

Molecular Mechanisms of Nematode-Nematophagous Microbe Interactions: Basis for Biological Control of Plant-Parasitic Nematodes

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Abstract

Plant-parasitic nematodes cause significant damage to a broad range of vegetables and agricultural crops throughout the world. As the natural enemies of nematodes, nematophagous microorganisms offer a promising approach to control the nematode pests. Some of these microorganisms produce traps to capture and kill the worms from the outside. Others act as internal parasites to produce toxins and virulence factors to kill the nematodes from within. Understanding the molecular basis of microbe-nematode interactions provides crucial insights for developing effective biological control agents against plant-parasitic nematodes. Here, we review recent advances in our understanding of the interactions between nematodes and nematophagous microorganisms, with a focus on the molecular mechanisms by which nematophagous microorganisms infect nematodes and on the nematode defense against pathogenic attacks. We conclude by discussing several key areas for future research and development, including potential approaches to apply our recent understandings to develop effective biocontrol strategies.

INTRODUCTION

Plant-parasitic nematodes (PPNs) bring serious economic burden to farmers worldwide, causing estimated crop losses worth more than US \$157 billion each year (1). On the basis of their feeding mechanisms, PPNs are classified into three broad groups: the migratory ectoparasites (e.g., *Belonolaimus* spp., *Xiphenema* spp., and *Trichodorus* spp.), the migratory endoparasites (e.g., *Pratylenchus* spp. and *Radopholus* spp.), and the sedentary endoparasites [e.g., the root-knot nematodes (RKNs) (*Meloidogyne* spp.) and the cyst nematodes (CNs) (*Heterodera* spp. and *Globodera* spp.)] (100). Among the PPNs, the RKNs and the CNs cause the most crop damage worldwide. For decades, the control of sedentary nematodes has relied heavily on chemical nematicides, although crop rotation and resistant crop cultivars have also been used as complementary methods. However, currently available chemical nematicides are being withdrawn from use because of their notorious toxicity to wildlife and human health. The effectiveness of crop rotation is limited in some cropping systems because of the wide host ranges or long-term survival rate of most PPNs. Furthermore, the high genetic diversity within/among nematode populations limits the effectiveness of nematode-resistant crops because of the limited genetic variation within most current crop cultivars. Consequently, global crop production remains under heavy threat from PPNs. There is an urgent need for finding novel, environmentally friendly, and effective management strategies to control PPNs.

Biological control has shown promise as an economically and ecologically friendly approach to reduce pest damages. Broadly defined, biological control refers to the use of living organisms or their metabolites to reduce the population density or disease impact of a specific pest organism (27). In the soil ecosystem, certain microorganisms, such as nematophagous fungi and bacteria, have sophisticated strategies for trapping, killing, and digesting PPNs, often targeting specific developmental stages of their life cycles. Their potent effects against PPNs make these soil-living species ideal biological control agents (BCAs). Indeed, several nematophagous microorganisms or their secondary metabolites with nematocidal activities have been developed into BCAs to control PPNs (**Table 1**). However, although showing great promise for antagonizing PPNs in the lab and contributing significantly to the reduction of chemical pesticide usage in the field, the effectiveness of the current BCAs is still limited in field applications. Many factors have likely contributed to the limited success in the field, and most of them are related to the complexity of interactions among the crops, PPNs, the BCAs, and the many abiotic environmental factors. At present, our understanding of these interactions is very limited at the ecosystem, organismal, cellular, and molecular levels among plants, nematodes, nematophagous microorganisms, and environmental factors. Elucidating the molecular mechanisms underlying the interactions between nematophagous microorganisms and nematodes is crucial for developing highly effective BCAs and providing novel biocontrol strategies to control PPNs in the future.

In this review, we summarize recent data on two major issues: (a) the pathogenesis of nematophagous microorganisms infecting nematodes and (b) nematode immune responses to pathogen attacks. It should be noted that although the ultimate applied goal of the research is to develop effective biological controls of PPNs, most current data on nematophagous microorganism-nematode interactions have come from studies using the free-living model nematode *Caenorhabditis elegans*.

NEMATOPHAGOUS FUNGI

Currently, more than 700 species of nematophagous fungi have been described. These fungi belong to diverse phylogenetic groups, including Ascomycota, Basidiomycota, Zygomycota, and Chytridiomycota. A few species of Oomycetes, traditionally included in the fungal kingdom but not

Table 1 Commercially available biological control products to control root-knot nematodes

Products	Active antagonist	Product form	Treatment form	Crop	Company or institution/country
Biocon	<i>Paecilomyces lilacinus</i>	Unspecified	Unspecified	Unspecified	Asiatic Technologies, Inc./Philippines
Bioact/Paecil		Water-dispersible granulate	Drench, drip irrigation	Vegetables	Prophyta/Philippines; Bayer CropScience/ United States
PIPlus		Wettable powder	Drip irrigation, drench	Vegetables, tobacco, banana, citrus	BCP/South Africa
Yorker		Unspecified	Unspecified	Vegetables, fruit trees	Agriland Biotech Limited/India
Miexianning		Wettable powder	Drip irrigation, drench	Tobacco	Agricultural Institute, Yunnan Academy of Tobacco Science/China
PL Gold		Granulate, powder	Drench, drip irrigation	Banana, tomato	BASF Worldwide/Germany
DiTera	<i>Myrothecium verrucaria</i>	Liquid, powder	Ground or chemigation	Vegetables, almonds, fruits	Valent Biosciences Corp./Canada
Xianchongbike	<i>Pochonia chlamydosporium</i>	Powder	Drench, drip	Tobacco, peanut, soybean, watermelon	Laboratory for Conservation and Utilization of Bio-resources, Yunnan University/China
Klamic		Granulate	Soil incorporation	Vegetables	Unspecified/Cuba
Bio-Nemax	<i>Bacillus firmus</i>	Liquid	Drench, spray	Vegetables	M.J. Exports/India
BioNem-WP		Wettable powder	Drip irrigation	Vegetables	Bayer/Germany
BioSafe			Drench	Vegetables	AgroGreen/Israel
VOTiVO		Wettable powder	Drench, drip	Vegetables, cotton, corn	Unspecified
Nortica		Wettable powder	Drench, drip	Turfgrass	Unspecified
Econem	<i>Pasteuria usgae</i>	Liquid, powder	Drench, drip irrigation	Vegetables, turf, soybean	Pasteuria Bioscience/United States; Nematech/Japan; Syngenta/Switzerland
Sudozome	<i>Pseudomonas fluorescens</i>	Liquid	Drench	Vegetables, fruit trees	Agriland Biotech Limited/India
Nemix	<i>Bacillus</i> sp.	Powder	Drench, drip	Vegetables, fruit trees	AgriLife/Brazil
Biostart	<i>Bacillus</i> spp. mixture	Liquid	Soil drench, irrigation	General use	Microbial Solutions/Southern Africa
BioYield	<i>Paenobacillus macerans</i> and <i>Bacillus amyloliquefaciens</i>	Liquid	Drip irrigation, drench	Tomato, bell pepper, strawberry	Unspecified
Deny	<i>Burkholderia cepacia</i>	Powder, solution	Seed treatment, irrigation	Vegetables, alfalfa, barley, beans, clover, cotton, peas, grain sorghum, wheat	Rinco Vilova/United States
Blue Circle					Stine Microbial Products/United States

true fungi, can also kill nematodes. The divergent phylogenetic distributions of nematophagous fungi suggest that the nematophagous behaviors have evolved independently multiple times in fungi through convergent evolution (30). On the basis of the mechanisms that they use to attack nematodes, nematophagous fungi can be categorized into four major groups: nematode-trapping fungi, endoparasitic fungi, egg-parasitic fungi, and toxin-producing fungi (**Table 2**) (139).

