MINIREVIEW

Bacteria used in the biological control of plant-parasitic nematodes: populations, mechanisms of action, and future prospects

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Received 6 November 2006; revised 19 April 2007; accepted 22 April 2007.
First published online July 2007.

DOI:10.1111/j.1574-6941.2007.00349.x

Editor: Peter Dunfield

Keywords
nematophagous bacteria; pathogenic mechanisms; molecular biological approaches; nematode management.

Abstract

As a group of important natural enemies of nematode pests, nematophagous bacteria exhibit diverse modes of action: these include parasitizing; producing toxins, antibiotics, or enzymes; competing for nutrients; inducing systemic resistance of plants; and promoting plant health. They act synergistically on nematodes through the direct suppression of nematodes, promoting plant growth, and facilitating the rhizosphere colonization and activity of microbial antagonists. This review details the nematophagous bacteria known to date, including parasitic bacteria, opportunistic parasitic bacteria, rhizobacteria, Cry protein-forming bacteria, endophytic bacteria and symbiotic bacteria. We focus on recent research developments concerning their pathogenic mechanisms at the biochemical and molecular levels. Increased understanding of the molecular basis of the various pathogenic mechanisms of the nematophagous bacteria could potentially enhance their value as effective biological control agents. We also review a number of molecular biological approaches currently used in the study of bacterial pathogenesis in nematodes. We discuss their merits, limitations and potential uses.

Introduction

Plant-parasitic nematodes cause serious crop losses worldwide and are among the most important agricultural pests (Koenning *et al.*, 1999). The management of nematodes is more difficult than that of other pests because nematodes mostly inhabit the soil and usually attack the underground parts of the plants (Stirling, 1991). Although chemical nematicides are effective, easy to apply, and show rapid effects, they have begun to be withdrawn from the market in some developed countries owing to concerns about public health and environmental safety (Schneider *et al.*, 2003). The search for novel, environmentally friendly alternatives with which to manage plant-parasitic nematode populations has therefore become increasingly important.

Nematodes in soil are subject to infections by bacteria and fungi. This creates the possibility of using soil microorganisms to control plant-parasitic nematodes (Mankau, 1980; Jatala, 1986). Bacteria are numerically the most abundant organisms in soil, and some of them, for example members of the genera *Pasteuria*, *Pseudomonas* and *Bacillus* (Emmert & Handelsman, 1999; Siddiqui & Mahmood, 1999; Meyer, 2003), have shown great potential for the biological control of nematodes. Extensive investigations have been conducted over the last twenty years to assess their potential to control plant-parasitic nematodes. These research efforts have found that nematophagous bacteria are distributed broadly, possess diverse modes of action, and have broad host ranges. A variety of nematophagous bacterial groups have been isolated from soil, host-plant tissues, and nematodes and their eggs and cysts (Stirling, 1991; Siddiqui & Mahmood, 1999; Kerry, 2000; Meyer, 2003). They affect nematodes by a variety of modes: for example parasitizing; producing toxins, antibiotics, or enzymes; interfering with nematode–plant-host recognition; competing for nutrients; inducing systemic resistance of plants; and promoting plant health (Siddiqui & Mahmood, 1999). These bacteria have a wide range of suppressive activities on different nematode species, including free-living and predatory nematodes as well as animal- and plant-parasitic nematodes (Mankau, 1980; Stirling, 1991; Siddiqui & Mahmood, 1999). They form a network with complex interactions among bacteria,

nematodes, plants and the environment to control populations of plant-parasitic nematodes in natural conditions (Kerry, 2000).

Understanding nematophagous bacterial populations and their mechanisms of action against nematodes at the molecular level will provide a basis for improving the pathogenic activity of potential biocontrol strains, for developing novel biological control strategies, and for exploring their roles in an integrated nematode management system. In this review we describe the known nematophagous bacteria and discuss their diverse mechanisms of action in reducing populations of nematodes. We pay specific attention to recent developments in the molecular biology of this group of bacteria. At present, based on their modes of action, the nematophagous bacteria include obligate parasitic bacteria, opportunistic parasitic bacteria, rhizobacteria, parasporal Cry protein-forming bacteria, endophytic bacteria, and symbiotic bacteria. This review also describes recent advances and molecular genetics approaches used in this field to facilitate future research.

Nematophagous bacteria and their modes of action against nematodes

Parasitic bacteria – *Pasteuria*

Taxonomy and host range of *Pasteuria*

Members of the genus *Pasteuria* are obligate, mycelial, endospore-forming bacterial parasites of plant-parasitic nematodes and water fleas (Sayre & Starr, 1985; Bekal *et al.*, 2001). A number of bacterial species in this genus have shown great potential as biocontrol agents against plant-parasitic nematodes. They occur worldwide and have been reported from at least 51 countries (Siddiqui & Mahmood, 1999). Members of the genus have been reported to infect 323 nematode species belonging to 116 genera, including both plant-parasitic nematodes and free-living nematodes (Chen & Dickson, 1998). The majority of economically important plant-parasitic nematodes have been observed to be parasitized (Bird *et al.*, 2003).

Pasteuria was first described as a protozoan and later classified into the bacterial genus *Bacillus* and then into *Pasteuria* (Sayre & Starr, 1985). At present, the taxonomy within the genus *Pasteuria* is based mainly on morphological and pathological characteristics, including the size and shape of sporangia and endospores, and ultrastructures, life cycles and host ranges (Atibalentja *et al.*, 2000). Over the last few years, a number of molecular biological analyses have been used in the identification and classification of this genus. Recent analysis of a portion of the 16S rRNA gene showed that the genes *Pasteuria* is a deeply rooted member of the *Clostridium–Bacillus–Streptococcus* branch of the Gram-positive Eubacteria (Anderson *et al.*, 1999). Charles *et al.* sequenced the genome of *Pasteuria panetrans*, performed amino acid-level analysis using concatenation of 40 housekeeping genes, and identified *Pas. penetrans* as ancestral to *Bacillus* spp. The results suggested that *Pas. penetrans* might have evolved from an ancient symbiotic bacteria associate of nematodes, possibly when the root-knot nematode evolved to a highly specialized parasite of plants (Charles, 2005; Charles *et al.*, 2005).

So far, four nominal *Pasteuria* species have been reported. Among them, *Pasteuria ramosa* has been described from water fleas (Ebert *et al.*, 1996). The other three nematode-infecting species are *Pas. penetrans*, which primarily parasitizes root-knot nematodes such as *Meloidogyne* spp.; *P. thornei*, which parasitizes root-lesion nematodes such as *Pratylenchus* spp.; and *Pas. nishizawae*, which occurs on cyst nematodes of the genera *Heterodera* and *Globodera* (Atibalentja *et al.*, 2000). Recently, based on morphological characteristics, host-specificity, and the analysis of 16S rRNA gene, Giblin-Davis *et al.* (2001, 2003) proposed that strain S-1, which parasitizes the sting nematode *Belonolaimus longicaudatus*, represents a novel *Pasteuria* species, Candidatus *Pasteuria usgae*.

