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REVIEW ARTICLE

EFFECT OF MICROBIAL SECRETION ON INHIBITORY EFFECT OF PHYTONEMATODE: A REVIEW

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ABSTRACT

Plant-parasitic nematodes cause significant damage to a broad range of agricultural crops throughout the world. For decades, the control of phytonematodes has relied heavily on chemical nematicides. Recently, the use of chemical nematicide has been restricted due to their toxic effect towards the physical environment. So an environment friendly alternative for nematodes control is increasingly important. As the natural enemies of nematodes, nematophagous microorganisms offer a promising approach to control the nematode pests (Tian *et al.,* 2007). Most of the microorganism released or secrets some metabolites which are toxin, antibiotic or inhibitory to phytonematodes and some have stimulatory effect. They act synergistically on nematode through direct suppression of nematodes, promoting plant growth, and facilitating the rhizosphere colonization. Some of the rhizobacterial species like *Bacillus subtillis*, *Pseudomonas florescence* produces hydrolytic enzyme such as protease, lipase, and cellulase which reduce the egg hatching, recognition and penetration (Siddiqui *et al.,* 2005). Avermectin MK-936 is an antibiotic produced by *Streptomyces avermitilus* found to kill infective juvenile and inhibit egg hatching. Mycoparasitic fungi and other egg and female parasitizing fungi also parasitize nematode egg and larva through extracellular enzymes, producing toxin and other metabolites (Li *et al.,* 2015). Researches on agents that work against phytonematode do not have detrimental impact on environment. Therefore increased understanding of the microbial secretion of the various mechanisms of beneficial microorganism could potentially enhance their value as effective biocontrol agent.

INTRODUCTION

Plant-parasitic nematodes (PPNs) bring serious economic burden to farmers worldwide and it is one most important agricultural pest, causing estimated crop losses worth more than US $125 billion each year. The management of nematodes is difficult because nematodes mostly inhabit the soil and usually attack the underground parts of the plants. It is small about 0.5 to 3mm unsegmented worm. Being a small habitat it affect the crop in many ways like it alter the normal root cell division, modifying plant cells for nutrient transfer and also transmitting virus by creating wounds. For decades, the control of phytonematodes has relied heavily on chemical nematicides, although chemical nematicides are effective, easy to apply, and show rapid effects, they have begun to be withdrawn from the use because of their notorious toxicity to physical environment and soil microflora and microfauna (Schneider *et al.,* 2003). Consequently, global crop production remains under heavy threat from PPNs.

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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. Soil is a complex environment and reserviour for microflora and microfauna that exist concomitantly. The root rhizosphere is the most intensified zone for microbial activity. In nature there are regulatory mechanisms through parasitic, predatory and pathogenic ability of other microorganism that prevent unlimited development of phytonematode. Besides direct parasitism, many of this rhizospheric organism are capable of producing naturally of substances (metabolites), which may be toxic, inhibitory or attractive to phytonematodes. There are several research report observation on the response of phytonematode to a variety of metabolites secreted by bacteria, fungi and actinomycetes isolated from soil and root rhizosphere. These bacteria, fungi and actinomycetes have been reported to influence nematode biological activity like

embryonic development, hatching, moulting, host finding and penetration and development and reproduction.

Microbes having inhibitory effect of metabolites on phytonematodes

Bacteria

Plant-parasitic nematodes co-exist in rhizosphere with biologically diverse bacterial communities. These bacteria impact the nematode life cycle as endoparasites or antagonists. Most of the antagonistic bacteria are saprophytes living in the rhizosphere. Many bacteria can produce and secrete toxins. The metabolites releases by soil microorganism are exceedingly variable and may be toxic, antibiotic or inhibitory to plant parasitic to nematode as they accumulate in soil. Bacteria whose secretions or metabolic products are harmful to nematodes are known as nematode toxin producing bacteria.