Nematode-trapping fungi share a unique ability to form specialized trapping structures (traps) to capture nematodes. Because of these easily identifiable features, these fungi have

Table 2 Taxonomy of nematophagous microorganisms and their infection modes

Fungi/bacteria	Genus	Typical species	Infection structures	Fungal group
Fungi/Ascomycota	<i>Arthrobotrys/Orbilia</i>	<i>Arthrobotrys oligospora</i> , <i>Arthrobotrys conoides</i> , <i>Arthrobotrys musiformis</i> , <i>Arthrobotrys superba</i>	Adhesive networks	Nematode-trapping fungi
	<i>Dactylellina/Orbilia</i>	<i>Dactylellina baptotyla</i>	Adhesive knobs and/or nonconstricting rings	Nematode-trapping fungi
	<i>Drechslerella/Orbilia</i>	<i>Drechslerella stenobrocha</i>	Constricting rings	Nematode-trapping fungi
	<i>Harposporium/Podocrella</i>	<i>Harposporium anguillulae</i> , <i>Harposporium cerberi</i>	Ingested conidia	Endoparasitic fungi
	<i>Drechmeria</i>	<i>Drechmeria coniospora</i>	Adhesive conidia	Endoparasitic fungi
	<i>Haptocillium/Cordyceps</i>	<i>Haptocillium balanoides</i>	Adhesive conidia	Endoparasitic fungi
	<i>Hirsutella</i>	<i>Hirsutella rboisiliensis</i> , <i>Hirsutella minnesotensis</i>	Adhesive conidia	Endoparasitic fungi
	<i>Pochonia/Metacordyceps</i>	<i>Pochonia chlamydosporia</i>	Appressoria	Egg- and female-parasitic fungi
	<i>Paecilomyces/Cordyceps</i>	<i>Paecilomyces lilacinus</i>	Appressoria	Egg- and female-parasitic fungi
	<i>Lecanicillium/Cordyceps</i>	<i>Lecanicillium psalliotae</i> , <i>Lecanicillium lecanii</i>	Appressoria	Egg- and female-parasitic fungi
	<i>Trichoderma</i>	<i>Trichoderma barzianum</i> , <i>Trichoderma pseudokoningii</i>		Mycoparasitic fungi
	<i>Acremonium</i>	<i>Acremonium</i> spp.		Endophytic fungi
	<i>Neotyphodium</i>	<i>Neotyphodium</i> spp.		Endophytic fungi
	<i>Fusarium</i>	<i>Fusarium oxysporum</i>		Endophytic fungi
	<i>Penicillium</i>	<i>Penicillium oxalicum</i>		Endophytic fungi
Fungi/Basidiomycota	<i>Nematotonus/Hobenbuebelia</i>	<i>Nematotonus concurrens</i> , <i>Nematotonus baptocladus</i>	Adhesive spores	Endoparasitic fungi
	<i>Pleurotus</i>	<i>Pleurotus ostreatus</i>	Toxic droplets	Toxin-producing fungi
	<i>Coprinus</i>	<i>Coprinus comatus</i>	Toxin, spiny structures	Toxin-producing fungi
	<i>Stropharia</i>	<i>Stropharia rugosoannulata</i>	Acanthocyte	
	<i>Glomus</i>			Arbuscular mycorrhizal fungi

(Continued)

Table 2 (Continued)

Fungi/bacteria	Genus	Typical species	Infection structures	Fungal group
Fungi/ Blastocladiomycota	<i>Catenaria</i>	<i>Catenaria anguillulae</i> , <i>Catenaria auxiliaris</i>	Zoospores	Endoparasitic fungi
Fungi/Oomycota	<i>Haptoglossa</i>	<i>Haptoglossa heterospora</i>	Gun cell, injection	Endoparasitic fungi
	<i>Myzocytiopsis</i>	<i>Myzocytiopsis glutinospora</i> , <i>Myzocytiopsis vermicola</i> , <i>Myzocytiopsis enticularis</i> , <i>Myzocytiopsis humicola</i>	Zoospores	Endoparasitic fungi
	<i>Nematophthora</i>	<i>Nematophthora gynophila</i>	Zoospores	Egg- and female-parasitic fungi
Fungi/Zygomycota	<i>Stylopage</i>	<i>Stylopage badra</i>	Adhesive hyphae	
	<i>Cystopage</i>	<i>Cystopage cladospora</i>	Adhesive hyphae	
Bacteria	<i>Bacillus</i>	<i>Bacillus thuringiensis</i> (Bt)	Cry proteins	Crystal-forming bacteria
		<i>Bacillus</i> sp. RH219	Toxins, reduced nematode attraction, induced systemic resistance	Opportunistic parasitic bacteria
		<i>Bacillus laterosporus</i>	Toxins, reduced nematode attraction, induced systemic resistance	Opportunistic parasitic bacteria
		<i>Bacillus nematodida</i> B16	Trojan horse	Opportunistic parasitic bacteria
		<i>Bacillus</i> spp. (approximately 15 species)	Toxins, reduced nematode attraction, induced systemic resistance	Rhizobacteria
	<i>Pseudomonas</i>	<i>Pseudomonas aeruginosa</i>	Toxins, reduced nematode attraction, induced systemic resistance	Rhizobacteria
		<i>Pseudomonas fluorescens</i>	Toxins, reduced nematode attraction, induced systemic resistance	Rhizobacteria
		<i>Pseudomonas protegens</i>	Toxins, reduced nematode attraction, induced systemic resistance	Rhizobacteria
		<i>Pseudomonas chlororaphis</i>	Toxins, reduced nematode attraction, induced systemic resistance	Rhizobacteria
	<i>Pasteuria</i>	<i>Pasteuria penetrans</i>	Parasitism	Endoparasitic bacteria
		<i>Pasteuria thornei</i>	Parasitism	Endoparasitic bacteria
		<i>Pasteuria nishizawae</i>	Parasitism	Endoparasitic bacteria
		<i>Pasteuria usgae</i>	Parasitism	Endoparasitic bacteria

attracted significant attention in recent years. All known nematode-trapping fungi belong to a monophyletic group in the order *Orbiliiales* (Ascomycota). These fungi can live saprophytically in the soil. However, in the presence of nematode prey, these fungi become predatory via production of specific traps, including constricting rings, adhesive knobs, adhesive networks, adhesive columns, and nonconstricting rings (**Figure 1**) (139). It has been estimated that the carnivorous features of nematode-trapping fungi probably appeared approximately 400–520 million years ago (Mya) (92, 125). Phylogenetic results based on the ribosomal RNAs (rRNAs) and protein-coding genes suggested that the trapping devices could serve as robust indicators for generic delimitation among these fungi (6, 59, 96). Accordingly, three new generic concepts of nematode-trapping fungi have been proposed: *Arthrobotrys* is characterized by adhesive networks, *Dactylellina* by stalked adhesive knobs and/or nonconstricting rings, and *Drechslerella* by constricting rings (59). This classification system differs from the traditional taxonomy based on morphological characteristics of the conidia. So far, approximately 100 nematode-trapping species (14, 28, and 54 species from *Drechslerella*, *Dactylellina*, and *Arthrobotrys*, respectively) have been morphologically described and illustrated (139). With the development of sequencing technology, the whole genomes of three nematode-trapping fungi [an adhesive networks-forming species, *Arthrobotrys oligospora*; an adhesive knobs-forming species, *Dactylellina baptotyla* (known as *Monacrosporium baptotylum*); and a constricting rings-forming species, *Drechslerella stenobrocha*] have been sequenced (**Table 3**) (66, 75, 130). Their genome sequences can provide valuable information for a comprehensive understanding of the biology of nematode-trapping fungi.

Endoparasitic fungi are a group of fungi that use their conidia or zoospores to infect nematodes. The conidia germinate rapidly and penetrate the nematode using assimilative hyphae (**Table 2**) (52). In contrast to nematode-trapping fungi, endoparasitic fungi have no or only a limited saprophytic phase and produce almost no mycelium in soil (78). Their limited saprophytic abilities in soil make endoparasitic fungi relatively narrow use in biocontrol applications. Among the endoparasitic fungi, *Drechmeria coniospora* is the most studied. It has been assumed that *D. coniospora* can produce a large number of adhesive conidia (as many as 10,000 conidia in a single nematode) to infect nematodes. Each mature conidium can form an adhesive bud at one end to adhere to the nematode cuticle or to the sensory structures in the head and vulva regions. After adhesion, an infection vesicle develops within the cuticle layers, and trophic hyphae are then produced inside the infected nematode. The fungal hyphae grow and digest the nematodes, typically within three days at which point conidiophores form new conidia and protrude from the nematode corpses (78).

Egg-parasitic fungi use appressoria, a specialized penetration peg, or lateral mycelial branches to infect nematode eggshells (**Table 2**) (52). Representative species of the nematode egg-parasitic fungi include *Pochonia chlamydosporia*, *Paecilomyces lilacinus*, *Clonostachys rosea*, and *Lecanicillium psalliotae*. These species all belong to the clavicipitaceous fungi in Ascomycota, showing close relationships with many entomopathogenic fungi, such as *Metarhizium* spp. Because the eggshells of nematodes mostly consist of protein and chitin organized in a microfibrillar and amorphous structure, extracellular hydrolytic enzymes, such as chitinases and proteases in egg-parasitic fungi, have been found to play important roles in the disintegration of eggshell layers (**Table 4**) (129). Recently, the genome of *P. chlamydosporia* has been sequenced, providing valuable information for understanding the transitions between the different trophic modes (pathogenic, endophytic, and saprophytic) in this fungus (53).