Mechanisms of infection

Pasteuria penetrans infects the root-knot nematode *Meloidogyne* spp. (Fig. 1). Spores of *Pasteuria* can attach to the cuticles of the second-stage juveniles, and germinate after the juvenile has entered roots and begun feeding. The germ tubes can penetrate the cuticle, and vegetative microcolonies then form and proliferate through the body of the developing female. Finally, the reproductive system of the female nematode degenerates and mature endospores are released into the soil (Mankau *et al.*, 1976; Sayre & Wergin, 1977).

Attachment of the spores to the nematode cuticle is the first step in the infection process (Davies *et al.*, 2000). However, spores of individual *Pasteuria* populations do not adhere to or recognize all species of nematode. The spores of each *Pasteuria* species usually have a narrow host range. For example, *Pas. penetrans* infects *Meloidogyne* spp., *Pas. thornei* infects *Pratylenchus* spp., and *Pas. nishizawae* infects the genera *Heterodera* and *Globodera* (Gives *et al.*, 1999; Atibalentja *et al.*, 2000). The specificity of spore attachment to the nematode cuticle has been intensively studied using biochemical and immunological methods. Monoclonal antibody studies have revealed a high degree of heterogeneity both within and among different populations of *Pas. penetrans* (Davies & Redden, 1997). The distribution on the spore of any particular epitopes that are thought to be involved in adhesion may differ among populations and species (Davies & Redden, 1997; Davies *et al.*, 2000). The distribution of an adhesin-associated epitope on

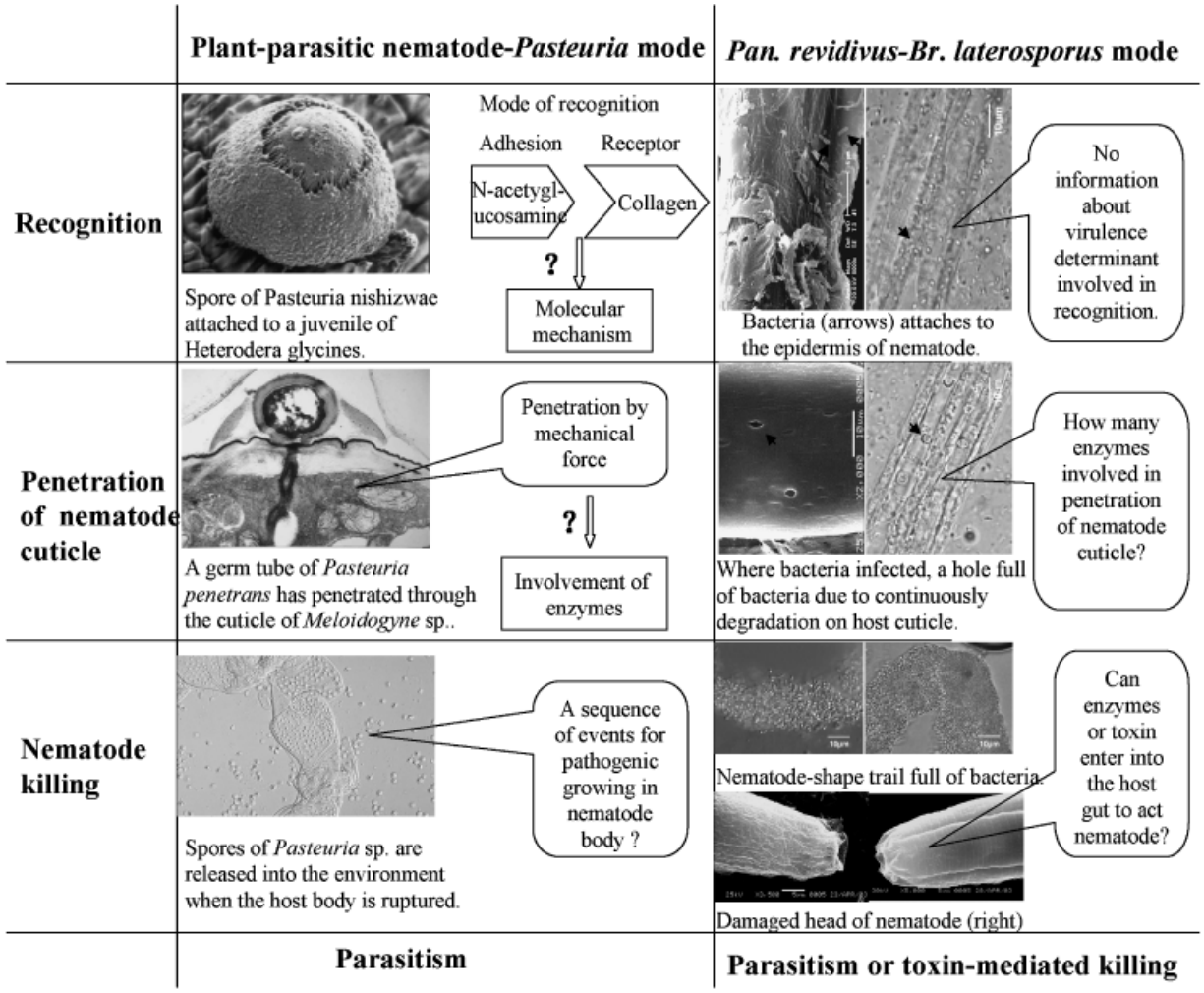

Fig. 1. Pathogenic mechanisms of typical bacterium–nematode interaction models (*Meloidogyne incognita–Pasteuria penetrans*; *Panagrellus redivius–Brevibacillus laterosporus*) (Mankau *et al.*, 1976; Sayre & Wergin, 1977; Morton *et al.*, 2004; Huang *et al.*, 2005).

polypeptides from different *Pasteuria* isolates provides an immunochemical approach to differentiating species and biotypes with specific host preferences (Preston *et al.*, 2003).

The processes associated with the initial binding of the endospores of *Pasteuria* spp. to their respective hosts have been explored by several laboratories (Stirling *et al.*, 1986; Persidis *et al.*, 1991; Davies & Danks, 1993; Charnecki *et al.*, 1996). These studies have led to a model in which a carbohydrate ligand on the surface of the endospore binds to a lectin-like receptor on the cuticle of the nematode host (Persidis *et al.*, 1991). The fibres surrounding the *Pasteuria* spore core are thought to be responsible for the adhesion of the spore to the host cuticle (Sayre & Wergin, 1977; Stirling *et al.*, 1986; Persidis *et al.*, 1991). Sonication can increase spore attachment by removing the sporangial wall and exposing the parasporal fibres (Stirling *et al.*, 1986). Removal of fibres from the bacterial surface was coupled with a loss of attachment ability, suggesting that the fibres are necessary for attachment (Persidis *et al.*, 1991). These fibres were shown to be beta-mercaptoethanol (BME)-soluble glycoproteins containing a high level of *N*-acetylglucosamine. *N*-acetylglucosamine, which is present on the spore surface, is thought to be involved in adhesion by interacting with a receptor on the nematode cuticle (Persidis *et al.*, 1991). The nature of the cuticle receptor(s) for *Pasteuria* adhesion is ambiguous. Persidis *et al.*, (1991) showed that collagen may be responsible for the recognition process, because cuticle components involved in attachment are sensitive to trypsin and endoglycosidase F, and because gelatin (denatured collagen) itself can inhibit spore attachment (Persidis *et al.*, 1991; Mohan *et al.*, 2001). However, the incubation of second-stage juveniles in the presence of

collagenases did not inhibit endospore attachment (Davies & Danks, 1993). Further molecular biological analysis of those cuticle components involved in attachment is required to elucidate the detailed mechanisms.

