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Secondary Metabolites Produced by *Heterorhabditis* Symbionts and Their Application in Agriculture: What We Know and What to Do Next

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Abstract: Gram-negative *Photorhabdus* bacteria have a dual lifestyle: they are mutualists of *Heterorhabditis* nematodes and are pathogens of insects. Together, this nematode–bacterium partnership has been used to successfully control a wide range of agricultural insect pests. *Photorhabdus* produce a diverse array of small molecules that play key biological roles in regulating their dual roles. In particular, several secondary metabolites (SM) produced by this bacterium are known to play a critical role in the maintenance of a monoxenic infection in the insect host and are also known to prevent contamination of the cadaver from soil microbes and/or predation by arthropods. A few of the SM this bacteria produce have been isolated and identified, and their biological activities have also been tested in laboratory assays. Over the past two decades, analyses of the genomes of several *Photorhabdus* spp. have revealed the presence of SM numerous gene clusters that comprise more than 6% of these bacteria genomes. Furthermore, genome mining and characterization of biosynthetic pathways, have uncovered the richness of these compounds, which are predicted to vary across different *Photorhabdus* spp. and strains. Although progress has been made in the identification and function of SM genes and gene clusters, the targeted testing for the bioactivity of molecules has been scarce or mostly focused on medical applications. In this review, we summarize the current knowledge of *Photorhabdus* SM, emphasizing on their activity against plant pathogens and parasites. We further discuss their potential in the management of agricultural pests and the steps that need to be taken for the implementation of *Photorhabdus* SM in pest management.

Key words: agricultural pests, bioactivity, genomes, *Photorhabdus*, secondary metabolites.

In the United States alone, plants are subject to attack by more than 50,000 different pathogens, primarily fungi, viruses, bacteria, and nematodes. Although a variety of chemical and other management tools are available, none is ideal with respect to environmental safety, efficacy, and/or costs (Pimentel et al., 1992, 2005; Pimentel and Greiner, 1997; Foster and Mourato, 2000; Roberts et al., 2003). Current chemical (including antibiotic) solutions increasingly lose their effectiveness because of emerging resistance (Hillocks, 2012). Moreover, the recent banning of several chemical nematicides and the loss of methyl bromide from the pest-control market compels the need for new and environmentally friendly methods to enhance current management systems (Kerry, 1998; Chitwood, 2003).

A promising approach to develop novel control methods is to study microorganisms and the SM they produce with biological activity against plant parasites and pathogens (Lange and Sanchez Lopez, 1996; Hattori, 2001; Webster et al., 2002; Stroebel, 2003; Berdy, 2005; Teasdale et al., 2009; Donadio et al., 2012). Indeed, many microorganisms are known to benefit plants by successfully controlling soil-borne pathogens, including plant-parasitic nematodes and plant-pathogenic microbes (Chen and Dickson, 1998; Kerry, 1998; Sikora and Hoffmann-Hergarten, 1993; Siddiqi and Mahmood, 1999). In addition, the bioactive metabolites from many of these beneficial microbes have shown to represent a valuable resource for the discovery of medical drugs and agricultural agents (Webster et al., 2002; Berdy, 2005). For example, SM of several *Pseudomonas* spp. are known to protect plants from diseases caused by various soil-borne pathogenic fungi (Haas et al., 1992). In particular, two SM, hydrogen cyanide and 2,4-diacetylphloroglucinol produced by *Pseudomonas fluorescens* strain CHA0, have been demonstrated to suppress tobacco black root rot (Voisard et al., 1989).

Many insect-pathogenic bacteria are also known to produce several natural compounds SM with broad biological activities. For instance, the screening of many microbial extracts from *Bacillus* spp., including *Bacillus thuringiensis* among others, has revealed an extraordinary large and structurally diverse number of natural compounds with antimicrobial, antiviral, immunosuppressive, and antitumor activities (Sansinenea and Ortiz, 2011). More recently, lipopeptides (surfactins, iturins, and fengycins) produced by *Bacillus* spp. have been shown to play a key role in protecting plants against a wide range of phytopathogens, including bacteria, fungi, and oomycetes (Ongena and Jacques, 2008). Furthermore, these molecules are also known to take part in beneficial interactions of *Bacillus* species with plants by stimulating host defense mechanisms (Mukherjee and Das, 2005).

Another insect-pathogenic bacterium, *Serratia marcescens*, has been proven to produce a variety of SM and other biomolecules with antimicrobial and antiprotozoal activities that are crucial for its success in the diverse environments they thrive (Williams, 1973; Kurtz et al., 1981). For example, prodigiosin, which is considered a multifaceted SM pigment, has been reported to have antifungal and immunosuppressive activities (Thomson et al., 2000).

