

Exploring the Role of Bacterial Bioagents in the Battle Against Plant-Parasitic Nematodes

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SUMMARY

Plant-parasitic nematodes (PPNs) are a severe threat to global agriculture, causing crop losses of over USD 173 billion each year. While chemical nematicides can offer immediate control, their environmental and health risks highlight the urgent need for safer, more sustainable solutions. Soil-dwelling bacteria offer a promising biocontrol strategy due to their diverse mechanism of action, ability to adapt genetically, and low risk of pests developing resistance. This article categorizes nematophagous bacteria based on their antagonistic strategies: some act as obligate parasites that interfere with nematode reproduction, others are opportunistic saprophytes that break down nematode cuticles with enzymes, and some are rhizobacteria or endophytes that boost plant defenses or compete with pests for resources. Specialized strains can produce toxic crystal proteins to damage nematode midguts, while symbiotic bacteria release lethal secondary metabolites to kill nematodes. Despite their potential, these biological controls face challenges in the field, such as sensitivity to environmental changes, limited host ranges, and high costs. Addressing these issues will require advanced multi-omics tools to understand their mechanisms, the discovery of broad-spectrum strains, and evaluating climate adaptability to ensure long-term efficacy and sustainability in diverse agricultural systems.

INTRODUCTION

Plant-parasitic nematodes (PPNs) pose a major threat to global agriculture by reducing the yield and quality of crops (Shakeel et al., 2022). They also play a significant role in disease complexes with other plant pathogens. In the field, the damage caused by nematodes is difficult to diagnose because of general symptoms (such as leaf yellowing, wilting, and stunting) that can easily be confused with other diseases or nutritional deficiency symptoms (Kumar et al., 2020). According to reports, plant-parasitic nematodes (PPNs) cause global crop yield losses estimated at over USD 173 billion (Elling et al., 2013). In India, recent studies indicate that annual crop losses due to pests and diseases reach approximately Rs. 500 billion, with nematodes accounting for 20.4% of the total losses, as reported by the All India Coordinated Research Project (AICRP) on Nematodes. *Meloidogyne graminicola*, the rice root knot nematode, is reported to be the most damaging PPN in India, causing ~23% loss of total (=Rs. 2346.7 Crores) in rice (Kumar et al., 2020). Management of plant parasitic nematodes presents greater challenges compared to other pests, as they dwell in the soil and primarily attack the underground parts of the plants (Stirling, 1991). Application of chemical nematicides has been proven to be the most effective for controlling PPNS, but it is hazardous to human health and the environment (Gao et al., 2016). This necessitates the need for an alternative, promising, and environmentally friendly approach. Various fungi and bacteria in the soil have been found to effectively control the populations of PPNS. They either directly affect the physiology of the host by preventing egg hatching, destroying females and second-stage juveniles (J2) by releasing toxic metabolites, or indirectly through enhancing plant defense responses (Migunova and Sasanelli, 2021; Ayaz et al., 2024). Therefore, soil microorganisms represent a reliable option for the management of these nematodes.

Bacteria as Biocontrol Agents of PPNS:

Bacteria are the most prevalent organisms in field soil. Although their total biomass is slightly less than that of fungi, it surpasses the combined biomass of algae, protozoa, and nematodes (Clark, 1967). Several bacterial genera, including *Pasteuria*, *Pseudomonas*, and *Bacillus*, have shown considerable potential for the biological control of nematodes (Emmert & Handelsman, 1999; Siddiqui & Mahmood, 1999; Meyer, 2003). Bacteria, as a bioagent, offer several advantages over fungi. Firstly, bacteria are eco-friendly and require a prolonged period for resistance to develop. Secondly, their simple genomes make them suitable for biotechnological interventions to produce more efficient strains.