Genomic project for *Pasteuria*

Recently, a project was initiated to sequence the *Pas. penetrans* genome (Bird *et al.*, 2003; Davies *et al.*, 2003; Davies, 2005). Because *Pasteuria* species cannot be cultured in the laboratory, to obtain their genomic DNA, the endospores of *Pas. penetrans* were first collected from *Pas. penetrans*-infected root-knot nematode females. Genomic DNA of bacteria was then extracted (Waterman *et al.*, 2006). Four genomic libraries from the broad-host-range *Pas. penetrans* strain RES147 have been constructed (Bird *et al.*, 2003; Davies *et al.*, 2003; Davies, 2005). The estimated *Pasteuria* genome was less than 4.2 Mb. The initial sequence assembled into about 1500 contigs and covers more than 2.5 Mb of the *Pas. penetrans* genome sequence. The genome has a GC content of 62% (Bird *et al.*, 2003; Davies, 2005). Preliminary analysis has shown that more than 50% of the sequences have significant similarities (*e*-value $< 1.0\,e^{-10}$) to known genes in the NCBI database, and significant genomic colinearity has been observed between *Pas. penetrans* and *Bacillus subtilis* in large contiguous sequences (Bird *et al.*, 2003; Davies *et al.*, 2003; Davies, 2005). A complete genome sequence will extend our knowledge of the biology and evolution of *Pas. penetrans*. Further comparative genomics will provide an invaluable tool for developing this organism into a biological control agent, and will provide important insights into the basic biology of this bacterium, especially the manner in which it functions as a nematode pathogen.

Opportunistic parasitic bacteria

In 1946, Dollfus investigated and documented bacteria within the body cavity, gut, and gonads of nematodes (Jatala, 1986). Other reports have since suggested the association of some bacteria with the nematode cuticle. However, these studies were unable to specify whether these bacteria were parasites or saprophytes (Jatala, 1986). In fact, most nematophagous bacteria, except for obligate parasitic bacteria, usually live a saprophytic life, targetting nematodes as one possible nutrient resource. They are, however, also able to penetrate the cuticle barrier to infect and kill a nematode host in some conditions. They are described as opportunistic parasitic bacteria here, represented by *Brevibacillus laterosporus* strain G4 and *Bacillus* sp. B16.

As a pathogen, *Br. laterosporus* has been demonstrated to have a very wide spectrum of biological activities. So far, it has been reported that four nematode species (three parasitic nematodes, namely *Heterodera glycines*, *Trichostrongylus colubriformis* and *Bursaphelenchus xylophilus*, and the saprophytic nematode *Panagrellus redivius*) could be killed by various *B. laterosporus* isolates (Oliveira *et al.*, 2004; Huang *et al.*, 2005). Among these isolates, *Br. laterosporus* strain G4, which was isolated from soil samples in Yunnan province in China and parasitizes the nematodes *Panagrellus redivius* and *Bursaphelenchus xylophilus*, has been extensively studied (Huang *et al.*, 2005). After attaching to the epidermis of the host body, *Br. laterosporus* can propagate rapidly and form a single clone in the epidermis of the nematode cuticle. The growth of a clone can result in a circular hole shaped by the continuous degradation and digestion of host cuticle and tissue (Fig. 1). Finally, bacteria enter the body of the host, and digest all the host tissue as nutrients for pathogenic growth (Huang *et al.*, 2005).

During bacterial infection, the degradation of all the nematode cuticle components around the holes suggests the involvement of hydrolytic enzymes (Cox *et al.*, 1981; Decraemer *et al.*, 2003; Huang *et al.*, 2005). Histopathological observations and molecular biological analyses have demonstrated that major pathogenic activity could be attributed to an extracellular alkaline serine protease, designated BLG4 (Huang *et al.*, 2005; Tian *et al.*, 2006). The most compelling evidence to support the role of protease as virulence factor was derived from studying protease-deficient mutants (Tian *et al.*, 2006). The BLG4-deficient strain BLG4-6 was only 43% as effective as the wild-type strain at killing nematodes, and showed only 22% as much cuticle-degrading activity. These results also suggest that BLG4 is not the only virulence factor responsible for nematicidal activities, and that other factors such as other extracellular enzymes or toxins are probably involved (Huang *et al.*, 2005; Tian *et al.*, 2006).

Several bacterial proteases have been shown to be involved in the infection processes against nematodes (Table 1). Among these, the bacterial serine protease genes from nematophagous bacteria isolated from a different area in Yunnan (*Br. laterosporus* strain G4, *Bacillus* sp. B16, *Bacillus* sp. RH219 and other *Bacillus* strains) have been isolated and compared in our laboratory (Niu *et al.*, 2005; Tian *et al.*, 2006). The amino acid sequences of these bacterial cuticle-degrading proteases have shown high sequence identity (97–99%). The consistency of these pathogenic proteases from the different nematophagous bacterial strains suggests that proteases are highly conserved in this group of bacteria.

Similar to the nemotode-pathogenic proteases from nematophagous fungi, the protease BLG4 also belongs to the family of subtilases (Segers *et al.*, 1999). Comparison of the deduced amino acid sequence of the protease BLG4 gene with other cuticle-degrading proteases from pathogenic fungi showed lower similarities than the above comparisons

Table 1. Reported bacterial extracellular enzymes involved in pathogenesis against nematodes

Enzyme	Microbial origin	Gene ID	Nematode target	Effects on nematodes	References
Extracellular alkaline protease BLG4	*Brevibacillus laterosporus* G4	AY720895	*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	DQ983787	*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)	AY708655	*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)	AY708654	*Panagrellus redivius* and *Bursaphelenchus xylophilus*	LC50 of purified Bae16 on *Pan. redivius* within 24 h is 1.69 $\mu g\,mL^{-1}$; on *Bu. xylophilus* it is 2.26 $\mu g\,mL^{-1}$	Niu *et al.* (2006)
Protease aprA inhibited by EDTA	*Pseudomonas fluorescens* CHA0	AY644718	*Meloidogyne incognita*	The aprA-deficient strain showed significant reduced activity on *M. incognita* in the field tests	Siddiqui *et al.* (2005)
Clostridial collagenase (Type III)	*Clostridial* (Sigma Chemical)	Unspecified	Cuticle of *Caenorhabditis elegans*	Collagenase can degrade the struts between the inner and outer layers, and make the outer layer become faint	Cox *et al.* (1981)
Pronase	Unspecified (Worhington Biochemical Corp)	Unspecified	Cuticle of *Caenorhabditis elegans*	As above. Moreover, pronase can digest the cuticle lining of the pharynx	Cox *et al.* (1981)

(35%). However, a high degree of similarity between the sequences was found in regions containing the active site residues Asp_{32}, His_{64} and Ser_{221}. The two blocks of side-chains that form the sides of the substrate-binding pockets in these serine proteases were also conserved in BLG4 as $Ser_{125}Leu_{126}Gly_{127}Gly_{128}$, and $Ala_{152}Ala_{153}Gly_{154}$, respectively (Niu *et al.*, 2005; Tian *et al.*, 2006).