Toxin-producing fungi are a group of fungi that can produce toxins to immobilize nematodes before hyphae penetrate through the nematode cuticles (**Table 2**) (52). More than 200 compounds with nematocidal activities have been identified from approximately 280 fungal species in 150 genera of Ascomycota and Basidiomycota (54, 55). These compounds belong to diverse chemical groups, including alkaloids, peptides, terpenoids, macrolides, oxygen heterocycle and benzo

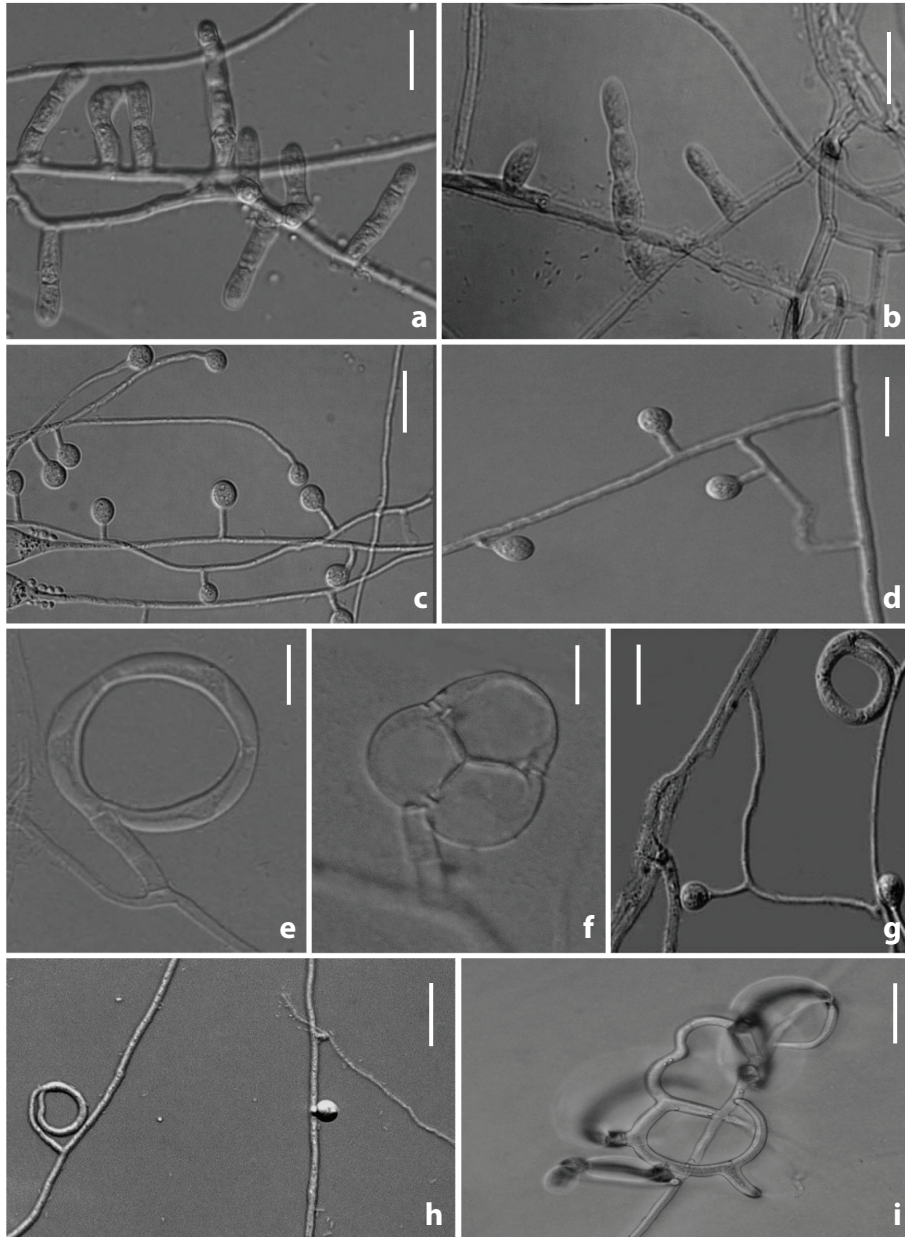


Figure 1

Morphology of traps produced by nematode-trapping fungi. (*a,b*) Adhesive hyphae. (*c,d*) Adhesive knobs. (*e,f*) Constricting rings. (*g,h*) Adhesive knobs and nonconstricting rings. (*i*) Adhesive networks. All scale bars are 10 μm .

Table 3 Available genomic information of nematophagous microorganisms

Features	<i>Drechslerella stenobrocha</i>	<i>Dactylellina baptotyla</i>	<i>Arthrobotrys oligospora</i>	<i>Pochonia chlamydosporia</i>
Traps	Constricting ring	Adhesive knobs	Adhesive networks	–
Assembled size (Mb)	29.02	40.4	40.1	41.2
Scaffolds (larger than 2 Kb)	134	–	–	–
Scaffolds (total)	142	–	–	–
Scaffold N50 (bp)	434,454	–	–	–
Coverage (fold)	80 × (Solexa)	28 ×	37 ×	136 ×
G + C content (%)	52.5	45.24	44.45	49.9
Repeat rate (%)	0.92	–	–	0.46
Coding rate (%)	41.55	–	–	42.1
Protein-coding genes	7,781	10,959	11,479	12,122
Gene density (genes per Mb)	268.3	271	286	294
GC exonic (%)	55.31	–	–	–
GC intronic (%)	49.24	–	–	–
Exons per gene	3.57	3.3	3.8	–
tRNA genes	82	149	154	45
Secreted proteins	–	1,666	1,568	2,485
Proteins with Pfam domain	7,036	7,455	7,555	–

compounds, quinones, aliphatic compounds, simple aromatic compounds, and sterols (54, 55). The discovery of these metabolic products with antagonistic activities against nematodes establishes a promising avenue for developing these chemicals as commercial BCAs. For example, thermolides A–F (1–6), a class of PKS–NRPS (polyketide synthase–nonribosomal peptide synthetase) hybrid metabolites that possess a 13-member lactam-bearing macrolactone, have recently been identified from a thermophilic fungus (*Talaromyces thermophilus*), and two of them (compounds 1 and 2) displayed potent nematocidal activities similar to commercial avermectins (38).

Interestingly, in addition to producing toxins, two basidiomycete fungi, *Coprinus comatus* and *Stropharia rugosoannulata*, can produce special nematode-attacking devices, namely spiny ball and acanthocyte, respectively (68, 69). The special sharp structures cause damages in the cuticle of nematodes and result in the leakage of nematode inner materials, implicating that mechanical forces can also function as a virulence factor (68, 69).

In addition to the four groups of fungi mentioned above, a wide variety of fungi also possess the capability to kill nematodes. For example, the mycoparasitic fungi *Trichoderma* spp. have been described as BCAs against PPNs, although the underlying mechanism remains largely unknown (3). *Trichoderma* can parasitize nematode eggs and larva through secreting extracellular hydrolytic enzymes, such as trypsin-like protease PRA1 (102), serine protease SprT (14), and chitinolytic enzymes *chi18-5* and *chi18-12* (104). Comparative analysis of protease expression profiles in *Trichoderma harzianum* revealed that 13 peptidase-encoding genes, including the acidic serine protease gene *PRA1*, the aspartic protease genes *P6281* and *P9438*, the metalloendopeptidase gene *P7455*, and the sedolisin serine protease gene *P5216*, are coexpressed during in vitro nematode egg parasitism, suggesting that these genes likely play pivotal roles in the egg-infection process (105). Furthermore, some nematocidal compounds have also been obtained from *Trichoderma* spp., such as trichodermin (134), β -vinylcyclopentane-1 α , 3 α -diol, 6-pentyl-2H-pyran-2-one, and 4-(2-hydroxyethyl) phenol (135). Some endophytic fungi (e.g., *Acremonium* spp. and *Neotyphodium*

Table 4 Partial properties of hydrophytic enzymes isolated from nematophagous microorganisms

Nematophagous fungi	Classification	Protease	Obtained mode	MW (kDa)	Inhibitors	pI	Optimum pH	Optimum T _m
<i>Arthrobotrys oligospora</i>	Serine protease	PII	Purified, cloned	35	PMSF, Chymostatin	4.6	7.0–9.0	–
	Serine protease	Aoz1	Purified, cloned	38	PMSF, SSI	4.9	6.0–8.0	45°C
<i>Monacrosporium microscaphoides</i>	Serine protease	Mlx	Purified, cloned	39	PMSF	6.8	9.0	65°C
<i>Dactylella shizishama</i>	Serine protease	Ds1	Purified, cloned	32	PMSF	–	10.0	55°C
<i>Arthrobotrys conoides</i>	Serine protease	Ac1	Purified, cloned	35	PMSF	–	7.0	53.2°C
<i>Dactylella varietas</i>	Serine protease	Dv1	Purified, cloned	30	PMSF	–	8.0	60.5°C
<i>Monacrosporium cystosporium</i>	Serine protease	Mc1	Purified, cloned	38	PMSF	–	7.0–9.0	56°C
<i>Monacrosporium megalosporum</i>	Serine protease	Spr1	Cloned	–	–	–	–	–
<i>Dactylella haprotyla</i>	Serine protease	Dha1	Cloned	–	–	–	–	–
<i>Dactylella cionopaga</i>	Serine protease	Dc1	Cloned	–	–	–	–	–
<i>Pochonia rubescens</i>	Serine protease	P32	Purified	32	PMSF, pCMB	–	8.5	–
	Chitinase	Chi43	Purified	43	–	–	5.2–5.7	–
<i>Pochonia chlamydosporia</i>	Serine protease	VCP1	Purified, cloned	33	PMSF	10.2	–	–
	Chitinase	Chi43	Purified	43	–	–	5.2–5.7	–
	Chitinase	PcChi44	Cloned	44	–	–	6.0	50°C
<i>Pacilomyces lilacinus</i>	Serine protease	pSP-3	Purified, cloned	33.5	PMSF	10.2	10.3	60°C
<i>Leaniciillium psalliotae</i>	Chitinase	Plc	Purified	45.8	–	–	–	–
	Serine protease	Ver112	Purified, cloned	32	PMSF	–	10.0	70°C
	Chitinase	LpChi1	Cloned	45	–	–	–	–
<i>Clonostachys rosea</i>	Serine protease	PrC	Purified, cloned	33	PMSF	10	9.0–10.0	60°C
	Chitinase	CrChi1	Purified	43.8	–	–	7.5	22°C

