insect, EPNs develop their life cycle, feeding on their partner bacteria and host tissues until the resources deplete. Next, the second-stage juveniles develop to the IJ stage, incorporate some of the symbiont bacteria and exit the CDI by thousands into the soil to begin a new life cycle (Fig. 5.2).

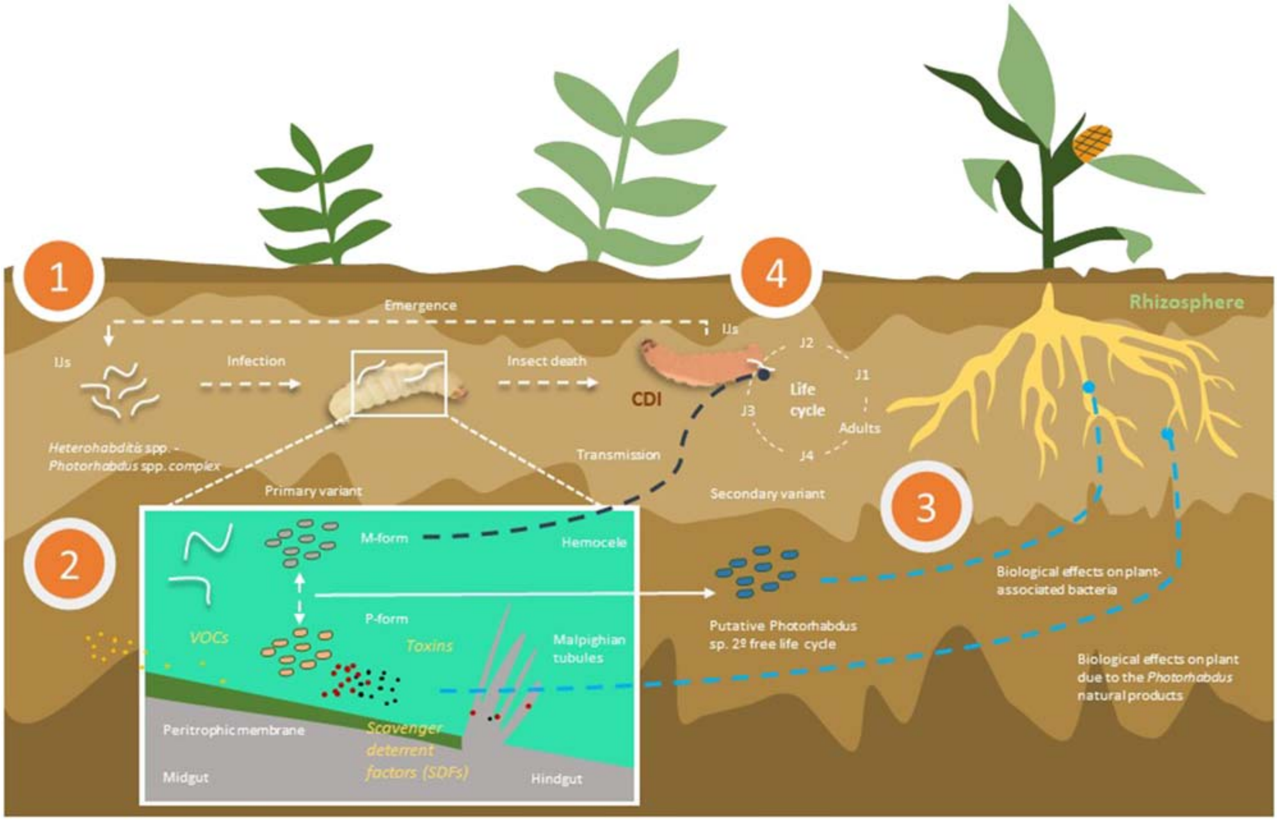

FIGURE 5.2

Model of life cycle of the entomopathogenic *Heterorhabditis-Photorhabdus*—complex belowground. **(1) Entomopathogenic nematode (EPN) infection**. During the infective juvenile stage (IJs), *Heterohabditis* spp. localize a host and release their symbiotic bacteria into the hemocoel completing the infection. **(2) *Photorhabdus* Primary variant**. The *Mutualistic*-form (*M*-form) of bacteria switching by a reversible promoter from an *M*-form to a *Pathogenic*-form (*P*-form). The bacteria replicate and kill the insect by the production of toxins. Once death comes to the insect, *P*-form is able to synthesize scavenger deterrent factors and different volatile organic compounds to control the scavengers. **(3) *Photorhabdus* Secondary variant**. After the cadaver decomposition island (CDI) of the victim is metabolized by the bacteria, 20–50% of the *Primary variant* cells switch to the *Secondary variant* phenotype. While the remaining *M*-form cells are reabsorbed by the nematodes. *Secondary variant* cells and metabolites interact with plant roots and defend them from attack by phytopathogens. If the secondary variant cells can convert back to primary variant cells is still unclear. **(4) EPNs life cycle**. EPNs develop their life cycle, feeding on their partner bacteria and degraded host tissues until the resources deplete. Next, the second-stage juveniles develop to the IJ stage, incorporate some of the symbiont bacteria (*M*-form), and exit by thousands into the soil to begin the life cycle anew.
]