At present, the majority of research efforts on opportunistic nematode-parasitic bacteria have concentrated on understanding pathogenesis using free-living nematodes as targets. Such studies should allow us to identify new pathogenic factors, and to learn more about infectious processes in nematodes. It is important to understand the mechanism that control s the switch from saprotrophy to parasitism in order to formulate effective commercial nematode control agents.

Rhizobacteria

Rhizobacteria have also been studied for the biological control of plant-parasitic nematodes (Sikora, 1992). Aerobic endospore-forming bacteria (AEFB) (mainly *Bacillus* spp.) and *Pseudomonas* spp. are among the dominant populations in the rhizosphere that are able to antagonize nematodes (Rovira & Sands, 1977; Krebs *et al.*, 1998). Numerous *Bacillus* strains can suppress pests and pathogens of plants and promote plant growth. Some species are pathogens of nematodes (Gokta & Swarup, 1988; Li *et al.*, 2005). The most thoroughly studied is probably *Ba. subtilis* (Krebs *et al.*, 1998; Siddiqui & Mahmood, 1999; Lin *et al.*, 2001; Siddiqui, 2002). In addition, a number of studies have reported direct antagonism by other *Bacillus* spp. towards plant-parasitic nematode species belonging to the genera *Meloidogyne*, *Heterodera* and *Rotylenchulus* (Gokta & Swarup, 1988; Kloepper *et al.*, 1992; Madamba *et al.*, 1999; Siddiqui & Mahmood, 1999; Insunza *et al.*, 2002; Kokalis-Burelle *et al.*, 2002; Meyer, 2003; Giannakou & Prophetou-Athanasiadou, 2004; Li *et al.*, 2005). Rhizosphere *Pseudomonas* strains also exhibit diverse pathogenic mechanisms upon interaction with nematodes (Spiegel *et al.*, 1991; Kloepper *et al.*, 1992; Kluepfel *et al.*, 1993; Westcott & Kluepfel, 1993; Cronin *et al.*, 1997a; Kerry, 2000; Jayakumar *et al.*, 2002; Siddiqui & Shaukat, 2002, 2003; Andreogloua *et al.*, 2003; Siddiqui *et al.*, 2005). The mechanisms employed by some *Pseudomonas* strains to reduce the plant-parasitic nematode population have been studied. These mechanisms include the production of antibiotics and the induction of systemic resistance (Spiegel *et al.*, 1991; Cronin *et al.*, 1997a; Siddiqui & Shaukat, 2002, 2003). Other rhizobacteria reported to show antagonistic effects against nematodes include members of the genera *Actinomycetes*, *Agrobacterium*, *Arthrobacter*, *Alcaligenes*, *Aureobacterium*, *Azotobacter*, *Beijerinckia*, *Burkholderia*, *Chromobacterium*,

Clavibacter, *Clostridium*, *Comamonas*, *Corynebacterium*, *Curtobacterium*, *Desulforibtio*, *Enterobacter*, *Flavobacterium*, *Gluconobacter*, *Hydrogenophaga*, *Klebsiella*, *Methylobacterium*, *Phyllobacterium*, *Phingobacterium*, *Rhizobium*, *Serratia*, *Stenotrotrophomonas* and *Variovorax* (Jacq & Fortuner, 1979; Kloepper *et al.*, 1991, 1992; Racke & Sikora, 1992; Guo *et al.*, 1996; Cronin *et al.*, 1997b; Duponnois *et al.*, 1999; Neipp & Becker, 1999; Siddiqui & Mahmood, 1999, 2001; Jonathan *et al.*, 2000; Tian & Riggs, 2000; Tian *et al.*, 2000; Meyer *et al.*, 2001; Mahdy *et al.*, 2001a; Hallmann *et al.*, 2002; Insunza *et al.*, 2002; Khan *et al.*, 2002; Mena & Pimentel, 2002; Meyer, 2003).

The rhizobacteria usually comprise a complex assemblage of species with many different modes of action in the soil (Siddiqui & Mahmood, 1999). Rhizobacteria reduce nematode populations mainly by regulating nematode behaviour (Sikora & Hoffmann-Hergarten, 1993), interfering with plant–nematode recognition (Oostendorp & Sikora, 1990), competing for essential nutrients (Oostendorp & Sikora, 1990), promoting plant growth (El-Nagdi & Youssef, 2004), inducing systemic resistance (Hasky-Günther *et al.*, 1998), or directly antagonising by means of the production of toxins, enzymes and other metabolic products (Siddiqui & Mahmood, 1999).

Most rhizobacteria act against plant-parasitic nematodes by means of metabolic by-products, enzymes and toxins. 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). Ammonia produced by ammonifying bacteria during decomposition of nitrogenous organic materials can result in reduced nematode populations in soil (Rodriguez-Kabana, 1986). *Pseudomonas fluorescens* controlled cyst nematode juveniles by producing several secondary metabolites such as 2,4-diacetylphloroglucinol (DAPG) (Cronin *et al.*, 1997a; Siddiqui & Shaukat, 2003). Mena *et al.* reported that *Corynebacterium paurometabolu* inhibited nematode egg hatching by producing hydrogen sulphide and chitinase (Mena & Pimentel, 2002). Some other rhizobacteria reduce deleterious organisms and create an environment more favourable for plant growth by producing compounds such as antibiotics or hydrogen cyanide (Zuckerman & Jasson, 1984).

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). Bacterial determinants of ISR include lipopolysaccharides (LPSs), siderophores and salicylic acid (SA) (Van Loon *et al.*, 1998; Ramamoorthy *et al.*, 2001). *Rhizobium etli* G12 has been repeatedly demonstrated to be capable of suppressing early infection by the potato cyst nematode *Globodera pallida* and the root-knot nematode *Meloidogyne incognita* (Hallmann *et al.*, 2001). LPS was identified as an inducing agent of the systemic resistance. The mechanism involved in resistance development seems to be directly related to nematode recognition and penetration of the root (Reitz *et al.*, 2000, 2001; Mahdy *et al.*, 2001b). However, Siddiqui *et al.* (Siddiqui & Shaukat, 2004) found that SA-negative or SA-overproducing mutants induced systemic resistance to an extent similar to that caused by the wild-type bacteria in tomato plants. They concluded that fluorescent pseudomonads induced systemic resistance against nematodes by means of a signal transduction pathway, which is independent of SA accumulation in roots.