(Continued)

Table 4 (Continued)

Nematophagous fungi	Classification	Protease	Obtained mode	MW (kDa)	Inhibitors	pI	Optimum pH	Optimum Tm
<i>Cordyceps sinensis</i>	Serine protease	Csp1	Cloned	36	PMSF	–	7.0	40°C
	Serine protease	Csp2	Cloned	35	PMSF	–	7.0	50°C
	Serine protease	Hnsp	Purified	32	PMSF	–	7.0	40°C
<i>Hirsutiella rhossiliensis</i>								
Serine protease	Hasp	Purified	33	PMSF	–	9.0	75°C	
<i>Hirsutiella minnesotensis</i>	Serine protease	Hm1	Cloned	–	–	–	–	–
<i>Duddingtonia flagrans</i>	Serine protease	Df1	Purified	38	PMSF	–	8.0	60°C
<i>Monacrosporium thaumasiium</i>	Serine protease	Mt1	Purified	40	PMSF	–	7.0–8.0	60°C
<i>Trichoderma pseudokoningii</i>	Serine protease	SprT	Cloned	31	–	–	8.5	–
<i>Paeclomyces variotii</i>	Chitinase	Chi32	Purified	32	–	–	2.5–3.0	60°C
<i>P. variotii</i>	Chitinase	Chi46	Purified	46	–	–	5.2–3.0	60°C
<i>Lecanicillium lecanii</i>	Chitinase	Chi2	Cloned	45.9	–	–	7.0	37.6°C
<i>Bacillus laterosporus</i>	Alkaline serine protease	BLG4	Purified, cloned	30	PMSF	–	10.0	50°C
<i>B. laterosporus</i>	Neutral protease	NPE-4	Purified, cloned	41	EDTA	–	–	–
<i>Bacillus nematocida</i> B16	Alkaline serine protease	Bace16	Purified, cloned	28	PMSF	–	10.0	50°C
<i>B. nematocida</i> B16	Neutral protease	Bae16	Purified, cloned	40	EDTA	–	6.5	55°C
<i>Bacillus</i> sp. RH219	Alkaline serine protease	Apr219	Purified, cloned	33	PMSF	–	10.0	60°C
<i>Bacillus</i> sp. RH219	Neutral protease	Npr219	Purified, cloned	41	EDTA	–	6.0	50°C
<i>Pseudomonas fluorescens</i>	Extracellular protease	AprA	Purified	47.1	EDTA	–	–	–
<i>Bacillus thuringiensis</i>	Metalloproteinase	Bmp1	Cloned	–	EDTA, PMSF	–	7.4	–

Abbreviations: EDTA, ethylenediaminetetraacetic acid; MW, molecular weight; pCMB, p-chloromercuribenzoate; PMSF, phenylmethylsulfonyl fluoride; SSI, streptomyces subtilisin inhibitor.

spp.) and arbuscular mycorrhizal (AM) fungi are capable of reducing nematode infestations and enhancing the growth of nematode-infected plants (116, 117). In addition, the nonpathogenic fungus *Fusarium oxysporum* has been reported to significantly suppress the populations of the PPNs *Pratylenchus goodeyi* and *Helicotylenchus multicinctus* and enhance the yield of tissue-cultured banana plants (121).

MOLECULAR MECHANISMS BY WHICH NEMATOPHAGOUS FUNGI INFECT NEMATODES

The infection process of nematophagous fungi in nematodes includes different stages: attraction/recognition, adhesion, penetration, and digestion (25). Attraction and recognition, the early steps during the infection process of nematophagous fungi, occur through cell-cell communication and involve a range of biochemical, physiological, or morphological interactions between fungi and nematodes. When nematodes pass by, nematophagous fungi can attach to nematodes via adhesive materials on the surface of the fungal traps and spores. The chemical composition of the surface fibrils of nematophagous fungi is not completely understood. However, the carbohydrate-binding proteins (lectins) are commonly found on the surface fibrils. After attaching to nematodes, the fungal hyphae penetrate the nematodes by breaking down the cuticles. Current evidence suggests that the penetration involves a combination of mechanical forces and extracellular hydrolytic enzymes, including serine proteases, chitinases, and collagenases (127). The hydrolytic enzymes are also important in the digestion phase of pathogenesis (**Figure 2**).

Attraction and Recognition

It has been known for a long time that the culture filtrates and living mycelia of several nematophagous fungi are attractive to nematodes. The attraction of the pinewood nematode (PWN) *Bursaphelenchus xylophilus* to an endoparasitic fungus *Esteya vermicola* leads to the hypothesis that certain volatile organic compounds (VOCs) continuously produced by the fungus are involved in this process (119). The identified VOCs include monoterpenes (α -pinene and β -pinene) and a terpenoid (camphor), similar to the volatile compounds emitted from the pine tree host of PWN, suggesting that *E. vermicola* mimics the scent of the pine tree host to attract PWN (63). However, relatively little is known about the compounds released by other nematophagous fungi to attract nematodes.

How fungi recognize nematodes and initiate morphological transition remains incompletely understood. It is believed that nematophagous fungi can sense certain substances produced by nematodes. For example, a putative morphogenic signal (nemin) produced by the nematode *Neoaplectana glaseri* has been demonstrated to cause a morphogenetic switch and trap formation in *Arthrobotrys conoides* (86), although the chemical composition of nemin remains unknown. A recent study reported that ascarioside, a type of small molecule constitutively released by many species of nematodes, could trigger trap formation in *A. oligospora* and several related species, producing three-dimensional adhesive networks (42). These results suggest that nematophagous fungi can recognize nematodes via chemical communication and then regulate morphogenesis.

In fungi, morphogenesis typically involves multiple signal transduction pathways to perceive environmental cues and transfer the signals to the cells. G proteins are a major class of sensors involved in a diverse group of biological processes in filamentous fungi, including development, pathogenesis, and response to environmental signals (57). In 2001, Chen et al. (15) reported that a heterotrimeric G-protein signal transduction pathway is essential for constricting-ring formation in *A. dactyloides*. In this fungus, a G-protein activator and the increased intracellular Ca^{2+} concentration stimulated the inflation of ring cells, whereas a G-protein inhibitor blocked

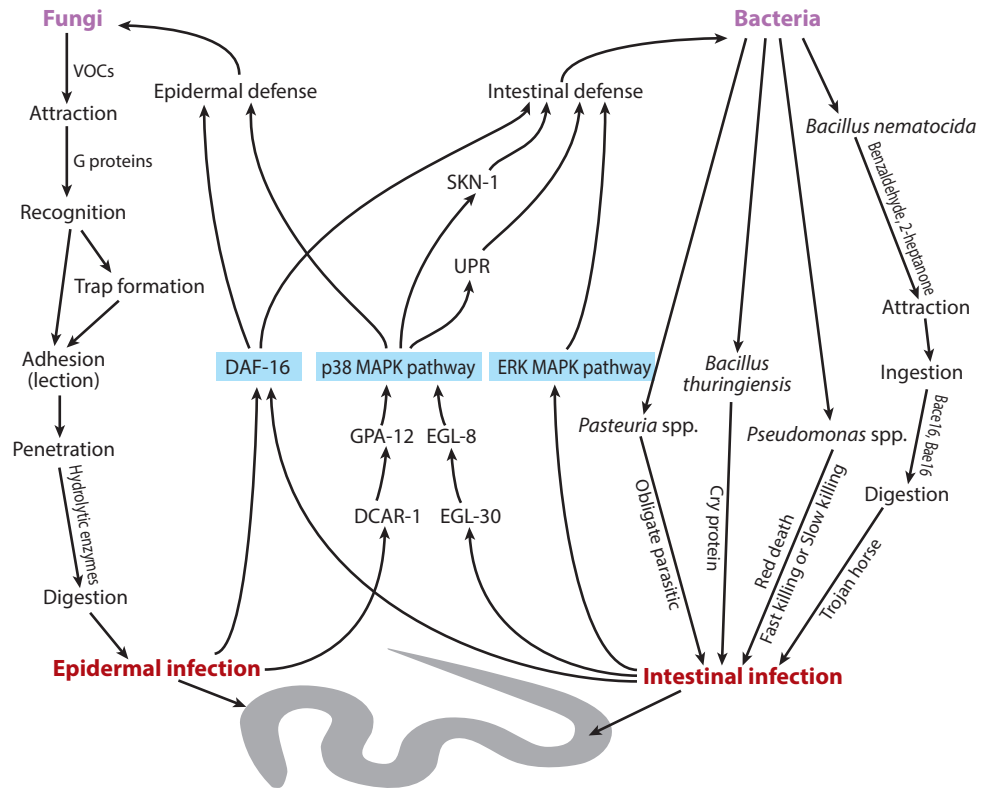


Figure 2

Interactions between nematophagous microbes and nematodes. Most nematophagous fungi and some pathogenic bacteria (e.g., *Microbacterium nematophilum*, *Xenorhabdus nematophila*, and *Yersinia pestis*) adhere to the cuticle or utilize mechanical force to immobilize nematodes and then infect the epidermis via hydrolytic enzymes. Most bacterial pathogens and several yeasts, including *Candida albicans*, are ingested and establish an infection in the intestine of nematodes. Several signaling cascades (blue), such as the p38 MAPK pathway, the DAF-2/DAF-16 pathway, and the ERK pathway, have been identified in *Caenorhabditis elegans* in its defense against pathogen attacks. Only representative interactions between nematophagous microorganisms and nematodes are shown. Abbreviations: ERK, extracellular signal-regulated kinase; MAPK, mitogen-activated protein kinase; UPR, unfolded protein response; VOC, volatile organic compound