Xenorhabdus- and *Photorhabdus*-based products: aims and scope

The development of antibiotic-resistant pathogens and the public concern about pesticides' toxicity and environmental impact promote novel biotechnological tools for pest/diseases management (Arthurs and Dara, 2019; Raymaekers et al., 2020). Identifying biological control agents and products thus forms a critical step in developing novel commercial biopesticides. Furthermore, their screening system has to differentiate between direct antagonism (e.g., parasitism, antibiosis, or competition) and biological agents or products that indirectly exert their action in the plant or even in the fruit resistance to the diseases insects or decay attack. Several research lines have targeted the entomopathogenic bacteria species in the genera *Xenorhabdus* and *Photorhabdus* during the last decades due to their potential in controlling the broad range of pests and diseases and the plasticity in the use of their derived products (Vicente-Díez et al., 2021a), the thermo-stability and shelf-life of their natural products (Cimen et al., 2021; Hazir et al., 2018), and their human health safety (Kusakabe et al., 2022). Researchers have proved their efficacy against insects (ffrench-Constant et al., 2007; Vicente-Díez et al., 2021b, 2021a; Vitta et al., 2018), bacteria (Muangpat et al., 2020), mites (Bussaman et al., 2009, 2012; Cevizci et al., 2020; Eroglu et al., 2019; Incedayi et al., 2021), fungi (Alforja et al., 2021; Chacón-Orozco et al., 2020; Cimen et al., 2021; Fang et al., 2011, 2014; Li et al., 2021), and root-knot nematodes (Abebew et al., 2022; Kepenekci et al., 2016; Kusakabe et al., 2022; Sayedain et al., 2019). Their plasticity lets also use them as biofactory of biochemical pesticides. Nevertheless, their isolated use as microbial pesticides or genetics in the production of PPP has not been deeply studied yet.

A single strain of *Xenorhabdus* or *Photorhabdus* may produce a broad range of antibiotic and insecticidal extracellular bioactive compounds (Dreyer et al., 2018). When producing antibiotics, it has been known that biochemistry engineering conditions are critical to the secondary metabolites production of microorganisms (Jiang and Zengyi, 2011). Even small changes in the culture medium can alter the quantities of certain compounds and modify the general metabolic profile of microorganisms, the microbial cellular networks, and fine-tuning physiological capabilities. Thereby, the industry can develop viable strains for producing natural and nonnatural value-added compounds. Different studies have enhanced the antibiotic activity of *Xenorhabdus* spp. and *Photorhabdus* spp. and optimized different bacterial strains. Factors such as environmental temperature, pH, rotatory speed, inoculation concentration, medium volume flask, fermentation time, aeration, the batch, the continuous stirred tank reactor, and other medium properties can condition the gene expressions and increase the production of product of antimicrobial interest. For example, Wang et al. (2011) tested the production of antibiotics of *Xenorhabdus bovienii* YL002 by medium optimization using response surface methodology. Their results pointed out that an optimized medium by adding glycerol and soytone increased antibiotic activity by 38%. However, previous one-factor-at-a-time assays with *X. nematophila* YL001 identified glucose and peptone as the best carbon and nitrogen sources that significantly affected antibiotic production (Wang et al., 2008). Recently, Booysen et al. (2021) suggested that the secondary metabolism of *X. khoisanae* J194 may be regulated by oxygen, water activity, or both. The dissolved oxygen level, tested only for *X. nematophila* YL001, was optimal when shifted during fermentation from 70% after the first 18 h to 50% for the remaining 54 h. Dreyer et al. (2018) and Booysen and Dicks (2020) summarized common trends for all the studies resumed in pH from 6.0 to 8.24, temperature range of 25−32°C, rotary speed of 150−220 rpm, inoculation volume of 4%−15%, a medium volume of 54−100 mL/250 mL flask, and a fermentation time of 54−72 h. Keskes et al. (2021) investigated the optimization of the culture conditions for enhancing *Photorhabdus temperata* biopesticide production using wastewater (WS4) as raw material and proved its viability. In this line, and in the context of the SDG, the research linking the production of biopesticides within a circular economy is very promising.