Nematophagous Bacterial Groups:

Parasitic Bacteria:

An obligate parasite is defined as a parasitic organism that is unable to survive independently of its host. *Pasteuria penetrans*, a mycelial and endospore-forming obligate parasitic bacterium, has shown significant potential as a

biological control agent against root-knot nematodes. The life cycle of *Pasteuria penetrans* consists of four distinct stages: spore germination, vegetative growth, fragmentation, and sporogenesis.

Mechanism of Action:-

Spores of *Pasteuria* attach to the cuticle of second-stage juveniles (J2) and typically germinate about eight days after the juvenile enters the roots and begins feeding (Sayre and Wergin 1977; Davies et al., 2001; Phani et al., 2018). Once germinated, the germ tubes penetrate the juvenile's cuticle, allowing vegetative microcolonies to proliferate throughout the developing female's body. This process leads to the degeneration of the nematode's reproductive system, after which mature endospores are released back into the soil (Mankau et al. 1976; Sayre and Wergin 1977; Tian et al., 2007).

Opportunistic Parasitic Bacteria:

Most nematophagous bacteria, except obligate parasites, typically exhibit saprophytic lifestyles and utilize nematodes as potential nutrient sources. Under certain conditions, these bacteria can penetrate the nematode's cuticle, infecting and killing the host. Such bacteria are classified as opportunistic parasitic bacteria, including *Brevibacillus laterosporus* strain G4 and *Bacillus sp.* B16.

Brevibacillus laterosporus exhibits a broad spectrum of biological activities as a pathogen. Previous studies have reported that isolates of *Br. laterosporus* are capable of killing four nematode species: three parasitic nematodes (*Heterodera glycines*, *Trichostrongylus colubriformis*, and *Bursaphelenchus xylophilus*) and the saprophytic nematode *Panagrellus redivivus* (Oliveira et al. 2004; Huang et al. 2005).

Mechanism of Action: -

Bacteria initially attach to the epidermis of the nematode host body, then rapidly propagate and form a single clone in the epidermis of the nematode cuticle. As the clone grows, it can create a circular hole by continuously degrading and digesting the host cuticle and tissue through hydrolytic enzymes (Cox et al. 1981; Decraemer et al. 2003; Huang et al. 2005). Eventually, the bacteria enter the host's body and digest all host tissue, using it as nutrients for pathogenic growth (Huang et al. 2005).

Plant Growth Promoting rhizobacteria:

Aerobic endospore-forming bacteria (AEFB), mainly *Bacillus spp.*, and *Pseudomonas spp.* are among the dominant populations in the rhizosphere that can antagonize nematodes (Rovira & Sands, 1977; Krebs et al., 1998), and the most thoroughly studied is probably *Bacillus subtilis* (Krebs et al., 1998; Siddiqui & Mahmood, 1999; Lin et al., 2001; Siddiqui, 2002).

Rhizobacteria primarily reduce nematode populations through several mechanisms, including regulation of nematode behavior (Sikora & Hoffmann-Hergarten, 1993), interference with plant-nematode recognition (Oostendorp & Sikora, 1990), competition for essential nutrients (Oostendorp & Sikora, 1990), promotion of plant growth (El-Nagdi & Youssef, 2004), induction of systemic resistance (Hasky-Günther et al., 1998), and direct antagonism via the production of toxins, enzymes, and other metabolic products (Siddiqui & Mahmood, 1999).

Parasporal Crystal-Forming Bacteria:

Bacillus thuringiensis is a spore-forming, aerobic, Gram-positive bacterium within the genus *Bacillus* and is recognized as a potential biocontrol agent due to its production of parasporal crystal inclusions (Cry or δ -endotoxins). Currently, six Cry proteins (Cry5, Cry6, Cry12, Cry13, Cry14, Cry21) have been identified as toxic to larvae of various free-living and parasitic nematodes (Alejandra et al., 1998; Crickmore et al., 1998; Marroquin et al., 2000; Wei et al., 2003; Kotze et al., 2005). In addition, other compounds produced by *B. thuringiensis*, including thuringiensin (Devidas and Rehberger 1992; Sánchez-Soto et al. 2015), chitinase (Zhang et al. 2014), and metalloproteinase (Luo et al. 2013b), exhibit nematicidal activity.