inflation (15). Most genes that encode for the components of common fungal signal transduction pathways have been identified in the genomes of nematophagous fungi (66, 75, 130). During the fungal trap formation, genes related to fungal signal transduction pathways were upregulated, including those for glycosylphosphatidylinositol-specific phospholipase C, mitogen-activated protein kinases (MAPKs), serine/threonine protein phosphatase 2A, calyculin binding proteins, and Ca^{2+} /calmodulin-dependent protein kinase, further suggesting the importance of signal sensing and transduction in nematophagous fungi (53, 130). However, the detailed regulation mechanisms and the subsequent cellular processes of these genes involved in the signal transduction pathways remain to be elucidated.

Adhesive Proteins

After successful recognition, the nematophagous fungi attach to their host nematodes via adhesive proteins (111). The adhesive proteins in nematophagous fungi are the major components of the

extracellular fibrillar polymers, and they accumulate on the outside surface of adhesive traps or spores, playing important roles for those fungi to adhere to nematode cuticles (112). Aside from adhesion, these proteins likely play roles in nutrient storage, trap development, nematode attraction and recognition, and defense against competitors (103). Although the exact chemical components of these adhesive substances remain largely unknown, recent works are providing clues to their structures and functions. Below, we describe the recent advances in this area.

Lectins are the first group of proteins identified from adhesive traps (84). On the basis of the presence of different glycosyls, such as N-acetyl-D-galactosamine (GalNAc), D-glucose, D-mannose, and L-fucose, in different adhesive network-forming species, lectins have long been suspected to function in fungal recognition of nematodes. However, a recent study showed that the deletion of a lectin gene (*AOL_s00080g288*) from *A. oligospora* did not affect either the fungal growth or its pathogenicity against nematodes (8). Also, none of the seven putative lectin genes showed significant expression changes in response to nematode extract (NE) induction (130). Furthermore, only two lectin genes (one GalNAc-binding lectin and one fucose-binding lectin) are found in the *D. stenobrocha* genome (66). Together, these results suggest that the role of lectins in pathogenesis might have been overestimated. It is possible that there are some unknown compensatory pathways for the deleted lectin genes in nematophagous fungi. Recently, two IgE-binding proteins (*AOL_s00076g13* and *AOL_s00083g104*), which are galactoside-specific lectins containing an S-type carbohydrate-recognition domain, have been identified using the liquid chromatography–mass spectrometry methods. However, their exact functions remain elusive (62).

Along with lectins, a large number of genes encoding putative fungal adhesions have also been found in nematode-trapping fungal genomes. For example, 12, 17, and 26 CFEM-containing proteins are identified in *D. stenobrocha*, *A. oligospora*, and *D. haptotyla*, respectively (66, 75, 130). These proteins function as cell-surface receptors or signal transducers, or as adhesion molecules in plant fungal pathogens (51). Similarly, 6, 6, and 28 GLEYA-containing proteins, which can bind to the lectin-like ligand domains (65), were also identified in *D. stenobrocha*, *A. oligospora*, and *D. haptotyla*, respectively (66, 75, 130). Moreover, qPCR analyses of 17 adhesion-associated protein-encoding genes in *A. oligospora* revealed that five genes (*AOL_s00076g567*, *AOL_s00043g50*, *AOL_s00007g5*, *AOL_s00210g231*, and *AOL_s00076g207*) were upregulated during trap formation (130). Among these five adhesive genes, *AOL_s00076g567* is homologous to *MAD1*, the only gene from filamentous fungi known to mediate the attachment of the entomopathogenic fungus *Metarhizium anisopliae* to insects (118).

Extracellular Enzymes Involved in Infection

It has been shown that extracellular enzymes, such as serine proteases, collagenases, and chitinases, can breakdown the physical and physiological integrity of nematode cuticles and eggshells, facilitating fungal penetration and colonization (**Table 4**) (127).

Serine proteases. Among extracellular enzymes secreted by nematophagous fungi, serine proteases are the most studied. P32 was the first serine protease identified from the egg-parasitic fungus *Pochonia rubescens* (also known as *Verticillium suchlasporia*) (67). So far, more than 20 serine proteases have been purified or identified from various nematophagous fungi (**Table 4**) (113, 128, 132). Functional analyses have revealed that these proteases can effectively degrade nematode cuticles; hence, they are also called nematode cuticle-degrading proteases. Phylogenetic analyses suggest that the cuticle-degrading proteases belong to two lineages: One lineage includes neutral proteases from nematode-trapping fungi and the other consists of those from nematode-parasitic

fungi with alkaline proteases (56). The neutral and alkaline cuticle-degrading proteases differ in the flexibility of substrate-binding sites and electrostatic surface potential distributions, contributing to the differences of their catalytic and nematocidal activities (60). Sequence analyses also suggest that positive selection might have played an important role in the evolution of cuticle-degrading proteases and the pathogenicity of nematode-trapping fungi (56). The crystal structures of two cuticle-degrading proteases, Ver112 (isolated from *Lecanicillium psalliotae*) and PL646 (isolated from *Paecilomyces lilacinus*), revealed that the different residues of the substrate binding sites, such as S1 and S4 pockets, contributed to their different hydrophytic activities (61). Improving the fungal pathogenicity through genetic modification has been successfully achieved in nematophagous fungi. For example, the virulence of the nematode-trapping fungus *A. oligospora* has been enhanced by introducing additional copies of the endogenous cuticle-degrading protease *P11* into its genome (4). Furthermore, overexpression of the *P. lilacinus* serine protease gene *Psp-3* or the *L. psalliotae* serine protease gene *ver112* in *P. lilacinus* resulted in higher nematocidal activities against *Panagrellus redivivus* and *C. elegans* than the wild-type parental strain (120, 131).

With more nematophagous fungi genome data available, an increasing number of genes encoding serine proteases have been identified. For example, 17, 24, and 32 putative serine protease encoding genes have been identified from *D. stenobrocha*, *A. oligospora*, and *P. chlamydosporia* genomes, respectively (53, 66, 130). However, only a few of these serine proteases are inducible by nematodes. For instance, the expressions of only 2 of the 24 genes (*P186*, *AOL_s00215g702* and *P12*, *AOL_s00170g103*) were significantly upregulated in *A. oligospora* when exposed to NE. Furthermore, disruption of *P186* greatly attenuated the pathogenicity of *A. oligospora*, suggesting that proteases *P12* and *P186* likely play an important role in the infection process (130). In addition, nitrogen sources, environmental pH, and/or other stress conditions can all regulate the expression of the cuticle-degrading protease gene *PrC* in *C. rosea* (143, 144, 146).

Chitinases. Chitin is the most abundant structural component (40% w/w) in nematode eggshells. It has been shown that egg-parasitic fungi can use chitinases to penetrate the nematode eggshell during infection. The first chitinase Chi43 was purified from two nematophagous fungi, *P. chlamydosporia* and *P. rubescens*, in 2002 (108). So far, nine chitinases of the glycosyl hydrolase 18 (GH18) family with highly conserved structures have been purified or cloned from different nematode-parasitic fungi (Table 4). Crystal structure analysis revealed that the chitinase CrChi1 of *C. rosea* has a DXDXE motif and the catalytic residue is Glu174 (31, 126). The substrate-binding domain can bind two caffeine molecules. Genomic analysis suggested that there are 22 GH18 chitinases in *P. chlamydosporia*, and 15 of them are putatively secreted (53). Although no chitinase has been purified from nematode-trapping fungi, 8 and 16 genes encoding putative GH18 chitinases are identified from the *D. stenobrocha* and *A. oligospora* genomes, respectively, suggesting the chitinases also have functions in nematode-trapping fungi (66).