Over the past two decades, a growing interest has been put in investigating SM produced by the bacterial symbionts of entomopathogenic nematodes (Steinernematidae and Heterorhabditidae), including *Xenorhabdus* and *Photorhabdus* spp. Many of the bioactive metabolites

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produced by these bacteria are perceived to have potential for their application in agriculture and medical arenas (Webster et al., 2002; Brachmann et al., 2007; Bode, 2009, 2011). Presently, many laboratories are dedicated to the study of small molecule SM natural products from these bacteria. However, despite these efforts, only a small fraction of the existing SM diversity has been examined or bioassayed for their value in controlling agricultural pests. In this review, we focus on *Photorhabdus* bacteria and summarize the research that has been done in relation to SM and their potential against agriculture pathogens and parasites.

The Dual Lifestyle of *Photorhabdus*

Photorhabdus are gram-negative bacteria that are pathogens to insects and have a mutualistic relationship with *Heterorhabditis* nematodes (Heterorhabditidae). The bacteria reside in the intestine of the only free-living stage of the nematodes, also known as the 3rd stage infective juvenile (IJ). IJ invade a susceptible insect host, seeking the hemolymph. Once in the hemocoel, the IJ release their symbionts. The released bacteria contribute to the killing of the insect host and grow to high density in the resulting cadaver. *Photorhabdus* are essential for nematode growth and development, presumably both by serving as a direct food source and by supplying nutrients through degradation of the insect carcass (Akhurst, 1982; Akhurst and Dunphy, 1993). When nematode numbers become high and nutrients become limiting in the insect cadaver, nematode progeny reassociate with bacteria and differentiate into the colonized, nonfeeding IJ form that emerges into the soil to forage for a new host. Because of their insecticidal capabilities, entomopathogenic nematode–symbiont bacteria pairs have been successfully implemented in biological control and integrated pest management programs worldwide (Kaya and Gaugler, 1993; Gaugler, 1999; Grewal et al., 2005).

This nematode–bacterium–insect system is also viewed as a tractable model system amenable to study the physiological, chemical, structural, and developmental aspects of beneficial symbiotic associations and their differences from pathogenic associations (Burnell and Stock, 2000; Goodrich-Blair and Clarke, 2007; Stock and Goodrich-Blair, 2008). *Photorhabdus* symbionts must evade the immune system of the insect; kill the insect host; and repel invading scavengers and other competitors, including other bacteria, fungi, nematodes, amoebae, insects, and even birds (Waterfield et al., 2009). Conversely, during the phase of mutualistic association with *Heterorhabditis* nematodes, *Photorhabdus* must refrain from producing toxic metabolites and has to evade the immune system of the nematode and avoid being used as a food source. These contrasting but very significant challenges associated with the different phases of *Photorhabdus* life cycle are met by the production of proteinaceous toxins, extracellular enzymes, and crystalline inclusion proteins, in addition to biosynthesizing a large variety of small-molecule SM with various biological activities. The switch between the pathogenic and the mutualistic phases in the life cycle of this bacterium (and by implication, the regulation of the production of their virulence factors and SM products) is presently being investigated (Somvanshi et al., 2012; Clarke, 2016).

Importantly, numerous bioactive SM belonging to diverse chemical classes have been reported (Table 1). Their biological activity is very diverse and includes antibiotic (Akhurst, 1982; McInerney et al., 1991a, 1991b) and nematicidal activities (Hu and Webster, 1995; Hu et al., 1996, 1998, 1999; Han and Ehlers, 1999), among others. Thus, *Photorhabdus* bacteria are now viewed as a rich source of novel classes of pharmacologically active compounds showing exciting biological activities.

Nematicidal Activity

Early studies by Hu and Webster (1995) showed that cell-free culture filtrates of several *Photorhabdus* spp. and/or strains were toxic to second-stage juveniles (J2) of the root-knot nematode, *Meloidogyne incognita*, as well as to fourth-stage juveniles (J4), as well as to adults of the pine wilt nematode, *Bursaphelenchus xylophilus*. In another study, Han and Ehlers (1999) tested the trans-specific nematicidal activity of *Photorhabdus luminescens* culture filtrates isolated from two *Heterorhabditis* species, *Heterorhabditis bacteriophora* H06 strain and *Heterorhabditis indica* LN2 strain against other entomopathogenic nematode species that were deprived of their native symbionts. The authors concluded that these filtrates had toxic effects on nonsymbiotic nematodes (trans-specific activity) and suggested they may have an impact on competitive interactions when one insect host is infected by different nematode species.