Cry proteins exert toxicity by targeting midgut intestinal epithelial cells, resulting in vacuole and pore formation, pitting, and eventual degradation of the intestinal tissue (Marroquin et al. 2000). The interaction between the pore-forming toxin and specific receptors on epithelial cells is a critical step in this process.

Endophytic Bacteria:

Endophytic bacteria are commonly found within the internal tissues of roots, stems, fruits, and vegetables in a wide variety of plant species, where they coexist without causing harm to their hosts (McInory & Kloepper, 1995; Hallmann et al., 1997, 1999; Azevedo et al., 2000; Hallmann, 2001; Surette et al., 2003). These beneficial microorganisms not only support plant growth but also help suppress diseases and nematode pests (Sturz &

Matheson, 1996; Hallmann et al., 1999; Azevedo et al., 2000; Munif et al., 2000; Shaukat et al., 2002; Sturz & Kimpinski, 2004).

For instance, Munif et al. (2000) found that 21 out of 181 endophytic bacteria isolated from tomato roots exhibited antagonistic activity against *Meloidogyne incognita* under greenhouse conditions. He also demonstrated the effectiveness of endophytic bacteria from *Tagetes sp.* to control *Meloidogyne spp.* infections in tomato plants (Munif et al., 2021). Additionally, various bacterial species have demonstrated efficacy against root-lesion nematode (*Pratylenchus penetrans*) in the soil surrounding potato roots. Ganeshan et al. (2024) showed that the endomicrobiome of guava plants produces various bioactive molecules that confer resistance against the guava root knot nematode, *Meloidogyne enterolobii*. Although they occupy distinct ecological niches, both rhizobacteria and endophytic bacteria utilize similar mechanisms, including niche competition, the production of inhibitory compounds, and the induction of systemic resistance in host plants, to promote growth and protect against phytopathogens (Hallmann, 2001; Compant et al., 2005).

Symbiotic Bacteria of Entomopathogenic Nematodes:

Xenorhabdus spp. and *Photorhabdus spp.* serve as bacterial symbionts of the entomopathogenic nematodes *Steinernema spp.* and *Heterorhabdus spp.*, respectively (Paul et al., 1981).

These symbiotic bacteria are believed to suppress plant-parasitic nematodes by producing defensive compounds (Samaliev et al., 2000). Several secondary metabolites, including ammonia, indole, and stilbene derivatives, have been identified as nematocidal agents (Hu et al., 1995, 1996, 1997, 1999). These compounds exhibit toxicity toward second-stage juveniles of root-knot nematode (*M. incognita*), as well as fourth-stage juveniles and adults of pine-wood nematode (*Bu. xylophilus*), and also inhibit egg hatching of *M. incognita* (Hu et al., 1999).

Challenges in Biocontrol of PPNs:

- One of the main challenges with using nematophagous bacteria as biocontrol agents is their often-limited host range, which restricts their effectiveness.
- Even though some biocontrol agents show strong nematocidal activity in the lab, they often do not perform well in field conditions.
- Factors like soil pH, temperature, and moisture can influence the effectiveness of biocontrol agents. For effective nematode management, these agents must remain in the soil for a prolonged period of time.
- Preparation of biocontrol formulation is challenging. High production costs, short shelf lives, and inconsistent results in the field all add to the challenge.

CONCLUSION:

Future studies should concentrate on the identification of strains with broad-spectrum activity. Omics study can provide a better understanding of the molecular mechanisms involved in biocontrol interactions, enabling researchers to develop effective management strategies for PPNs in agricultural systems. To ensure long-term sustainability and efficacy against PPNs, research evaluating the performance and adaptability of biocontrol agents across various climate scenarios will be crucial. Training programmes, seminars, and public campaigns can aid in bridging the knowledge gap and promote the adoption of biocontrol methods for PPNs management

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