Once optimized the production of the bioactive compounds, the main possibilities for formulations are as follows: the hole cell suspension as unfiltered ferment (UF), the cell-free metabolites recovered in the supernatants (CFS), or the crude isolated bacteria cells extracts (Fig. 5.3). Bussaman et al. (2012) compared the three possibilities obtained from the fermentation of *X. stokiae* against the mushroom mite (*Luciaphorus* sp.). They observed higher efficacies when using the cell-free supernatants than the unfiltered ferment or the crude extract. Conversely, Vicente-Díez et al. (2021a) registered higher mortalities of *Lobesia botrana* when the UF *X. nematophila* and *P. laumondii* were applied rather than with the CFS. Hence, the use of the bacteria combined or not with their products are also candidates for searching bio-tools. Most studies have employed CFS due to several practical advantages: (1) homogeneous metabolites, (2) thermo-stability, and (3) longtime proper/storage. Abd-Elgawad (2021) established a list of possible scopes of application of the CFS of *Photorhabdus* spp. Spraying the CFS in the leaves has been proved by Eroglu et al. (2019) as an acaricide against *Tetranychus urticae*. Similarly, the cell-free filtrate of *X. nematophila* culture showed significant inhibitory effects (90%) on mycelial growth on postharvest tomato pathogens when sprayed over the fruits (Fang et al., 2014). Studies by Rajagopal et al. (2006) showed that *P. luminescens* encapsulated in alginate beads could infect *Spodoptera litura* and induce 100% mortality in 48 h.

Other possibilities could be employing the bacteria or their derivates as elicitors (low molecular weight compounds that activate a signal cascade and trigger plant immune response) or synergizing with other biopesticides. Indeed, the secondary metabolites have been shown to complement the action of other biopesticides such as *B. thuringiensis* (Jung and Kim, 2006; Park et al., 2016). However, even if their use as elicitors could extend their action range in crop protection programs, the required fundamental knowledge is still beyond comprehension.

Besides their straight use as toxic compounds, entomopathogenic bacteria produce chemical compound(s) that deter scavengers from feeding on nematode-

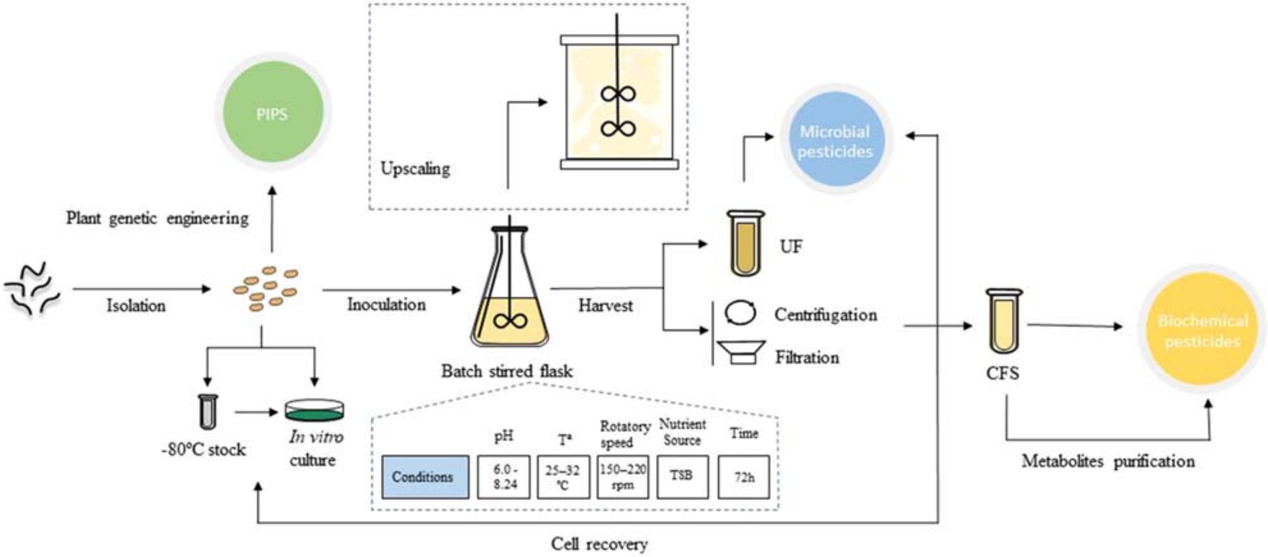

FIGURE 5.3

Simplified schematic of in vitro production of bioactive compounds from *Xenorhabdus* spp. and *Photorhabdus* spp.