Collagenases and glycoside hydrolases. Because collagens are the main components of nematode cuticles, collagenases from nematophagous fungi have long been suspected to function during nematode infection. Schenck et al. (94) found that eight nematophagous fungi could secrete extracellular collagenases with high hydrolytic activities in collagens. Furthermore, Tosi et al. (109) found that all the species within *Arthrobotrys* genus could produce collagenases. However, although putative collagenase genes have been found in the genomes of the three nematode-trapping fungi (i.e., EGX43606 in *A. oligospora*), their definitive functions have not yet been experimentally confirmed (130). Interestingly, along with the extracellular enzymes, many gene families related to pathogenicity in other fungi were found to have significantly expanded in the genomes of nematophagous fungi (75, 130). These results suggested that these genes are probably important

for adaptation to parasitism in the nematophagous fungi. Among these expanded gene families, glycoside hydrolases (GHs) are very noteworthy. The GHs function to degrade cellulose, lignocellulose, hemicellulose, xylans, and other constituents of the cell wall (33). On the basis of the carbohydrate-active enzymes (CAZymes) database, a total of 147, 226, and 305 GHs are predicted in *D. stenobrocha*, *A. oligospora*, and *P. chlamydosporia* genomes, respectively (53, 66, 130). The potential roles of these GHs in nematophagous fungi remain to be experimentally confirmed.

Trap Formation of Nematode-Trapping Fungi

Traps are not only used as tools by nematode-trapping fungi to capture nematodes but also are important indicators for switching from the saprophytic to the predacious lifestyles (130). These distinct structures represent remarkable morphological adaptations of these fungi to environmental fluctuations. A molecular phylogenetic analysis suggests that traps have evolved along two major lineages, one with constricting rings and the other with adhesive traps that include three-dimensional networks, knobs, and branches. With the lineage producing adhesive traps, those species that form adhesive knobs and adhesive networks are clustered into a sister clade separated from those that form adhesive branches (133). In addition, nematodes are only one of many factors capable of inducing traps. Along with nemin and ascarosides, mentioned above, several types of materials, such as small peptides with a high proportion of nonpolar, aromatic amino acids or their amino acid constituents and abscisic acid, can also induce trap formation effectively (29, 71, 124, 130). For example, the mycelia of *Monacrosporium lysipagum* produced knobs with the addition of amino acids such as phenylalanine and valine (48). Interestingly, at a certain concentration range, the addition of NH₃ or steroids, including lanosterol, ergosterol, phytosterol, β -sitosterol, and cortisone acetate, to culture media stimulated trap formation under laboratory conditions (45, 79). Recently, two metabolites (paganins A and B) isolated from the nematode-trapping fungus *Arthrobotrys entomopaga* were shown to induce the formation of adhesive knobs (123).

Trap formation is a complex process. An ultrastructure analysis suggested that numerous cytosolic organelles named electron dense bodies (~0.35 μ m in average) filled the trap cells but were not present in vegetative hyphae (26). An investigation of the catalase and D-amino acid oxidase activities within the dense bodies indicated that the formation of dense bodies was likely related to the peroxisome biogenesis (24). Although the exact biochemical properties and biogenesis mechanism of the dense body remain to be elucidated, the upregulation of the peroxisomal proteins in traps illustrates that the peroxisome may have functions in trap formations (130). Similarly, genes related to regulating morphogenesis, cell polarity, stress response, protein synthesis, and degradation, transcription, and carbon metabolism are also differentially expressed during the formation of adhesive knobs in *D. baptotyla* (5). Recently, by monitoring the gene expression patterns after the addition of NE, Yang et al. (130) found that at least 90 genes related to translation, amino acid metabolism, carbohydrate metabolism, cell wall biosynthesis, cell division, and membrane biogenesis were significantly upregulated at the early stage of trap formation. On the basis of the combined genomic, proteomic, and qPCR data, a model was proposed to explain how the adhesive networks are formed in the fungus *A. oligospora* (130). In this model, the signal transduction pathways activated by nematodes regulate downstream cellular processes such as translation, post-translational modification, amino acid metabolism, carbohydrate metabolism, energy conversion, and cell wall and membrane biogenesis, finally leading to the shift from a saprophytic to a carnivorous lifestyle in *A. oligospora* (130). In addition, transcriptional analysis of *D. stenobrocha* during the nematode-fungal interaction suggested that the genes related to rapid cell growth, intracellular signal transduction, and protein degradation may also be involved in the formation of trapping structures (66).

In addition to those genes identified by transcriptomics, proteomics, and qPCR, the disruption of some genes resulting in the failure of trap production confirmed the involvement of these specific genes during trap formation. For example, the *atg8* gene, an essential gene related to the autophagic pathway in fungi, was found to be involved in trap formation in *A. oligospora* (16). The absence of a malate synthase (*Mls*) gene *AoMls* leads to retarded trap formation and significant reductions in conidiation and fatty acid and sodium acetate utilization abilities (141).

NEMATOPHAGOUS BACTERIA

Nematophagous bacteria are another important group of soil microorganisms that are capable of suppressing a wide range of nematode species, including free-living and predatory nematodes as well as animal- and plant-parasitic nematodes. According to their modes of action against nematodes, nematophagous bacteria are classified into the following groups: obligate parasitic bacteria, opportunistic parasitic bacteria, rhizobacteria, parasporal Cry protein-forming bacteria, endophytic bacteria, and symbiotic bacteria (107). However, organisms belonging to the genera *Bacillus*, *Pseudomonas*, and *Pasteuria* represent the dominant populations of nematophagous bacteria in soil.

Cry Protein-Mediated Infection: *Bacillus thuringiensis*

Bacillus thuringiensis (*Bt*) is a ubiquitous spore-forming bacterium that produces proteinaceous protoxin crystals (called crystal protein or Cry protein) during sporulation (95). The Cry proteins show specific toxin activity on caterpillars, beetles, and nematodes but do not affect vertebrates; thus, *Bt* has been described as an ideal biopesticide. In 1972, Prasad et al. (87) reported for the first time that the populations of *Meloidogyne incognita* were significantly reduced by treatment with *B. thuringiensis* var. *thuringiensis*. Since then, several studies have demonstrated the success of using *Bt* to control PPNs in organic agriculture. Indeed, various Cry proteins have been expressed in transgenic plants, and these plants are widely applied around the world to protect crops from nematode infestations (58, 93). Currently, three families of Cry proteins have been found to exhibit potent activities against the larvae of nematodes (Cry5, Cry12, Cry13, Cry14, and Cry21 in the Cry5 family, Cry6 in the Cry6 family, and Cry55 in the Cry55 family) (58, 70, 122). Cry5B is the most extensively studied Cry protein. After ingestion by nematode larvae, Cry5B exerts its effects by interacting with specific receptors located on the membrane of gut epithelial cells, resulting in the formation of lytic pores and causing lysis of the intestine and nematode death (37, 114). Different from Cry5B, the Cry6Aa2 protein causes detrimental effects on *C. elegans*, including growth inhibition, reduced brood size, and abnormal motility (70). The combination of Cry6A and Cry5B proteins showed synergistic activity against *C. elegans*, providing a highly effective strategy for biocontrol of PPNs (137). To search for novel Cry toxins, the genomes of several nematocidal *Bt* strains have been sequenced and three Cry-like genes belonging to the Cry21 family are identified from the genome of *Bt* strain DB27 (44). In addition to the well-known crystal toxins, *Bt* also produces additional virulence factors with insecticidal/nematocidal activity (81). *Bt*'s pathogenic activity is specific toward a narrow range of insect or nematode species; thus, its application is likely harmless to humans and other mammals. One downside of *Bt* strains is that they are highly susceptible to solar irradiation and many environmental chemicals. This susceptibility limits its application as a BCA in agricultural fields (89).

Trojan Horse: *Bacillus nematocida*

Bacillus nematocida (B16), an endospore-forming bacterium, was isolated from a forest soil sample in Yunnan, China. It has high nematocidal activity against the nematode *Panagrellus redivivus* (43).

This bacterium lures nematodes to their death using a Trojan horse mechanism (82). The attraction is mediated by VOCs, such as benzaldehyde and 2-heptanone, produced by the bacterium. Once the bacterium is consumed by nematodes as food, it secretes extracellular proteases, such as an extracellular alkaline serine protease Bace16 and a neutral protease Bae16, to attack the host intestinal tissues, eventually killing the hosts. The Trojan horse mechanism of *B. nematocida* B16 pathogenesis to nematodes expands our understanding of the diverse pathogenic mechanisms used by bacteria (82). The process of B16 infecting nematodes is analogous to the social behavior exhibited by many bacteria and is modulated by a quorum sensing (QS) system (34). This system enables bacterial cells to sense each other and instigate population-level responses. Indeed, a subsequent investigation has revealed that the ComP-ComA system, a conserved QS system in the genus *Bacillus*, is involved in pathogenesis by *B. nematocida* B16 (23).