Except for the nematophagous fungi and actinomycetes, rhizobacteria are the only group of microorganisms in which biological nematicides have been reported. Deny is a commercial biocontrol nematode product based on a natural isolate of the bacterium *Burkholderia cepacia*. This bacterium has been shown to reduce egg hatching and juvenile mobility (Meyer & Roberts, 2002). There are two commercial bionematicidal agents based on *Bacillus* species. Through a PGPR research program of the ARS (Agriculture Research Service, USA), a commercial transplant mix (Bio Yield^{TM}, Gustafson LLC) containing *Paenobacillus macerans* and *Bacillus amyloliquefaciens* has been developed to control plant-parasitic nematodes on tomato, bell pepper and strawberry (Meyer, 2003). Another product, used in Israel, is BioNem, which contains 3% lyophilized *Bacillus firmus* spores and 97% nontoxic additives (plant and animal extracts) to control root-knot nematodes as well as other nematodes (Giannakou & Prophetou-Athanasiadou, 2004). In extensive testing on vegetable crops (tomato, cucumber, pepper, garlic and herbs), BioNem preplant applications significantly reduced nematode populations and root infestation (galling index), resulting in an overall increase in yield (Giannakou & Prophetou-Athanasiadou, 2004). BioNem showed a higher effectiveness against root-knot nematodes in the field than did *Pas. penetrans*. However, the excellent biocontrol effects of BioNem can be partially attributed to the stimulating effect that the animal and plant additives contained in the bio-nematicide formulation have on the microbial community of the rhizosphere. Previous studies have shown that the addition of manure or other organic amendments stimulate the activity of the indigenous soil

microbial community (Giannakou & Prophetou-Athanasiadou, 2004).

Cry protein-forming bacteria

Bacillus thuringiensis (Bt) produces one or more parasporal crystal inclusions (Cry or δ-endotoxins), which are known to be toxic to a wide range of insect species in the orders Lepidoptera (butterflies and moths), Diptera (flies and mosquitoes), Coleoptera (beetles and weevils) and Hymenoptera (wasps and bees) (Schenpe *et al*., 1998; Maagd *et al*., 2001). Some Cry proteins are also toxic to other invertebrates such as nematodes, mites and protozoans (Feitelson *et al*., 1992). To date, there are six Cry proteins (Cry5, Cry6, Cry12, Cry13, Cry14, Cry21) known to be toxic to larvae of a number of free-living or parasitic nematodes (Alejandra *et al*., 1998; Crickmore *et al*., 1998; Marroquin *et al*., 2000; Wei *et al*., 2003; Kotze *et al*., 2005). On the basis of amino acid sequence homology, these nematode-affecting Cry proteins (except for Cry6A) were assigned to a single cluster in the main Cry lineage, parallel to other main groups (Bravo, 1997; Marroquin *et al*., 2000). Separate phylogenetic analysis of the three domains of Cry protein also generated a consensus tree result. The domain I and domain II trees showed that nematode-specific toxins (Cry5, Cry12, Cry13, Cry14 and Cry21) were arranged together in a single branch (Bravo, 1997). Domain III from all the nematode-specific toxin trees are also clustered together (Bravo, 1997).

Nematicidal and insecticidal toxins of Bt are believed to share similar modes of action. Cry protein exerts its effects by forming lytic pores in the cell membrane of gut epithelial cells (Crickmore, 2005). After ingestion of toxin by target nematode larvae, the crystals dissolve within the gut of the nematode, and this is followed by proteolytic activation (Crickmore, 2005). Cry toxicity is directed against the intestinal epithelial cells of the midgut and leads to vacuole and pore formation, pitting, and eventual degradation of the intestine (Marroquin *et al*., 2000). The binding of pore-forming toxin to a receptor in the epithelial cell is a major event. In order to determine host receptors, a mutagenesis screen was performed with the genetically well-characterized nematode *Caenorhabditis elegans*. This screen obtained five *bre* mutants that failed to internalize toxin because they lacked the receptor. The *bre* gene encodes a glycosyltransferase, which is responsible for synthesizing a carbohydrate receptor glycolipid. Convincing evidence exists for the involvement of a set of glycolipids as receptors of Bt toxins (Huffman *et al*., 2004; Crickmore, 2005). A detailed understanding of how the Bt toxins interact with nematodes should facilitate the production of more effective Bt biocontrol agents.

Other than Cry toxin, previous studies using *Ba. thuringiensis* israelensis, *Ba. thuringiensis* kurstaki and another parasporal crystal-forming bacterium, *Bacillus sphaericus,* showed that some strains had significant activity on the eggs and larvae of the parasitic nematode *Trichostrongylus colubriformis* (Bottjer *et al*., 1986; Bowen *et al*., 1986a, b; Bowen & Tinelli, 1987; Meadows *et al*., 1989). The toxicities of these strains were inhibited by antibiotics and did not correspond to the sporulation phase of the bacteria, nor to their resistance to alkaline pH and heat, demonstrating that the pathogenic factors were not the parasporal crystal (Bottjer *et al*., 1986; Bowen *et al*., 1986a, b; Bowen & Tinelli, 1987; Meadows *et al*., 1989). Subsequently, an unknown Bt isolate was also reported to have toxicity to root-lesion nematodes (Bradfish *et al*., 1991). However, the pathogenic factors of this strain have not been discovered.

Other nematophagous bacterial groups

Endophytic bacteria

Endophytic bacteria have been found internally in root tissue, where they persist in most plant species. They have been found in fruits and vegetables, and are present in both stems and roots, but do no harm to the plant (McInory & Kloepper, 1995; Hallmann *et al*., 1997, 1999; Azevedo *et al*., 2000; Hallmann, 2001; Surette *et al*., 2003). They have been shown to promote plant growth and to inhibit disease development 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 example, Munif *et al*., (2000) screened endophytic bacteria isolated from tomato roots under greenhouse conditions. They found antagonistic properties towards *M. incognita* in 21 out of 181 endophytic bacteria. Several bacterial species have also been found to possess activity against root-lesion nematode (*Pratylenchus penetrans*) in soil around the root zone of potatoes. Among them, *M. esteraomaticum* and *K. varians* have been shown to play a role in root-lesion nematode suppression through the attenuation of host proliferation, without incurring any yield reduction (Munif *et al*., 2000). Despite their different ecological niches, rhizobacteria and endophytic bacteria display some of the same mechanisms for promoting plant growth and controlling phytopathogens, such as competition for an ecological niche or a substrate, production of inhibitory chemicals, and induction of systemic resistance (ISR) in host plants (Hallmann, 2001; Compant *et al*., 2005).