More recently, Orozco et al. (2016) showed that crude extracts of *Photorhabdus l. sonorensis* (CH35 strain) inhibited *M. incognita* J2 and that mortality of nematodes was concentration dependent. The authors also tested crude extracts on nontarget species, including *Steinernema carpocapsae* and *Caenorhabditis elegans*, demonstrating a very low nematicidal activity against them (Orozco et al., 2016).

Until now, only two SM molecules, a stilbene derivative (3,5-dihydroxy-4-isopropylstilbene) and indole, had been found to have nematicidal activity (Hu et al., 1996). Interestingly, *Photorhabdus* is the only organism outside the plant kingdom to produce this stilbene. Hu et al. (1996) found that a stilbene derivative (3,5-Dihydroxy-4-isopropylstilbene) at a concentration of 200 μg/ml was toxic to a selection of bacterial- and fungal-feeding nematodes, including *C. elegans*, *B. xylophilus*, *Bursaphelenchus mucronatus*, and *Aphelenchoides rhytium*.

TABLE 1. Presently identified *Photorhabdus* secondary metabolites (SM) and their biological activity.

	SM	Activity	Target organism	Reference
Anthraquinone and its derivatives	1,3,8-trihydroxy-9, 10-anthraquinone	Insecticidal	*Culex pipiens*	Brachmann et al. (2007), Ahn et al. (2013)
	1,8-dihydroxy-3-methoxy-9, 10-anthraquinone[a]	Weak antibiotic activity		Li et al. (1995), Hu et al. (1998), Richardson et al. (1988), Challinor and Bode (2015)
	3,8-dihydroxy-1-methoxy-9, 10-anthraquinone			
	3-methoxychrysazine	Insecticidal	*C. pipiens*	Ahn et al. (2003)
Benzaldehyde[a]		Antimycotic	*Phytophthora capsici*, *Rhizoctonia solani*, and *Corynespora cassiicola*	Ullah et al. (2015)
		Insecticidal	*Galleria mellonella*	
Carbapenem	1-carbapen-2-em-3-carboxylic acid	Antibiotic	*Escherichia coli*, *Klebsiella pneumoniae*, and *Enterobacter cloacae*	Derzelle et al. (2002)
GameXpeptides		Cytoxic	Eukaryotic cells	Bode et al. (2012)
Glidobactin/Cepafungin I			None	Theodore et al. (2012), Dudnik et al. (2013), and Stein et al. (2012)
Indigoidine		Unknown	None	Brachmann et al. (2012)
Indole[a]		Nematicidal	*Caenorhabditis elegans*, *Meloidogyne incognita*, *Bursaphelenchus xylophilus*, and *Bursaphelenchus mucronatus*	Hu et al. (1996, 1998)
Kollisin A		Unknown	None	Bode et al. (2015)
Lumizinone A		Cytotoxic	Human plasma	Park and Crawford (2015)
Phurealipids		Unknown	None	Nollmann et al. (2015)
Probactin		Antibiotic	None	Ciche et al. (2003)
Pyrone		Antimycotic, Cytotoxic	None	Brachmann et al. (2013)
Rhabduscin		Insecticidal potential	None	Crawford et al. (2012)
Stilbene and its derivatives	3,5-Dihydroxy-4-isopropyl stilbene (syn. 2-isopropyl-5-[(E) - 2-phenylethenyl] benzene-1,3-diol)[a]	Nematicidal	*C. elegans*, *B. xylophilus*, *B. mucronatus*, and *Aphelenchoides rhytium*	Hu et al. (1996), Paul et al. (1981), Chen (1996), Shi et al. (2012)
		Antibacterial	*Bacillus subtilis*	
		Antimycotic	*Aspergillus flavus*, *Aspergillus fumigatus*, *Botrytis cinerea*, *Candida tropicales*, *Cryptococcus neoformans*, *Pythium aphanidermatum*, *R. solani*, *Exserohilum turcicum*, and *Fusarium oxysporum*	
		Insecticidal potential		

(*Continued*)

TABLE 1. Continued.