Obligate Parasites of Nematodes: *Pasteuria penetrans*

Pasteuria penetrans, an obligate, Gram-positive, endospore-forming parasitic bacterium, can colonize more than 300 nematode species, including the majority of important PPNs and free-living nematodes. The obligate nature makes *P. penetrans* a promising agent for biological control of PPNs. In addition to *P. penetrans*, three other *Pasteuria* species can also infect nematodes [*Pasteuria thornei* infects *Pratylenchus* spp. (root lesion nematodes), *Pasteuria nishizawae* parasitizes *Heterodera* spp. and *Globodera* spp. (CNs), and *Pasteuria usgae* infects *Belonolaimus* spp.] (83, 88).

P. penetrans is closely related to *Bacillus*. Phylogenetic analysis suggests that *P. penetrans* has likely evolved from an ancient symbiotic bacteria associated with nematodes, with the obligate parasitic behavior originating when the RKNs evolved to parasitize plants (13). The life cycle for *Pasteuria* consists of three phases: (a) attachment and germination, (b) rhizoid production and exponential growth, and (c) sporogenesis. This shows a high degree of similarity to the developmental stages in *Bacillus* spp. (22). *P. penetrans* can produce highly resistant endospores that can adhere to the cuticles of the second-stage juveniles (J2) and germinate with an infection peg to penetrate the nematode cuticle after the J2 nematodes invade plant roots and begin feeding. The terminal region of the infection peg then extends into the pseudocoelom of nematodes, branches dichotomously, and produces a mycelial ball, or microcolony. The growth of the microcolony of *P. penetrans* in female nematodes causes significant decreases in their fecundity. Finally, *P. penetrans* undergoes sporogenesis, and the female nematodes eventually die. Approximately 10^6 endospores can be released into the soil from each female nematode cadaver.

The attachment of endospores to the nematode cuticle is the first step in the infection process. Three nonmutually exclusive mechanisms of the endospore attachment have been proposed (11, 17, 107). The first mechanism suggests that carbohydrate ligands on the surface of the endospore are responsible for adhesion to the lectin-like receptors on the cuticle of nematodes (101). The second proposes a velcro-like model that involves collagen-like fibers on the surface of the endospore interacting with mucins on the nematode cuticle (20). The third suggests that gelatin-like proteins play critical roles in endospore attachment. This is because the pretreatment of *Pasteuria* endospores with either the heparin-binding domain (HBD) or the gelatin-binding domain (GBD) of nematode fibronectin significantly inhibit endospore attachment (77).

Although the use of *P. penetrans* to control RKN is promising, its fastidious nature (the inability to grow outside its hosts and its host specificity) limits its commercial application as an effective BCA (20). Because this bacterium cannot be cultured in the laboratory, its complete genome sequence is not yet available. Recently, a strategy to collect the genomic DNA from the *Pasteuria*-infected root-knot nematodes was adopted, and four genomic libraries from the *P. penetrans* strain RES147 were constructed (9). These libraries should allow us to obtain its complete genome sequence from this species. A complete genome sequence of *P. penetrans* and further genomic

comparison may facilitate the understanding of the *P. penetrans*--nematode interaction and help in developing this bacterium as a BCA.

***Pseudomonas* spp.**

The Gram-negative bacteria *Pseudomonas* spp. can use a wide variety of compounds as energy and carbon source and be highly adaptive to various environmental niches. Some of the species in *Pseudomonas* are saprophytes, whereas others are plant pathogens, opportunistic human pathogens, or root-colonizing members. Some strains of the species *Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, *Pseudomonas protegens*, and *Pseudomonas chlororaphis* have been found capable of suppressing diverse groups of soilborne plant pathogens, including fungal, bacterial, viral, and oomycete pathogens, as well as some PPNs. Although the hosts for *Pseudomonas* are highly diverse, ranging from plants to mammals and nematodes, the virulence factors and pathogenesis strategies are highly similar among the *Pseudomonas* species. Hence, *Pseudomonas* species are unlikely to act as ideal organisms for biological control of PPNs. However, the *C. elegans*–*P. aeruginosa* pathogenesis model has facilitated the systematic dissection of both host and pathogen genes involved in the pathogenesis, providing valuable information for understanding the molecular mechanisms of host-pathogen interaction (73).

P. aeruginosa strain PA14 can kill *C. elegans* through either the toxin-mediated fast-killing model or the cell growth-mediated slow-killing model (106). In the fast-killing model, PA14 kills worms within 4–24 hours via low-molecular-weight diffusible toxins called phenazines (12, 73, 106). A recent study further revealed that the bacterial siderophore pyoverdine causes worm death by disrupting host iron homeostasis and triggering a hypoxic response (50). In the slow-killing model, PA14 kills *C. elegans* over a period of two to three days by an infection-like process that causes the accumulation of the bacteria in the intestine. In addition, iron acquisition by pyoverdine is involved in the red death phenomenon observed in *C. elegans* infected with *P. aeruginosa* PAO1 (138). The red death response involves three pathways (the phosphate signaling/PhoB pathway, the MvfR-PQS pathway, and the pyoverdine iron acquisition system) and requires a red-colored $\text{PQS}+\text{Fe}^{3+}$ complex (138).

It has been reported that the QS system is also an important mechanism for regulating the pathogenesis of *P. aeruginosa* infections (46). *Pseudomonas* spp. use the *las-rhl* system and the *GacS/GacA* regulators to regulate the production of different virulence factors. Although quorum-controlled virulence genes vary from one species to another, a subset of quorum-controlled genes is often shared among *P. aeruginosa* strains, including a number of extracellular factors encoding genes, LasA and LasB proteases, the ClpP2 protease, an alkaline protease, hydrogen cyanide, and the antibiotic methoxyvinylglycine (18). Recently, a genome-wide screening of virulence factors has revealed that many putative virulence factors contribute to the pathogenesis of *P. aeruginosa* against *C. elegans*, and several of them are related to quorum sensing (28).

Although *Pseudomonas* spp. are known to cause high mortality in PPNs (7), the mechanism of this mortality rate in *Pseudomonas*-PPNs interactions remains poorly understood. The extracellular protease AprA identified from *P. fluorescens* strain CHA0 showed a significant biological activity against *M. incognita* (98). In addition, several secondary metabolites from *P. fluorescens* CHA0, such as 2,4-diacetylphloroglucinol (2,4-DAPG), are key factors in killing nematodes and have shown potential in controlling cyst and root-knot nematodes (99).

NEMATODE RESPONDS TO PATHOGEN ATTACK

Nematodes have evolved specific defense mechanisms against pathogen attack. Up to now, studies using *C. elegans* as a model have revealed the involvement of several signaling pathways in the innate

immune responses to microbial infections. The majority of signal pathways involved in innate immune response identified in a different nematode *Meloidogyne* spp. are similar to those found in *C. elegans*, suggesting that the underlying mechanisms of nematode-pathogen interactions may be highly conserved (21). However, some immune effectors, such as lysozymes, C-type lectins, and chitinases, are much less abundant in *Meloidogyne* spp. than in *C. elegans* (1). In addition, the antibacterial genes (*abf* and *spp*) and the antifungal *nlp*, *cnc*, *fip*, and *fipr* gene families are absent from the *M. incognita* genome (1, 36). Moreover, unlike free-living nematodes, the sedentary endoparasitic PPNs spend the majority of their life cycle inside plant root tissues and feed on the cytoplasm of root cells. As a result, the endoparasitic nematodes may not be exposed to the diversity of microbes, making many of the antibacterial and antifungal genes unnecessary. The availability of the genome and transcriptomic data of more PPNs can help understand the genes and molecular mechanisms involved in the diversity of nematophagous microbes and PPN interactions.

Physical Barriers to and Behavioral Avoidance of Defense Microorganisms

As some pathogens need to adhere to and penetrate the surface of nematodes to initiate infection, the nematode epidermis acts as a physical barrier and represents the first line of defense against pathogens (35). Internally, the pharynx within the nematode mouth can break up bacteria, and the intestine contains many hydrolytic proteins (e.g., lysozymes, lipases, lectins, and some proteases). Both the pharynx and the intestine are also important physical barriers to protect the nematode against invading microorganisms (74).

In addition, nematodes have evolved a sophisticated nervous system to recognize and avoid chemical, physical, and biological cues against dangerous pathogens. When encountering pathogens, nematodes can either move away from these potential toxic pathogens or reduce their ingestion rate. These behavior responses seem crucial for nematodes to survive in the soil and, obviously, are an extremely economic alternative for escaping infections. The avoidance behavior has been observed in *C. elegans* in its response to many pathogens, such as *Bt* (40), *P. aeruginosa* (106), *Salmonella typhimurium* (2), *Serratia marcescens* (85), and *Microbacterium nematophilum* (136). For instance, activation of AWB (amphid wing B) chemosensory neurons in worms causes avoidance by detecting serrawettin W2 produced by the pathogenic bacterium *S. marcescens* (85). *C. elegans* learns to avoid the odors of pathogenic bacteria if worms are raised in the presence of pathogens (140). In addition, *C. elegans* can escape from the constricting rings when encountering the nematode-trapping fungus *Drechlerella doedycoides* (72). After a worm enters the ring, activation of the anterior touch sensory neurons can suppress head movements and induce an escape response before the ring is inflated. It has been demonstrated that a Toll-like receptor *TOL-1* and the insulin/insulin-like growth factor-1 (IGF-1) receptor DAF-2 signaling play essential roles for the behavioral avoidance of pathogenic bacteria (40, 85). Moreover, the G protein-coupled receptors in several olfactory neurons are also involved in mediating the behavioral avoidance in *C. elegans* (39, 85, 90).