Rhizobacteria

Soil microbiota is attracted to roots. Root exudates are excellent food source for soil organisms that accumulate around the roots. Diversity of microbes in this area called the rhizosphere transcends the diversity in bulk soil. The bacteria that colonize the rhizosphere of the host plant are called rhizobacteria. These are mostly non-pathogenic bacteria. Most rhizobacteria which are known to be detrimental to plant parasitic nematode act by metabolic-by-product, enzymes and toxin rather by parasitism. The effects of these toxins include the suppression of nematode reproduction, egg hatching and juvenile survival, as well as direct killing of nematodes (Zuckerman & Jasson, 1984; Siddiqui & Mahmood, 1999). The nematicidal activity of these bacteria may due to antibiotic, which they produce in agar medium (Becker *et al.,* 1988) Most frequently studied antagonistic rhizobacteria to affect the RKN are *Bacillus subtilis*, *B. firmus, B. thurengensis* and *Pseudomonas fluorescens* (Becker *et al.,* 1988; Sikora, 1992; Tian *et al.,* 2007).

Bacillus subtilis

It is one of the important bacterium most widely used across the world. The isolation and selection of *Bacillus subtilis* strain exhibit a broader spectrum of activity against phytonematodes. The strain produce hydrolytic enzyme such as protease, lipases, b-gluconase and cellulase. In soil, this species interfare in the reproductive cycle of nematode, acting on the larvae orientation to the host plant. On the other hand it also act as plant growth promoter and help in control of phytopathogenic fungi such as *Rhizoctonia solani*.

Bacillus firmus

*Bacillus firmu*s is a Gram-positive, endospore producing soil bacterium sparsely represented in nature. It is one of the most potential nematicidal bacterium, has been widely described and characterized in recent years. Many studies have demonstrated that B. firmus is effective against Meloidogyne spp., Ditylenchus dipsaci (Mendoza *et al.* 2008), Rodopholus similis (Mendoza *et al.,* 2008), Heterodera spp., Tylenchulus semipenetrans, Xiphinema index (Keren-Zur *et al.* 2000). Not all strains exhibit nematicidal activity. Those exhibit nematicidal property destroy egg of *Meloidogyne* spp. by colonising egg sacs (Keren-Zur *et al.,* 2000), some have also suggested the involvement of toxins (Mendoza *et al.,* 2008) and this toxin damage the external egg pellicle of gall forming nematode, inhibit the hatching.

***Bacillus thurengensis* (BT) (Cry Protein–Mediated Infection):**

Bacillus thuringiensis was first discovered in Japan (Ishiwata, 1901) from diseased silk-worm larvae identified as sotto disease of silk worm. A German biologist Berliner (1911) isolated it from pupae of Mediterranean flour moth *Ephastia kuchmiella* living in stored grains in the city of Thuringen and hence named it as *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. Delta endotoxins and thuringiensis (beta-exotoxin) are derived from certain strains of the bacterium, *Bacillus thuringiensis*. They interfere with RNA transcription by inhibiting RNA polymerase.

Plant growth promoting Rhizobacteria (PGPR): Pseudomonas fluorescence

Pseudomonas spp. is aerobic, gram-negative bacteria, ubiquitous in agricultural soils, and is well adapted to growing in the rhizosphere. Produce a wide spectrum of bioactive metabolites (i.e., antibiotics, siderophores, volatiles, and growth-promoting substances). Fluorescent Pseudomonads have property to form a ferric-siderophore complex. The bacteria utilizing this complex prevent the availability of iron to other microorganisms. Antibiotic mediated suppression compound such as phenazine, pyrolnitrin, tropoplene, pyocyanin and 2,4-diacetylphloroglucinol have been isolated from fluorescent pseudomonads. This antibiotic may have lethal effect on plant parasitic nematode in rhizosphere. Pseudomonas strains are commonly uses to treat seeds or roots of plants before planting. They can also be used to treat tubers and bulbs. *Pseudomonas fluorescens* controlled cyst nematode juveniles by producing several secondary metabolites such as 2, 4-diacetylphloroglucinol (DAPG) (Cronin *et al.,* 1997; Siddiqui & Shaukat, 2003).