SM	Activity	Target organism	Reference
3-hydroxy-2- isopropyl-5-phenethyl phenyl carbamate[a]	Antimycotic	*P. aphanidermatum*, *R. solani*, *E. turvicum*, and *F. oxysporum*	Shi et al. (2012)
2-isopropyl-5-(3-phenyl-2-oxiranyl) -benzene-1,3-diol (syn. 2-Isopropyl-5-(3-phenyl-2-oxiranyl)-1,3-benzenediol)	Antibacterial	*B. subtilis*, *E. coli*, *Streptococcus pyogenes*, and *Staphylococcus aureus*	Hu et al. (2006)
Trans-cinnamic acid	Antimycotic	*Fusicladium effusum*	Bock et al. (2014)

[a] Tested activity against plant pathogens or parasites.

However, the authors showed that at the same concentration, this SM had no toxicity to second-stage juveniles of then root-knot nematode, *M. incognita* (Hu et al., 1996). This study clearly showed that SM may have different effects on different nematode species and highlights the need to further test the bioactivity of stilbene derivatives against other plant parasitic nematodes.

Another compound, isolated from several *Photorhabdus* strains with shown nematicidal activity is indole (Hu et al., 1996, 1998). This molecule had activity against three plant parasitic nematode species: *M. incognita*, *B. xylophilus*, and *B. mucronatus* at concentrations greater than 200 μg/ml. Specifically, indole caused high levels of paralysis of *M. incognita* and *Bursaphelenchus* spp. at a concentration of 100 to 300 μg/ml.

Both stilbene and indole also inhibited egg hatching of *M. incognita* (Hu and Webster, 1995; Hu et al., 1999). Another molecule with nematicidal activity was 2-stilbenol, which showed to be more toxic to *B. xylophilus* than stilbene, causing 100% mortality at 6.25 to 12.5 μg/ml (Hu et al., 1996).

Antibacterial Activity

During its life cycle, *Photorhabdus* produce several broad-spectrum antibiotics, which are secreted into the insect hemolymph when the bacteria enter the stationary-phase condition, preventing the putrefaction of the infected cadaver (Webster et al., 2002). Early studies conducted by Poinar et al. (1980) showed that *P. luminescens* inhibited *Bacillus cereus* subsp. *rnycoides* and *Bacillus subtilis*. In addition, Akhurst (1982) demonstrated that both in vivo and in vitro cultures of a selection of *P. luminescens* strains had activity against several bacteria species including the plant pathogen *Erwinia carotovora*.

Paul et al. (1981) were the first to isolate and structurally characterized two antibiotic molecules from *P. luminescens* pure cultures. Specifically, the authors reported two trans-stilbene derivatives, compounds V and VI. Compound V was isolated as 20% of the extract of *H. bacteriophora* and identified them as, 3,5-dihydroxy-4-isopropyl-trans-stilbene. Compound VI which was isolated as 6% of the extract was characterized as 3,5-dihydroxy-4-ethyl-trans-stilbene.

Later studies by J. Webster's team found that hydroxylated stilbene congeners, produced by *Photorhabdus* spp., also display antibiotic activities. Specifically, Chen (1996) reported that 2-isopropyl-5-(3-phenyl-2-oxiranyl)-benzene-1,3-diol (syn. 2-Isopropyl-5-[3-phenyl-2-oxiranyl]-1,3-benzenediol) stilbene had antibiotic effect against the gram-positive *B. subtilis*. In addition, Hu et al. (2006) also isolated, identified, and chemically synthesized a novel antimicrobial compound, 1,2-isopropyl-5-(3-phenyl-oxiranyl)-benzene-1,3-diol (syn. 2-Isopropyl-5-(3-phenyl-2-oxiranyl)-1,3-benzenediol) from the larvae of *G*alleria *mellonella* infected with the *Heterorhabditis megidis*

90–*P. luminescens* C9 complex. This molecule was tested against several clinical isolates of gram-negative and gram-positive bacteria, showing potent antimicrobial activity at different minimum inhibitory concentrations ranging from 6.25 to 12.5 μg/ml, to 100.0 μg/m. However, these SM have not yet been evaluated against plant pathogenic bacteria.

Photorhabdus are also known to produce several other SM compounds with broad-spectrum antibiotic properties (McInerney et al., 1991a, 1991b; Maxwell et al., 1994; Webster et al., 2002; Eleftherianos et al., 2007; Waterfield et al., 2009; Inman and Holmes, 2012; Clarke, 2016). One of them is carbapenem molecule, which is a prominent class of β-lactam antibiotic, which was first identified and characterized by Derzelle et al. (2002). Many naturally occurring carbapenems have been reported thus far, mostly all originated from streptomycetes and with wide broad-spectrum activity against many gram-positive and gram-negative bacteria. Carbapenems have gained clinical prominence to treat infections with rapidly spreading multidrug-resistant human pathogens such as methicillin-resistant *Staphylococcus aureus* (Coulthurst et al., 2005; Huber et al., 2009). Yet, the activity of these SM against plant pathogenic microbes has not been elucidated.