Symbionts of entomopathogenic nematodes

Xenorhabdus spp. and *Photorhabdus* spp. are bacterial symbionts of the entomopathogenic nematodes *Steinernema* spp. and *Heterorhabdus* spp., respectively (Paul *et al*., 1981). They have been thought to contribute to the symbiotic

association by killing the insect and providing a suitable nutrient environment for nematode reproduction (Boenare *et al.*, 1997). In recent years, a potentially antagonistic effect of the symbiotic complex on plant-parasitic nematodes has been reported (Bird & Bird, 1986; Grewal *et al.*, 1997, 1999; Perry *et al.*, 1998; Lewis *et al.*, 2001). Further investigation demonstrated that the symbiotic bacteria seemed to be responsible for the plant-parasitic nematode suppression via the production of defensive compounds (Samaliev *et al.*, 2000). To date, three types of secondary metabolites have been identified as the nematicidal agent: ammonia, indole and stilbene derivative (Hu *et al.*, 1995, 1996, 1997, 1999). They were toxic to second-stage juveniles of root-knot nematode (*M. incognita*) and to fourth-stage juveniles and adults of pine-wood nematode (*Bu. xylophilus*), and inhibited egg hatching of *M. incognita* (Hu *et al.*, 1999).

Understanding bacterial pathogenesis in nematodes at the molecular level

Molecular genetic techniques used in studying bacterial pathogenesis in nematodes

A number of bacteria have been shown to exhibit a variety of effects on nematodes in natural environments and laboratory conditions. However, studies on the mechanisms of bacterial pathogenicity have lagged behind those assessing their roles in biological control and resource potential. Over the past few years, a number of molecular genetic methods in bacterial pathogenicity have been developed, and it is now possible to introduce these successful techniques to the study of bacterial pathogenesis in plant-parasitic nematodes (Hensel & Holden, 1996; Aballav & Ausube, 2002; Tan, 2002; Barker, 2003). We briefly summarize these methods here, and review their merits and limitations (Table 2). Although some technologies have been reported not to be successful in studying plant-parasitic nematodes, knowledge from studying bacterial pathogens of *C. elegans* and other animal pathogens may enhance knowledge of bacterial pathogenesis in plant-parasitic nematodes, and provide a basic methodology for studies on plant-parasitic nematodes.

Reverse genetics is a common approach in identifying and determining functions of virulence determinants. This method involves the isolation of virulence proteins involved in pathogenicity, and cloning of the corresponding genes. The functions of virulence proteins are further confirmed by their expression in other organisms, by the inactivation of the gene in a wild-type strain, or by immunological techniques (Huang *et al.*, 2005; Tian *et al.*, 2006). For example, studies on the bacterial proteases of *Br. laterosporus* G4 serving as pathogenic factors in nematode infection used reverse genetics methods (Huang *et al.*, 2005; Tian *et al.*,

Table 2. Available molecular biology approaches for studying nematophagous bacteria

Approach	Advantages	Disadvantages	Successful examples in studies of bacterial pathogenesis in nematodes	References
Reverse genetics	It is easy to identify and determine the important virulence factors	It is labour- and time-consuming and difficult to isolate minor toxins	The proteases serving as factors have been isolated, and their actions against nematodes identified	Huang *et al.* (2005), Tian *et al.* (2006), Niu *et al.* (2005, 2006)
Directed mutagenesis	Directly clarifies roles of suspected virulence determinants	Need information about suspected virulence determinants	Constructed aprA-deficient strains show significantly reduced activity on *M. incognita* in the field tests	Siddiqui *et al.* (2005)
Random mutagenesis	No assumptions have to be made about the roles of specific genes	Screening is labour-intensive and requires a tractable molecular genetic system	A mutant library of *Pseudomonas* sp. BG33R generated five mutants lacking ovicidal activity	Wechter *et al.* (2001, 2002)
Genomic sequences comparison	No complex genetics manipulation. Rapid cloning	Requires complete genomic sequences of target pathogen and non-pathogens	Comparison of partial genomic sequence of *Pasteuria* with *Bacillus* showed significant colinearity in larger contiguous sequences	Bird *et al.* (2003), Davies *et al.* (2003), Davies (2005), Hensel & Holden (1996)
In vivo expression technology (IVET)/differential fluorescence induction (DFI)	Both can sensitively and directly monitor gene expression during infection. DFI is more sensitive	Requires a tractable genetics system and further experiments to verify the roles of obtained genes. Moreover, some virulence expressed *in vitro* may be missed	–	Hensel & Holden (1996), Valdivia & Ramakrishnan (2000)
Subtractive hybridization/ different display	Both can directly monitor and measure gene expression during infection. Subtractive hybridization is more sensitive	Requires further experiments to verify the roles of obtained genes. Moreover, some virulence expressed *in vitro* may be missed	–	Harakava & Gabriel (2003), Ogawa *et al.* (2000)

2006). However, the path of discovery from proteins to genes is very labour-intensive.

Mutational analysis is another popular technique for identifying pathogenic determinants. This tool can be divided into directed and random mutagenesis. In directed mutagenesis, a putative virulence determinant encoding a gene postulated to be responsible for a certain pathogenic trait is disrupted or replaced to construct a mutant strain. The mutant and the wild-type strain are then compared to determine the importance of the suspected virulence determinant. Siddiqui *et al.* (2005) constructed mutants of the Gac-controlled *aprA*, which encodes a major extracellular protease in *Ps. fluorescens* CHA0, by inserting a suicide plasmid into the site of the chromosomal *aprA* gene. The mutant showed significantly reduced biocontrol activity against *M. incognita* during tomato and soybean infection (Siddiqui *et al.*, 2005). Much current research is instead, however, based on the use of random mutagenesis. *Pseudomonas* sp. BG33R can suppress multiplication of *M. xenoplax* and inhibit egg hatching. To investigate the pathogenic factors, Wechter *et al.* (2001, 2002) utilized Tn5 transposon-mediated mutagenesis to construct a mutant library and generate five BG33R mutants that lacked ovicidal activity. ORF analysis and amino acid comparative database searches of the Tn5 insertion sites in the five mutants revealed a high degree of homology to several putative regulatory genes (Wechter *et al.*, 2001, 2002). It is time-consuming to identify a mutant with attenuated virulence within a large population of mutants. In future, signature-tagged mutagenesis (STM) may be introduced to allow mutants to be differentiated from each other by the tagging of a unique sequence for every individual transposon (Hensel *et al.*, 1995).