Possible mechanism of nematode suppression

Non parasitic rhizobacteria which colonize the rhizophere of host plant. Two mechanism of action are thought to be responsible for reduction of nematode infection of roots by production of metabolites which reduce hatch and attraction and another is degradation of specific root exudates which control nematode behaviour. The effect of non parasitic rhizobacteria on penetration of nematode is possibly due to bacteria binding to lectins on the root surface and surface carbohydrate of nematodes. In degradation it blocks the lectin-

carbohydrate recognition site therefore interfering with host recognition by the nematodes which control nematode behavior and inhibit egg hatching and induce larval mortality. In aerobic soil, ammonia is usually produced by ammonifying bacteria during decomposition of nitrogenous organic material and this has resulted in reduced nematode population. Another important bacterium *Clostridium butyricum* produced butyric acid (Rodriguez-kabana, 1986) while hydrogen sulphide was produced by *Desulfovibrio desulfuricans* and this resulted in reduced nematode multiplication. Another plant growth promoting bacteria *Pseudomonas fluorescens* generally act through direct antagonism to pathogen, through antibiotic production (Rodriguez-Kabana, 1986).

Recently, rhizobacteria-mediated induced systemic resistance (ISR) in plants has been shown to be active against nematode pests (Van Loon *et al.,* 1998; Ramamoorthy *et al.,* 2001). Plant growth-promoting rhizobacteria (PGPR) can bring about ISR by fortifying the physical and mechanical strength of the cell wall by means of cell-wall thickening, deposition of newly formed callose, and accumulation of phenolic compounds. They also change the physiological and biochemical ability of the host to promote the synthesis of defence chemicals against the challenge pathogen (e.g. by the accumulation of pathogenesis-related proteins, increased chitinase and peroxidase activity, and synthesis of phytoalexin and other secondary metabolites) (Van Loon *et al.,* 1998; Siddiqui & Mahmood, 1999; Ramamoorthy *et al.,* 2001).

Actinomycetes

Another important group of soil microorganism with potent antagonistic activity toward *Meloidogyne spp*. are actinomycetes. Actinomycetes have potential for use in biological control as they are known to produce antibiotics. It is possible that such antibiotics and/or other microbial metabolites produced by the soil microflora have adverse effects on nematodes. These are also known producers of secondary metabolites with antibiotic activity towards many fungi and bacteria. The nematicidal properties of avermectins produced by actinomycetes (Streptomyces sp.) have stimulated interest in 'natural' nematicides. Work by Mishra *et al.,* (1987) tested 800 actinomycete strains for nematicidal activity and approximately 2% were found to be positive. The production of avermectins by a species of Streptomyces shows that soil-borne organisms can produce highly nematicidal compounds. Most studied are *Streptomyces* species that act against various fungal species and *Meloidogyne* spp. (Krechel *et al.,* 2002). *S. avermitilis* produces antibiotic compounds avermectins that are the most effective nematicides. Avermectin (Mk-936) is a macrocyclic lactone derived from actinomycete *Streptomyces avermitilus*. This antibiotic kills infective juveniles, reduces egg hatching, and it has been suggested recently that avermectins inhibit RNA synthesis (Takatsu *et al.,* 2003). A commercial product available on the market is Avicta (Syngenta, Switzerland) used as a seed treatment for vegetables and cotton. In insect it blocks the neurotransmitter GABA (gamma aminobutric acid). It has also been widely used to control nematode parasites of animal.