Photorhabdus, also produce 1-carbapen-2-em-3-carboxylic acid, which has shown antibiotic activity against *Escherichia coli*, *Klebsiella pneumoniae*, and *Enterobacter cloacae* (Derzelle et al., 2002). However, at present, no studies have been undertaken to test the activity of this molecule against phytopathogenic bacteria.

Anthraquinone molecules are considered a rarity in gram-negative bacteria, but have been identified in *Photorhabdus*. Recent studies have shown that Type II polyketide synthase enzymes are involved in the production of these SM (Brachmann et al., 2007). Two anthraquinone derivatives have been identified in *P. luminescens*, 1,3,8-trihydroxy-9,10-anthraquinone and two of its monomethyl ether derivatives, 1,8-dihydroxy-3-methoxy-9,10-anthraquinone and 3,8-dihydroxy-1-methoxy-9,10-anthraquinone (Richardson et al., 1988; Sztaricskai et al., 1992; Li et al., 1995; Hu et al., 1998). Although the antibiotic activity of these SM has been reported as 'weak' (Richardson et al., 1988; Challinor and Bode, 2015), further studies should be conducted to consider a wider repertoire of bacteria species and/or strains.

Another compound, a catecholate siderophore (photobactin) was reported to play a role in antibiosis in the insect cadaver by sequestering iron from invading microbes (Ciche et al., 2003). Furthermore, a few studies showed that extracts of several *Photorhabdus* spp. have bactericidal activity against *Erwinia amylovora*, the causative agent of fire blight disease (Hevesi et al., 2004; Hu et al., 1996). More recently, Uma et al. (2010) showed the potential of *P. luminescens* to control two important plant pathogens: *Xanthomonas* and *Pseudomonas*. Despite this knowledge, no efforts have been placed on the testing of purified *Photorhabdus* SM on phytopathogenic bacteria.

Antimycotic Effects

Inside the insect host, *Photorhabdus* spp. produce antimycotic compounds that help prevent invasion of the cadaver by soil fungal competitors (Chen et al., 2008; Webster et al., 2002). Many studies have been undertaken to assess the antimycotic properties of crude cell-free extracts. For example, Chen et al. (2008) were the first to test the activity of *P. luminescens* (from *H. megidis*) against 32 species of fungi from a range of habitats. Their study showed that cell filtrates of this bacterium completely inhibited the growth of various plant pathogenic fungi spp., including *Botrytis cinerea*, *Ceratocystis ulmi*, *Ceratocystis dryocoetidis*, *Mucor piriformis*, *Pythium coloratum*, *Pythium ultimum*, and *Trichoderma pseudokingii*, among others.

Shapiro-Ilan et al. (2009) also evaluated crude extracts of several *Photorhabdus* spp. and strains against a selection of fungal plant pathogens, including *Glomerella cingulata*, *Phomopsis* sp., *Phytophthora cactorum*, and *Fusicladosporium effusum*, which are fungal or oomycete pathogens of pecan, and *Monilinia fructicola*, a fungal pathogen of peach, considering in planta and in vitro assays. Their study showed that the crude extracts had moderate effect of the tested fungi.

Similarly, San-Blas et al. (2012) reported strong antifungal effects of metabolites from Venezuelan strains of *Photorhabdus*, against the causative agent of the cacao frosty pod rot disease, *Moniliophthora roreri*. In another study, Hazir et al. (2016) evaluated the potency of cell-free supernatants of three *Photorhabdus* strains, *Photorhabdus temperata*, *P. luminescens* (VS) and *P. luminescens* (K122) against several plant pathogenic fungi. Their results demonstrated that these cell-free culture filtrates can inhibit the vegetative growth of a variety of economically important phytopathogenic fungi, including *Fusicladium carpophilum* (peach scab), *F. effusum* (pecan scab), *M. fructicola* (brown rot), *G. cingulata* (anthracnose), and *Armillaria tabescens* (root rot). Recently, Orozco et al (2016) evaluated crude extracts of *P. l. sonorensis* against two fungal pathogens, *Fusarium oxysporum* (f.sp. *asparagi*) and *Alteraria alternata*, based on in vitro inhibition assays. Although the extracts inhibited growth of these fungi, they were considered to have moderate to low effect.