Innate Immune Response

Most of the nematophagous fungi and some pathogenic bacteria (*M. nematophilum*, *Xenorhabdus nematophila*, and *Yersinia pestis*) infect nematodes mainly by first adhering to nematode cuticles. In contrast, most bacterial pathogens and several yeasts such as *Candida albicans*, infect nematodes by first colonizing the intestine of *C. elegans* (Figure 2). Thus, both the epidermal and intestinal innate immune responses are important for defense against microbial pathogens (Figure 2). Several signaling cascades have been identified in *C. elegans* in its defense against pathogenic

bacteria. These pathways include the p38 MAPK PMK-1, the extracellular signal-regulated kinase (ERK) MAPK MPK-1, the protein kinase D DKF-2, the G protein-coupled receptor FSHR-1, the DAF-2/DAF-16 pathway, and the transforming growth factor- β -like family (35, 36).

The p38 MAPK pathway, which functions via the NSY-1/SEK-1/PMK-1 axis, plays a key role in both the epidermal and intestinal innate immune responses in *C. elegans* against bacterial and fungal infections (49). Several upstream regulators of this cascade have been identified. Ausubel's lab first identified that *tir-1*, a gene encoding a highly conserved Toll/IL-1 resistance (TIR) domain protein, is required for activation of PMK-1 in response to bacterial infection. TIR-1 is also required for resistance to the nematophagous fungus *D. coniospora* (19). Epistasis analyses suggest that the G12 α protein GPA-12, through phospholipase C β EGL-8, acts upstream of protein kinase C δ , which in turn activates TIR-1, during *D. coniospora* infection (142). A recent study further demonstrated that the G protein-coupled receptor (GPCR) DCAR-1 together with its ligand 4-hydroxyphenyllactic acid (HPLA) activates GPA-12 after *D. coniospora* infection (147). The NSY-1/SEK-1/PMK-1 p38 MAPK pathway is also activated by the Gq α protein EGL-30 through β EGL-8 after infection of the pathogenic bacterium *P. aeruginosa* (47, 142). However, the two conserved transcription factors ATF-7 and SKN-1 have been identified as downstream signaling molecules of the p38 pathway toward bacterial infection (97, 115). In addition, the endoplasmic reticulum unfolded protein response (UPR) is also a downstream signaling component of the p38 MAPK pathway in response to *P. aeruginosa* infection or to the pore-forming toxins produced by human pathogens, such as *Staphylococcus aureus*, *Streptococcus pyogenes*, and *Aeromonas hydrophila*. Lack of either the *ire-1-xbp-1* or the *atf-6* branches of the UPR renders *C. elegans* hypersensitive to *P. aeruginosa* and pore-forming toxins (10, 91).

The second major cascade involved in the innate immunity against pathogens is the DAF-2/DAF-16 signaling pathway. DAF-2 is a negative regulator of DAF-16, the ortholog of mammalian forkhead box O (FOXO) transcription factors. Reduction in the DAF-2 signaling cascade leads to the dephosphorylation of DAF-16, resulting in its nuclear translocation and transcriptional activation (41, 64). It has been shown that *daf-2* mutants are more resistant to several pathogenic bacteria than wild-type worms (32). However, the pathogen-resistant phenotype of *daf-2* mutants is completely abolished by mutations in *daf-16*, indicating that DAF-16 plays an important role in innate immunity against bacteria. Interestingly, *daf-16* mutants with or without *daf-2* mutations exhibit similar sensitivity to pathogenic bacteria as wild-type worms, suggesting that DAF-16 is not normally activated during bacterial infection in wild-type worms. In contrast, DAF-16 in the epidermis is required for worm survival upon infection by nematophagous fungi, such as *D. coniospora* and *C. rosea* (145). The EGL-30-Ca²⁺-DUOX-1-ROS-CST signaling regulates DAF-16 activity after fungal infection, which is functionally independent of the DAF-2 insulin-like signaling pathway.

The third major cascade implicated in *C. elegans* innate immunity is the ERK MAPK pathway. The role for ERK/MPK-1 in worm defense was first discovered in *C. elegans* when infected by *M. nematophilum* (80). This Gram-positive bacterium can colonize the rectum and postanal cuticle in worms and induce a pronounced swelling of the surrounding hypodermal cells. The ERK cascade mediates this tail swelling response, thereby protecting *C. elegans* from severe constipation caused by the bacterium (80). Mutations in the core components of the ERK signaling also enhanced susceptibility to *P. aeruginosa* PA14, which infects the intestine of worms. Unlike the p38 MAPK pathway, which fights off infection by upregulating secreted immune response genes, including C-type lectins, lysozymes, and antimicrobial peptides (110), the ERK signaling pathway functions to elicit autophagy, which in turn protects worms against organismal damage triggered by *P. aeruginosa* (80).

CONCLUSION AND PERSPECTIVE

Over the past decade, significant progress has been made in our understanding of the molecular mechanisms of interaction between model nematodes and nematophagous microorganisms. These understandings have provided exciting new targets and guidance for developing effective strategies for biological control of PPNs. With the development of molecular biology and biotechnology, as well as the increasing availability of -omics data from both PPNs and their interacting microorganisms, we expect greater progress will be made in this field over the next few years. Identifying the functions of those key genes/factors in determining the mode of actions of BCAs should lead to the improvement of their nematicidal potential through targeted genetic manipulations, enhancing their biological control efficiency in PPN management.

For decades, researchers studying the mechanisms of microbe-nematode interactions have mainly focused on the interaction between two species. However, both the nematodes and microbes live in the complex soil ecosystem as part of the soil food web. Within this web, plants are the primary producers, whereas other organisms (bacteria, fungi, plant-feeding nematodes, and root-grazing insects) either feed on or decompose plant materials to obtain nutrients and become food and energy sources for organisms at higher trophic levels. Obviously, there are many interactions among organisms rather than the simple interactions between one pathogen and one host. For example, we have recently identified that certain food bacteria can release urea and trigger the morphogenetic switch of nematode-trapping fungi from a saprophytic to a predacious form that in turn can capture and kill the nematode predators of bacteria (148). Our finding suggests that the multiple predator-prey interactions are likely very common and important for maintaining population balance within the complex microbial community.

Although many nematicidal metabolites have been identified from microorganisms and some have been developed as BCAs to control PPNs, the mechanisms involved in the biocontrol activity and their biosynthetic pathways are still poorly known. The availability of the genomic and metabolomics data of various species should contribute to the identification of novel metabolites and their biosynthetic genes (PKSs, NRPSs, and P450s). Moreover, with the identification of novel metabolites with high nematicidal activities and the understanding of the molecular interactions during infections, some of these metabolites with high nematicidal efficiencies will be developed or improved in the near future.

Currently, a number of studies have reported that combining different organic amendments and biological control organisms may provide greater nematode suppression than using a single biological control agent (76). Strategies that use multiple microbial control agents with complementary and synergistic modes of action, and integrate biological control agents with other control methods, such as chemical nematicides, nematode-resistant cultivars, and crop rotations, could be highly effective in reducing pest nematode populations. Greater understanding of the molecular mechanisms of microbe-nematode interactions will provide further guidance from which to develop more effective strategies.

SUMMARY POINTS

1. Elucidating the molecular mechanisms between nematophagous microorganism–nematode interactions is crucial for developing highly effective BCAs and provides novel biocontrol strategies for controlling PPNs. Although significant progresses have been made in our understanding of the molecular mechanisms of nematophagous fungi–nematodes interactions, much remains unknown.

2. Most nematophagous fungi colonize nematodes by penetrating the cuticle, whereas nematophagous bacteria use a diversity of modes to infect nematodes.
3. The pathogenesis of nematophagous fungi against nematodes includes the following stages: attraction/recognition, adhesion, penetration, and digestion.
4. Nematodes have evolved specific defense mechanisms against pathogen attacks. However, the majority of studies on the molecular mechanisms of nematode defense against microbes have used *C. elegans* as the model host.

FUTURE ISSUES

1. There is an urgent need for a comprehensive understanding of the underlying mechanisms of microorganism-nematode interactions. Improving the nematocidal potential of BCAs through targeted genetic manipulations could enhance the potential application of biological control in PPN management.
2. Multiple predator-prey interactions may be important for understanding the composition and population dynamics of nematodes and microorganisms in the soil ecosystem.
3. The identification of an increasing number of novel metabolites with high nematocidal activities and the understanding of their biosynthetic pathways, as well as the mechanisms of their toxicity to nematodes, would enhance the development of novel metabolites as BCAs.
4. Strategies that use multiple microbial control agents with complementary and synergistic modes of action and integrate biological control agents with other control methods, such as chemical nematicides, nematode-resistant crops, and crop rotations, could contribute to reducing nematode pest populations.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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