Fungi

Fungi have an antagonistic action against the nematodes. The nematode cuticle and egg walls play an important role in the infestation by the fungi. The cuticle is made up of proteins (chitin, collagen, fibers) and can play the role of a precursor in the invasion of the nematode by nematophagous fungi (Huang *et al.,* 2005). Some important group of fungi whose secretion reduce nematode population are:

Toxin-producing fungi

Toxin-producing fungi are a group of fungi that can produce toxins to immobilize nematodes before hyphae penetrate through the nematode cuticles (Lòpez-Llorca *et al.,* 2008). Nematophagous fungi secreted a number of compounds *in vitro* that may have nematicidal or nematostatic traits. The *in vivo* role of such compounds is usually not well known but *Pleurotus ostreatus* produces droplets of a potent toxin that quickly immobilizes nematodes (Thorn and Barron., 1984) and has the structure of trans-2-decenedioic acid (Kwok *et al.,* 1992). More than 200 compounds with nematicidal activities have been identified from approximately 280 fungal species in 150 genera of Ascomycota and Basidiomycota (Li G and Zhang K.Q., 2014).

These compounds belong to diverse chemical groups, including alkaloids, peptides, terpenoids, macrolides, oxygen heterocycle and benzocompounds, quinones, aliphatic compounds, simple aromatic compounds, and sterols (Li G and Zhang K.Q., 2014). 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) and 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 nematicidal activities with LC50 values of 0.5-1 μg/mL as similar to commercial avermectins (Guo *et al*, 2012). Antibiotic (nematicidal and antifungal) activities have been demonstrated for *Drechmeria coniospora*, *Harposporium anguillulae* (Barron, 1977), *Lecanicillium*, *Paecilomyces lilacinus* (Jatala., 1996), and *Pochonia* (Seger *et al.,* 1998). *Paecilomyces lilacinus* secretes acetic acid that paralyzes juvenile nematodes (Djain *et al.,* 1991).

The mycoparasitic fungi *Trichoderma* spp

The mycoparasitic fungi *Trichoderma* spp. has been described as BCAs against PPNs, although the underlying mechanism remains largely unknown. The biocontrol activities exhibited by *Trichoderma* are:

Mycoparasitism: Tricoderma is able grow on the cyst surface and penetrate through the cyst wall and egg shell of cyst nematode. The fungus has been reported to secret many lytic enzymes. Chitinase of many trichoderma spp. Help parasitism of nematode such as *Meloidogyne* spp.

Antibiosis: It produces antibiotic like trichodermin, dermadin, tricoviridin and a sesquiterpene-heptalic acid.

Competition: They have high rhizosphere competency and easily colonize the root. This may reduce the feeding site for nematode.

Table 1. Bacterial antagonists affect of different developmental stages of *Meloidogyne* spp (Hallmann *et al.,* 2009)

Developmental stage	Nematode behavior Intercepted	Mode of action	Place of action	Examples of Bacteria	References
Egg or egg mass	Development, hatching	Toxins, lytic enzymes, parasitism	Soil	*Telluria chitinolytica, Bacillus firmus*	Spiegel *et al*.,1991; Wilson and Jackson, 2013
Infective juveniles	Vitality, host attraction, host recognition, penetration	Toxins, lectins, degradation of root exudates, induced resistance	Soil, rhizosphere	*Pseudomonas fluorescens, Pseudomonas aeroginosa, Rhizobium etli*	Krechel *et al.,* 2002; Siddiqui and Shaukat (2003); Siddiqui *et al.,* 2006; Sikora *et al.,* 2007; Oliveira *et al.,* 2007
Sedentary juvenile	Formation of feeding site, development	Toxins, induced resistance	Endorhiza	*R. etli*	Reitz *et al.,* 2002

Table2. Reported bacterial extracellular enzymes involved in pathogenesis against nematodes