A few efforts have also been placed into the testing of specific SM molecules. For instance, Li et al. (1995) were the first ones to report the activity of specific metabolites, including 3,5-dihydroxy-4-isopropylstilbene, against several fungal spp. of medical and agricultural importance, including *Aspergillus flavus*, *Aspergillus fumigatus*, *B. cinerea*, *Candida tropicales*, and *Cryptococcus neoformans*. The authors showed that this SM had strong fungicidal

activity and suggested that it may probably function as an antagonistic agent preventing the insect cadaver to be attacked by fungal saprobes.

Shi et al. (2012) isolated and identified seven metabolites from *P. temperata* SN259 strain. Three of these compounds were depicted as novel stilbene derivative molecules. The activity of these metabolites was evaluated against four plant pathogenic fungi, including *Pythium aphanidermatum*, *Rhizoctonia solani*, *Exserohilum turcicum*, and *F. oxysporum* in in vitro assays. Two stilbene derivatives, 3-hydroxy-2-isopropyl-5-phenethyl phenyl carbamate and 2-isopropyl-5-([E]-2-phenylethenyl) benzene-1,3-diol (syn. 3,5-dihydroxy-4-isopropyl stilbene), showed strong inhibition against *P. aphanidermatum* with an effective concentration (EC50) values of 2.8 and 2.7 μg/ml, respectively.

Bock et al. (2014) also purified and identified *trans*-cinnamic acid from *P. luminescens* and tested it against a pecan fungal pathogen, *Fusicladium effusum* showing an inhibitory activity at a concentration of 148 to 200 μg/ml.

Ullah et al. (2015) isolated and characterized benzaldehyde, an aromatic aldehyde, isolated from *P. temperata* M102. The authors tested the activity of this molecule against three fungal plant pathogens, *Phytophthora capsici*, *R. solani*, and *Corynespora cassiicola*, demonstrating its ability to inhibit their growth in in vitro assays.

Insecticidal Activity

A few studies have demonstrated the insecticidal activity of cell-free filtrates of various *Photorhabdus* spp. and/or strains. For example, Shrestha and Lee (2012) tested the oral toxicity of *P. l. laumondii* (TT01 strain) crude extracts against the sweet potato whitefly, *Bemisia tabaci*, and adults, showing they were completely lethal at 60 hr posttreatment.

Orozco et al. (2016) also showed that crude extracts of *P. l. sonorensis* have insecticidal activity against the corn earworm *Helicoverpa zea*. Their study showed that the crude extracts had low insecticidal activity, killing only 11% to 37 % of *H. zea* neonates.

It is well recognized that *Photorhabdus* produce a wide array of toxins that contribute to the killing of an insect host (Waterfield et al., 2009). But in addition, many SM molecules, including stilbene derivatives and anthraquinone derivatives, genistine (a furan derivative), and a phenol derivative, have been identified from in vitro cultures, showing insecticidal activity (Eleftherianos et al., 2007; Chalabaev et al., 2008).

Photorhabdus anthraquinone has been reported to have bird and ant deterrent properties (Baur et al., 1998). Ahn et al. (2013) also demonstrated that two anthraquinone molecules, 1,3-dimethoxy-8-hydroxy-9,10-anthraquinone and 3-methoxychrysazine, isolated from *P. temperata* had mosquitocidal activity against *Culex pipiens* larvae.

Eleftherianos et al. (2007) also reported that stilbenes are involved in intercepting the immune system of the insects by inhibiting phenoloxidase (PO), an insect enzyme involved in the production of melanotic nodules, using *Manduca sexta* larvae as the model system. Crawford et al. (2012) also reported that the production of another molecule, rhabduscin, an amidoglycosyl- and vinyl-isonitrile-functionalized tyrosine derivative in *P. luminescens*, has immunosuppressive activity against PO.

Ullah et al. (2015) showed that another compound, benzaldehyde, exhibited insecticidal activity against *G. mellonella* larvae in a dose-dependent manner. For example, the authors reported that at a 8 mM concentration, they observed 100% insect mortality at 108 hr after injection. Furthermore, in vivo assays showed that benzaldehyde also has an effect on the insect immune response by inhibiting PO activity and nodule formation.

Culture Conditions and SM Production

Several studies have shown that culture conditions play a critical role in determining the quantity and quality of SM molecules produced by *Photorhabdus*. For example, Hu et al. (1998) showed that two stilbene derivatives, 5-dihydroxy-4-isopropylstilbene and 3,5-dihydroxy-4-ethylstilbene, can be isolated from extracts collected from nematode–bacterium infected cadaver cultures. However, only 3,5-dihydroxy-4-ethylstilbene was only isolated from the extracts derived from *Photorhabdus*-only culture broth. Similarly, these authors showed that three anthraquinone pigments were isolated when extracts were obtained from nematode-bacterium infected *G. mellonella* cadavers.