Comparative genomics can identify pathogenic genes by comparing genomic sequences of pathogenic and nonpathogenic strains, or other sequences from strains of interest of the same genus. Similarly, a genomic-bioinformatic approach might further define the evolutionary relationships among the various pathogenic and nonpathogenic bacteria (Hensel & Holden, 1996). For example, a comparison of the genomes of the obligate nematode parasite *Pas. penetrans* with those of other closely related bacteria, such as *Bacillus anthracis* and *Bacillus cereus* (facultative mammalian pathogen), and *Bacillus haladurans* and *Ba. subtilis* (free-living), have shown significant colinearity in larger contiguous sequences among these species. Amino acid level analysis using concatenation of 40 housekeeping genes revealed that *Pas. penetrans* is more closely related to the saprophytic species *Ba. haladurans* and *Ba. subtilis* than to the pathogenic species *Ba. anthracis* and *Ba. cereus* (Bird *et al.*, 2003; Preston *et al.*, 2003; Charles, 2005; Charles *et al.*, 2005; Davies, 2005). A genomic-bioinformatic approach will also be useful for studying the processes of host recognition and attachment. Collagen is a filamentous protein that contains a G-x-y repeated structure. These proteins were thought to be restricted to animals; however, collagen-like proteins were recently identified in the genome of *Pas. penetrans*. They are similar to those in other species of bacilli, and are likely to be responsible for endospore attachment. Four separate nucleotide sequences, Pcl.C1, Pcl.C336, Pcl.C374 and Pcl.C384, were identified in the *Pas. penetrans* genome. Other proteins containing collagen-like sequences from other bacilli were obtained from the NCBI public database. A preliminary analysis of these collagens has shown that *Pasteuria* collagens are most closely related to *Ba. thuringiensis* and *Ba. cereus* collagens rather than to those in *Ba. anthracis* (Charles, 2005; Davies, 2005; Davies & Opperman, 2006).

Some techniques have not yet been employed in the study of bacterial infection against plant-pathogenic nematodes but may prove useful, for example *in vivo* expression technology (IVET), differential fluorescence induction (DFI), subtractive hybridization and differential display etc. All these techniques are able to monitor bacterial gene expression during infection in a living organism. IVET has allowed the identification of hundreds of *in vivo* induced (*ivi*) genes in bacterial pathogens (Hensel & Holden, 1996). The DFI technique can be applied to more complex environments for easy isolation of GFP-expressing bacteria. However, these approaches may miss some virulence genes whose promoters do not express during certain stages of infection, or genes that are expressed only *in vitro* (Valdivia & Ramakrishnan, 2000). Subtractive hybridization and differential display approaches are techniques based on the comparison of mRNA profiles (Ogawa *et al.*, 2000; Harakava & Gabriel, 2003). The ability to synthesize cDNA from RNA populations isolated from infected hosts permits differential screening to identify genes that are specifically expressed during infection.

The subtractive hybridization and differential display approaches that have been developed have been used to study nematophagous fungi. Recently, Ahrén *et al.* (2005) compared the gene expression patterns in traps and in the mycelium of the nematode-trapping fungus *Monacrosporium haptotylum* . Despite the fact that the knobs and mycelium were grown in the same medium, there were substantial differences in the patterns of genes expressed in the two cell types. A number of the genes that were differentially expressed in trap cells are known to be regulated during the development of infection structures in plant-pathogenic fungi (Ahrén *et al.*, 2005). Therefore, the techniques used to differentiate bacterial gene expression during infection are useful tools for studying stage-specific functional genes. For example, studies on the infection processes of nematodes revealed that a series of enzymes such as protease, collagenase, chitinase, lipase etc. are

involved in bacterial penetration of the nematode cuticle (Cox *et al.*, 1981; Morton *et al.*, 2004; Huang *et al.*, 2005). However, which enzymes are involved in infection and when these pathogenic factors are expressed remain largely unknown. IVET, DFI and subtractive hybridization and differential display are appealing methods to answer these questions owing to their ability to monitor gene expression during infection or directly to measure transcription levels of genes.

Developing available models for studying bacterial pathogenesis in plant-parasitic nematodes

At present there is limited knowledge of the genetics of the interactions between nematode hosts and their pathogens. It is necessary to develop an alternative model for obligate bacterial parasites to understand bacterial pathogenesis in plant-parasitic nematodes at a molecular level. Unlike *Pasteuria*, opportunistic parasitic bacteria can be easily cultured and manipulated for genetic studies, so they can be used as models to gain an understanding of bacterial infection processes in nematodes. During a study of the infection of *Br. laterosporus* against freeing-living nematodes (*Panagrellus redivius*) and pine-wood nematodes (*Bu. xylophilus*), the extracellular protease BLG4 that served as a pathogenic factor during infection was first identified using the free-living nematode *Pan. redivius* as a model. Subsequently, its role in infection against the parasitic nematode *Bu. xylophilus* was confirmed, indicating that it is feasible to identify pathogenic factors and define their roles in the infection of plant-parasitic nematodes using an easily tractable *Br. Laterosporus–Pan. redivius* model (Huang *et al.*, 2005; Tian *et al.*, 2006). Furthermore, *Br. laterosporus* strain G4 and its spores can also attach to nematode cuticles. *Brevibacillus laterosporus–Pan. redivius* could be used as a model to understand the recognition mechanism between *Pasteuria* spores and parasitic nematode cuticles in future research.

Another reference for plant-parasitic nematode–pathogen interactions is the use of *C. elegans* as a high-throughput screening model to facilitate the identification of virulence determinants (Davies, 2005). To date, there are some 20 species of bacteria that are known to be pathogens of *C. elegans,* of which six are Gram-positive and the remainder are Gram-negative (Couillault & Ewbank, 2002; Ewbank, 2002). *Caenorhabditis elegans* is currently being used as a model for defining bacterial virulence factors and nematode defence response factors (Ewbank, 2002; Gravato-Nobre & Hodgkin, 2005; Gravato-Nobre *et al.*, 2005). During the identification of the bacterial virulence factors that are required for the killing of *C. elegans* by the human opportunistic pathogen *P. aeruginosa* PA14, a random insertion library was generated using Tn5-based transposon mutagenesis. Following mutagenesis, mutants were analysed either individually or in pools for attenuated or increased virulence. By this means, five structural genes involved in 'fast killing' and eight involved in 'slow killing' were identified (Aballav & Ausube, 2002; Tan, 2002).

The major question is whether the pathogenic factors identified in these models can be used to explain pathogenesis in plant-parasitic nematodes. Until recently, it was believed that animals did not share similar virulence factors. However, the existence of a universal virulence factor has been clearly demonstrated in the case of *P. aeruginosa*. Among eight bacterial mutants with reduced pathogenicity against *C. elegans*, six in an insect model and seven in a mouse model also showed attenuated virulence (Couillault & Ewbank, 2002). Moreover, the enzyme-mediated infection in the *Br. Laterosporus–Pan. redivius* model has been extensively studied and confirmed to be similar to the fungal penetration of plant-parasitic nematode cuticles. It is therefore feasible to understand pathogenic mechanisms in plant-parasitic nematodes using tractable models such as the *Br. Laterosporus–Pan. redivius* model or the bacterium–*C. elegans* model. These models of pathogenicity have been intensively studied, including the stages of attraction and attachment between bacteria and their hosts, entry into the host through nematode stoma or penetration of the nematode body wall, and parasitism or toxin-mediated host death (Fig. 1) (Sayre & Wergin, 1977; Tan, 2002; Huang *et al.*, 2005).