Enzyme	Microbial origin	Nematode target	Effects on nematodes	References
Extracellular alkaline protease BLG4	Brevibacillus laterosporus G4	Panagrellus redivius and Bursaphelenchus xylophilus	Purified BLG4 killed 71% of the tested nematodes within 24 hours. BLG4-deficient strain had a significantly reduced activity against nematodes; only 43% of the nematodes were killed and 22% of the cuticles of dead nematodes were degraded	Huang et al. (2005), Tian et al. (2006)
Neutral protease NPE-4 inhibited by EDTA	Brevibacillus laterosporus G4	Panagrellus redivius	Addition of NPE-4 in the extracellular crude protein extracts from wild Br. laterosporus strain G4 enhanced activity against nematodes. After 24 h, striaes were clear, but the lateral sections of cuticle in the treatments of NPE-4 became irregular and the whole body of cuticle appeared faint	Tian et al. (2007)
Alkaline serine protease	Bacillus nematocida (Bacillus B16)	Panagrellus redivius	Purified protease killed about 90% of the nematodes within 24 h. After 48 h, all the tested nematodes were killed	Niu et al. (2005)
Neutral protease Bae16	Bacillus nematocida (Bacillus B16)	Panagrellus redivius and Bursaphelenchus xylophilus	LC50 of purified Bae16 on Pan. redivius within 24 h is 1.69 μg mL^{-1}; on Bu. xylophilus it is 2.26 μg mL^{-1}	Niu et al. (2006)
Protease aprA inhibited by EDTA	Pseudomonas fluorescens CHA0	Meloidogyne incognita	The aprA-deficient strain showed significant reduced activity on M. incognitain the field tests	Siddiqui et al. (2005)

Enzyme production: *Trichoderma* can parasitize nematode eggs and larva through secreting extracellular hydrolytic enzymes, such as trypsin-like protease PRA1 (Suarez B *et al.,* 2004), serine protease SprT (Chen L *et al.,* 2009), and chitinolytic enzymes *chi18-5* and *chi18-12* (Szabo M *et al.,* 2012).

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 (Szabo M *et al.,* 2013). Furthermore, some nematicidal compounds have also been obtained from *Trichoderma* spp., such as trichodermin (Yang Z *et al.,* 2010), β-vinylcyclopentane-1α, 3α-diol, 6-pentyl-2H-pyran-2-one, and 4-(2-hydroxyethyl) phenol (Yang Z *et al.,* 2012). Jonathan, 2010 reported on *Trichoderma viridae* that secretion of lytic enzyme chitinaze help parasitism of Meloidogyne and *Globodera* egg. Also releases dermadin which destruct the nematode cuticle and reduced the feeding site for nematode by rhizospheric competition.

Nematode egg and female parasites

Nematode egg and female parasites target the immobile stages of economically important genera of cyst and rootknot nematodes such as *Globodera*, *Heterodera*, *Rotylenchulus*, *Tylenchulus*, and *Meloidogyne*. *Pochonia chlamydosporium* produce Phomalactone at a concentration of 500 mg/l exhibited promising inhibitory activity against the second stage larvae of *M. incognita* (Khambay *et al*. 2000). *Paecilomyces lilacinus* form cuticle degrading crystal protein. Enzymes chitinase, protease and acetic acid produced by *P. lilacinus* were applied to *M. javanica* eggs, and it resulted degradation of eggshell and reduction in egg hatching (Anita *et al.,* 2010). Park *et al.(*2004) studied the influence of leucinostatins, a secondary metabolite produced by *P. lilacinus*, in the colonization of *M. javanica* eggs, revealing positive results.

Extracellular Enzymes Involved in Infection

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 (Huang *et al.* 2004; Yang *et al.* 2007)

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*). Functional analyses have revealed that these proteases can effectively degrade nematode cuticles; hence, they are also called nematode cuticle–degrading proteases.

Chitinases: Chitin is themost 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. So far, nine chitinases of the glycosyl hydrolase 18 (GH18) family with highly conserved structures have been purified or cloned from different nematodeparasitic fungi.

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.* (1994) found that eight nematophagous fungi could secrete extracellular collegenases with high hydrolytic activities in collagens.

Conclusion

Microbial control will never be a substitute for chemical control because of its inherent limitations: inconsistency and lower effectiveness. But, its added value on a long-term scale is much higher: clean environment, safe food and water, and most importantly healthy people. Based on current knowledge we have a long road ahead. Fortunately, the use of microbial agents is widely accepted among the growers, which is a strong stimulus for a continued research.

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