Moreover, Hu et al. (1996, 1998) reported that stilbene was commonly produced in *G. mellonella* cadavers infected by different *Photorhabdus* spp. In particular, they showed that stilbene produced by *P. luminescens* C9 strain (bacterial symbiont of *H. megidis* 90 strain). Specifically, it was observed that after 24 hr of nematode infection, the stilbene derivative, 3,5-dihydroxy-4-isopropylstilbene, produced by *P. luminescens* C9, increased rapidly at 2 to 5 d postinfection and remained at a level of 3,000 to 3,600 μg/g wet larvae for about 21 d and decreased gradually thereafter. The authors concluded that the early production and continued presence of a relatively large amount of 3,5-dihydroxy-4-isopropylstilbene in the infected cadavers support the hypothesis that the antibiotics produced by *Photorhabdus* help minimize competition from other microorganisms and prevent the putrefaction of the nematode-infected insect cadaver (Hu et al., 1996). Following up on these findings, Hu et al. (1998) also studied the metabolic composition of the *Photorhabdus–Heterorhabditis–Galleria* interaction and found that stilbene, 3,5-dihydroxy-4-ethylstilbene, and several anthraquinone derivatives were major

metabolic components of *G. mellonella* cadavers infected by *H. megidis* 90.

Recently, Orozco et al. (2016) also showed that fewer signals were detected in thin layer chromatography analyses when extracts were obtained from in vitro cultures. These results confirm the premise that synthesis of *Photorhabdus* metabolites is dependent not only on the conditions and the signals the bacteria encounter in the insect cadaver or under in vitro conditions, but it is also depended on the presence and/or the absence of their nematode hosts.

Photorhabdus Genome and SM Discovery

Natural products have traditionally been identified from a top–down perspective, but more recently genomics- and bioinformatics-guided bottom–up approaches have provided powerful alternative strategies. High throughput sequencing has become a fast and affordable approach for investigating bacteria as a source of novel toxins, metabolites, and enzymes for use in agriculture and pharmaceutical applications (Duchaud et al., 2003; fFrench-Constant et al., 2003, 2007; Kontnik et al., 2010; Edwards and Holt, 2013).

Genome mining is another valuable approach to identify the genes and/or gene clusters involved in the production of new antibiotics and the discovery of cryptic products. For example, genomic analysis of *P. luminescens* TT01 strain (genome size: 5.5 Mb) revealed that nearly 6% of its genome is involved in the production of SM (Duchaud et al., 2003; Clarke, 2008). The reported SM proportion in this bacterium is higher than the 3.8% observed in *Streptomyces coelicolor,* the model organism for studying SM production, suggesting that *Photorhabdus* has either the same or higher total coding capacity.

Mining of *Photorhabdus genomes* has also revealed the presence of several genes involved in the production of insecticidal proteins and hydrolytic enzymes, including proteases, lipases, and chitinases. In addition, many gene clusters involved in the biosynthesis of SM, including isopropylstilbenes, ethyl stilbenes, anthraquinones, siderophore photobactin, and carbapenems, have been identified setting the path for further research on their roles and functionality in the dual life cycle of this bacterium.

Recent studies have also identified more than 20 loci in the genomes of *Photorhabdus*, which are thought to be involved in the synthesis of antibiotic peptide molecules (Bode, 2009). However, until now, only three loci have been characterized, including those involved in the synthesis of stilbene, carbapenem, and anthraquinone (Brachmann et al., 2007; Joyce et al., 2008; Derzelle et al., 2002). For example, Brachmann et al. (2007) identified the biosynthesis gene cluster *plu4186–plu4194* that is responsible for the production of anthraquinone in *P. luminescens* (TT01 strain). This pigment is produced by proteins, which are encoded in the 9-gene antA-I locus (Brachmann et al., 2007). Genes at both ends of this locus (plu4185 and plu4195) were also predicted to encode transcriptional regulators, although the role for these genes in the regulation of anthraquinone production remains obscure.

Easom and Clarke (2008) showed that there is another transcriptional regulator, HdfR, which was originally identified during a screen for *Photorhabdus* mutants that were unable to colonize their nematode host. The gene acts as a repressor of antA-I expression and anthraquinone production. However, the role of this SM during nematode colonization was not verified.