Conclusion

Over the past twenty years a large number of studies have been undertaken to investigate the use of microorganisms as biocontrol agents against nematode pests. More and more bacteria have been identified as pathogens of plant-parasitic nematodes and have shown suppression effects on nematode pest populations (Table 3). However, only a few commercial biocontrol products from the bacteria with nematicidal potentials have been developed (http://www.oardc.ohio-state.edu/apsbcc/productlist.htm) and used in the agriculture system (Whipps & Davies, 2000; Gardener, 2004; Schisler *et al.*, 2004). The development of biocontrol agents is often unpredictable and too variable for large-scale implementation (Meyer, 2003). No matter how well suited a commercial nematode antagonist is to a target host in a laboratory test, in order to realize ideal biocontrol effects in practice an intensive exploration of the mechanisms of the antagonist against nematode populations, and a thorough understanding of the interactions among biocontrol strains, nematode target, soil microbial community, plant and environment must be developed.

Table 3. Reported bacterial groups with pathogenic activity against nematodes

Nematophagous bacterial group	Genus and species	Target nematodes	Pathogenic effects on nematodes	Action mode	References
Parasitic bacteria	Four species: *Pasteuria. penetrans*; *P. thornei*; *P. nishizawae;* Candidatus *Pasteuria usgae*	323 nematode species of 116 genera	Major economic important plant-parasitic nematodes have been observed to be parasitized by *Pasteuria*	Parasitism	Siddiqui & Mahmood (1999), Bekal *et al.* (2001), Chen & Dickson (1998), Bird *et al*. (2003), Giblin-Davis *et al*. (2003, 2001)
Opportunistic parasitic bacteria	*Bacillus nematocida* (*Bacillus* sp. B16); *Brevibacillus laterosporus*. *Bacillus* sp. RH219 etc	*Panagrellus redivius* and *Bursaphelenchus xylophilus*	*Br. laterosporus* strain G4 could penetrate the nematode (*Pan. redivius* and *Bu. xylophilus*) cuticles and eventually digest the target organism in the laboratory	Parasitism, production of enzymes and toxin	Huang *et al.* (2005), Tian *et al*. (2006, 2007), Niu *et al.* (2005, 2006)
Rhizobacteria	Distribution in more than 29 genera. *Bacillus* (more than 15 species) and *Pseudomonas* (more than 11 species) are two of the most dominant populations	Reduce nematode populations in soil	Different rhizobacteria showed different degrees of suppression on nematodes in various conditions. Three commercial bionematicides from bacteria all belong to this group	Interfering with recognition, production of toxin, nutrient competition, plant-growth promotion; induction of systemic resistance	Siddiqui & Mahmood (1999, 2001), Meyer (2003), Insunza *et al*. (2002), Kloepper *et al*. (1992), Jonathan *et al*. (2000), Mahdy *et al.* (2001a), Cronin *et al*. (1997b), Tian & Riggs (2000), Tian *et al*. (2000), Guo *et al*. (1996), Duponnois *et al*. (1999), Mena & Pimentel (2002), Meyer *et al.* (2001), Khan *et al*. (2002), Racke & Sikora (1992), Kloepper *et al.* (1991), Neipp & Becker (1999), Hallmann *et al.* (2002), Jacq & Fortuner (1979)
Parasporal crystal-forming bacteria	*Bacillus thuringiensis* (Cry5, Cry6, Cry12, Cry13, Cry14, Cry21)	*Trichostrongylus colubriformis*; *Caenorhabditis elegans*; and *Nippostrongylus brasiliensis*	These Cry proteins showed toxicity to larval stages of free-living and parasitic nematodes	Cry proteins caused damage to the intestines of nematodes	Kotze *et al.* (2005), Wei *et al*. (2003), Marroquin *et al.* (2000), Crickmore *et al.* (1998)
Endophytic bacteria	The majority of rhizobacteria can also be identified as endophytic bacteria	Root-knot nematode and root-lesion nematode etc	Suppress root-knot nematodes and root-lesion nematode etc	Rhizobacteria and endophytic bacteria use some of the same mechanisms	Hallmann (2001), Shaukat *et al*. (2002), Munif *et al.* (2000), Sturz & Kimpinski (2004), Compant *et al*. (2005)
Symbiontic bacteria of entomopathogenic nematodes	Two genera: *Xenorhabdus*. and *Photorhabdus*	*Bursaphelenchus xylophilus*; *M. incognita* and their eggs	Toxic to juveniles of root-knot and pine-wood nematodes and inhibit egg hatch	Toxin production (ammonia, indole and stilbene derivative)	Bird & Bird (1986), Grewal *et al*. (1999, 1997), Lewis *et al*. (2001), Perry *et al*. (1998), Samaliev *et al.* (2000), Hu *et al.* (1995, 1996, 1997, 1999)

Recently, interactions among the microorganism, nematode target, plant and environment have been well reviewed and emphasized (Kerry, 2000; Barker, 2003; Davies, 2005; Dong & Zhang, 2006). Sustainable working methodologies have been proposed, including integrated pest management (IPM). The goal of IPM is to combine biocontrol and other methods, such as green manure, organic or inorganic soil amendments, resistant plant cultivars, hot-water treatment and crop rotation, so that they act synergistically on nematodes through the direct suppression of nematodes, promotion of plant growth, and facilitation of rhizosphere colonization and activity of the microbial antagonists (Akhtar, 1997; Barker & Koenning, 1998; Meyer & Roberts, 2002; Barker, 2003). For this goal to be achieved, however, accurate knowledge is needed of the ecology, biology, and mechanisms of action of the populations of nematophagous bacteria.

An increased understanding of the molecular basis of the various bacterial pathogenic mechanisms on nematodes not only will lead to a rational nematode management decision, but also could potentially lead to the development of new biological control strategies for plant-parasitic nematodes. For example, it has been recognized that the attraction between bacteria and their hosts is governed by chemotactic factors emanating from the hosts or pathogens (Zuckerman & Jasson, 1984). Knowledge of these mechanisms could be used to attract or target nematodes intentionally by modified nematicidal bacteria or to regulate nematode populations by the chemotactic factors produced by these nematophagous bacteria.

Advances in molecular biology have allowed us to obtain important information concerning molecular mechanisms of action, such as the production of nematotoxins, the signalling pathways that induce the host-plant defence mechanism, and the infection process. Such information should provide novel approaches to improving the efficacy of nematophagous bacteria for biological control applications, to increasing the expression of toxins or enzymes from the microorganisms, and to formulating commercial nematicidal agents. For example, the developing genomic-bioinformatic approach may help to solve the difficulty of culturing the nematode parasite *Pasteuria in vitro*. This may allow mass-production of spores for commercial use.

Acknowledgements

We thank Professor Jianping Xu (McMaster University, Canada), Dr Peter Dunfield and the reviewers for their help and invaluable advice in preparing this manuscript. The work was funded by projects from National Natural Science Foundation (approved nos 30500338, 30630003) and the Department of Science of Technology of Yunnan Province (approval nos 2005NG05, 2005NG03).

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