killed insects. These scavenger deterrent factors play an essential role in the survival and persistence of EPNs in soils, even if not all scavengers are affected by them (Gulcu et al., 2012; Ulug et al., 2014). Compounds such as xenocoumacins (Shi and Bode, 2018) and fabclavines (Fuchs et al., 2014; Wenski et al., 2020) are well-known natural products that protect the insect cadaver from opportunists. The fabclavines are hybrid secondary metabolites derived from NRPS, polyunsaturated fatty acid, and PKS, which deserve special attention due to their potent mosquito feeding-deterrents activity (Kajla et al., 2019). There is diverse fabclavines derivate from different *Xenorhabdus* strains, whose importance lies in their antibiotic abilities, as in the case of other compounds of low volatility, which can be applied to suppress plant diseases (Chacón-Orozco et al., 2020). Many microbial volatile organic compounds have been closely associated with insect feeding behaviors (Tasin et al., 2011), but some are also powerful repellants (Jaffuel et al., 2022). In situ emissions from microorganisms may signal aspects of habitat suitability or potential exposure to entomopathogens (Davis et al., 2013). Although their use is today restricted to mosquito repellency (Da Silva et al., 2020; Kajla et al., 2019), exploring their potential in crop protection could enhance the eco-friendly management of pests and diseases.

Traditionally, EPN applications were the only strategy to utilize the pathogenic capabilities of *Xenorhabdus* and *Photorhabdus* in biological control programs (Askary and Abd-Elgawad, 2021). Their direct use as biocontrol agents represents a great opportunity but faces critical impediments, mainly cost and reliability. Although it has not been studied in-depth, some studies have addressed this issue. Thus, according to Vicente-Díez et al. (unpublished data), *P. laumondii* develops antifungal activity against *Botrytis cinerea* but not *X. nematophila*. Similarly, Mohan and Sabir (2005) observed that *P. luminescens* causes mortality of eggs of the parasitoid *Trichogramma* sp., and Rajagopal et al. (2006) assayed the direct infection of *Spodoptera litura* by *P. luminescens* encapsulated in alginate beads. Their results showed 100% mortality in 48 h by encapsulated *P. luminescens*, while the use of alginate-encapsulated *Heterorhabditis* nematodes resulted in 40% mortality after 72 h.

Genetic engineering offers opportunities to develop insect-resistant plants by inserting or expressing entomopathogenic proteins *in planta*. Several gene clusters for secondary metabolite biosynthesis have been identified in genome sequences of entomopathogenic bacteria (Bode, 2009). Specifically for *Xenorhabdus* and *Photorhabdus* species, several new secondary metabolites have already been identified that are currently isolated (Bode, 2009). In addition, the design and synthesis of some plant-incorporated protectants have been completed. For example, Zhang et al. (2012) expressed a nematode symbiotic bacterium-derived protease inhibitor protein in tobacco, enhancing its tolerance against *Myzus persicae*. This study suggested that the EPN-symbiotic bacterium complex is another valuable resource of protease inhibitors to be engineered into plants for insect pest management. Despite these advances, the mechanisms behind the production of PIPs by *Xenorhabdus* and *Photorhabdus* are still poorly understood.

Conclusions and future perspectives

The next-generation agriculture is avid of new approaches and products. Knowing how soil organisms can contribute to maintaining productive and healthy crops is crucial in the sustainable paradigm of current social demand. Some initiatives focus their effort on exploring the best combination of several well-known beneficial soil organisms, such as EPN, AMF, and Pseudomonas (Imperiali et al., 2017; Jaffuel et al., 2019), but the discrepancies in their success highlight the necessity of a comprehensive and holistic understanding of the complex agroecosystem. Similarly, searching for new organisms with possible new action mechanisms or production with novel active material is a must to provide alternatives to the dominated market by *Bt*. The present overview of the status of research and application of the entomopathogenic bacteria *Xenorhabdus* and *Photorhabdus* illustrates their vast potential and the substantial limitation for the feasible released as commercial products shortly. Besides the basic knowledge on which are the best species of these two bacteria for the production of certain active materials (Bode, 2009), still in the early stage of accounting for the overall potential, the scale to commercial production requires a deep investigation of the best conditions for their fermentation (production) and formulation (commercial product). Finally, as demonstrated in the combination of well-known biological control agents, fine-tuning for successful application to their full potential will require years of study (probably decades) until it becomes a reality. As learned from the findings and development of other bioproducts, such as the *Bt* paradigm, the starting points are always complex and challenging. However, the current research on the possibilities of *Xenorhabdus* and *Photorhabdus* is launched by various research programs worldwide, and the goal, driven by the SDG, is clear and achievable in the medium term. Hence, we envision that the bacteria *Xenorhabdus* and *Photorhabdus* will produce final commercial products and successful applications in one or more of the approaches (Fig. 5.1), providing novel tools for preserving future generations and protecting the Earth.

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