Joyce et al. (2008) described the biosynthesis pathways of stilbene, a multipotent molecule with roles in both the pathogenic and mutualistic lifestyles. The authors reported the proteins involved in stilbene production are encoded in genes that are located in at least four different genetic loci. It was also hypothesized that these different loci may be regulated independently from each other and that the flux toward stilbene synthesis may involve both.

Derzelle et al. (2002) identified and characterized a cluster of eight genes (named *cpmA* to *cpmH*), which were found to be responsible for the production of a carbapenem-like antibiotic *P. luminescens* strain TT01. This gene cluster is apparently different in several aspects from *car* operons in other bacteria. This study also showed that *cpm* mRNA peaks during the exponential growth phase of the bacterium and is regulated by a Rap/Hor homolog identified in the *P. luminescens* genome. Marker-exchange mutagenesis of this gene resulted in a decrease of antibiotic production. Furthermore, Derzelle et al. (2002) also showed that regulation of the *cpm* operon also influences the *luxS*-like signaling mechanism of quorum sensing.

Brachman et al. (2009) reported that many SM loci are cryptic in *Photorhabdus* and therefore the genes are apparently not expressed under normal laboratory conditions. For example, it has been shown that indigoidine synthetase is functional but silent in *P. luminescens* (Brachman et al., 2009). For example, through heterologous gene expression of *indC* in *E. coli* and considering a promoter exchange approach, the authors demonstrated that indigoidine can be produced in *Photorhabdus*.

Conclusions

It is now widely accepted that the intensive use of chemical pesticides for control of plant parasitic nematodes and other plant pests (including bacteria and fungi) has led to severe negative environmental impacts (1988; Pimentel et al., 1992; Foster and Mourato, 2000; Stickle, 2003). The EPA is in the process of reviewing the use of organophosphate and carbamate pesticides with the intention of phasing out obsolete and toxic chemicals,

as has already happened with methyl bromide—a soil fumigant that had been widely used for controlling plant-parasitic nematodes and other soil-borne pathogens. Furthermore, resistance to both antibiotics such as streptomycin and oxytetracycline, presently licensed for use used in crop protection, has been spreading rapidly in plant pathogenic bacteria of economic significance (Vidaver, 2002). Thus, there is a great need for the discovery and use of pesticides that are not only highly effective but also have low toxicity and reduced impact in the environment (Pearce and Koundouri, 2003).

In this respect, the use of microorganisms and their natural products has emerged as a promising alternative for more rational and safe crop management. Furthermore, modern omics, genetics, and biochemical tools have made substantial contributions toward the unraveling of the biological activity of microbial SM (Demain and Fang, 2000). Furthermore, current automated bioinformatics platforms have enabled the prediction of gene pathways involved in the production of SM natural products and also to understand the function of these molecules in the biology of the microorganisms that produce them.

Over the past two decades, significant progress has been made in the mining of *Photorhabdus* genomes. Comparison across different species and strains has revealed the presence of several conserved biosynthesis gene clusters that are involved in the biosynthesis of diverse natural products (Tobias et al., 2016). Furthermore, these bioactive small molecules have been shown to play key roles in the pathogenic and mutualistic phases of this bacterium.

Despite this progress, there are yet many critical steps that must be pursued before their consideration of *Photorhabdus* SM as agricultural pesticides. Most of what is known regarding the bioactivity of this bacterium's SM is based on structure comparisons and/or similarities with molecules of known antimicrobial, insecticidal, or cytotxic activity.

Only a few SM have been tested under laboratory conditions with a limited number of targeted pathogens. In particular, more bioassays should be carried out to test the bioactivity of specific SM molecules, specially increasing the range of targeted plant pathogens and parasites. These assays should be followed by pilot greenhouse trials to assess the effectiveness of the studied SM in more natural scenarios. Focus should also be placed on understanding their specificity; biosafety, and environmental impact to nontarget organism. Specifically, studies should be conducted to evaluate their allergenicity and toxinogencity on plants, animals, and humans.

Moving toward the commercial delivery of *Photorhabdus* SM as pesticides, efforts should be placed on their production at an industrial scale. Considerations should be made toward the evaluation of their shelf life and on their compatibility with additives and/or adjuvants that could potentially improve their application and stability as a final product (Montesinos, 2003).

There is no doubt that *Photorhabdus* represent an abundant and valuable source of bioactive and chemically novel compounds with potential for exploitation in agriculture. Indeed, we are yet in an infancy stage, but the promise that *Photorhabdus* SM warrant will continue to lead the path for further discoveries and applications into pest management.

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