Chapter 13
Bacteria in the Management of Plant-Parasitic Nematodes

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13.1 Introduction

Nematodes are one of the most important constraints to crop productivity and cause 12 % annual loss in the yield of important food and fiber crops on a worldwide basis (Sasser and Freckman 1987; Barker et al. 1994). The control of nematode is far more difficult than any other kind of pest because they inhabit soil and usually attack the underground parts of plants. On account of eco-friendly plant protection drive, great emphasis has been given to the exploitation of potential bioagents for controlling nematodes. Soils being a complex environment, housing various flora and fauna, nematodes are generally exposed to many enemies. The most widely found enemies are fungi and bacteria, of which bacteria as a bioagent have several advantages, followed by fungi. Not only is bacterium eco-friendly, but it also takes a long time to develop resistance. Besides, biotechnological interventions for evolving efficient strains are possible in the organism as they possess a simple genome.

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 and Handelsman 1999; Siddiqui and Mahmood 1999; Meyer 2003), have shown great potential for the biological control of nematodes. Extensive investigations have been conducted over the last few years to assess their potential to control plant-parasitic nematodes.

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13.2 Bacteria in Nematode Control

Bacterial antagonists of plant-parasitic nematodes are grouped under the following categories: obligate parasites, antagonistic bacteria, and other soil bacteria.

13.2.1 Obligate Parasites

An obligate parasite is a parasitic organism that cannot live independently of its host. Members of the genus *Pasteuria* are obligate parasites of plant-parasitic nematodes. *Pasteuria penetrans* is a mycelial, endospore-forming, bacterial parasite that has shown great potential as a biological control agent of root-knot nematodes. Considerable progress has been made during the last 10 years in understanding its biology and importance as an agent capable of effectively suppressing root-knot nematodes in field soil. The biological control potential of *Pasteuria* spp. has been demonstrated on 20 crops; host nematodes include *Belonolaimus longicaudatus*, *Heterodera* spp., *Meloidogyne* spp., and *Xiphinema diversicaudatum*. The potential of predacious and nematoxic fungi and bacteria for the biological control of nematode parasites may offer a cheaper and more sustainable approach to reducing the damage caused by phytonematodes.

13.2.1.1 *Pasteuria penetrans*

Members of the genus *Pasteuria* are obligate, mycelial, endospore-forming bacterial parasites of plant-parasitic nematodes and water fleas (Sayre and 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 and Mahmood 1999).

Taxonomy and Host Range of *Pasteuria penetrans*

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 and 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 and 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 genus

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 *Pas. penetrans*, 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.; *Pas. 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

The life cycle of *Pas. penetrans* is completed in four stages, viz., spore germination, vegetative growth, fragmentation, and sporogenesis. *Pasteuria penetrans* infects the root-knot nematode *Meloidogyne* spp. Spores of *Pasteuria* can attach to the cuticles of the second-stage juveniles and germinate about 8 days after the juvenile has entered roots and begun feeding (Sayre and Wergin 1977). 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 and Wergin 1977). Attachment of the spores to the nematode cuticle is the first step in the infection process (Davies et al. 2001). 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 and 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 and Redden 1997; Davies et al. 2001). The distribution of an adhesin-associated epitope on 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 and Danks 1993; Charnecki 1997). 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 fibers surrounding the *Pasteuria* spore core are thought to be responsible for the adhesion of the spore to the host cuticle (Sayre and Wergin 1977; Stirling et al. 1986; Persidis et al. 1991). Sonication can increase spore attachment by removing the sporangial wall and exposing the parasporal fibers (Stirling et al. 1986).

13.2.1.2 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, targeting 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 an 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 redivivus*) could be killed by various *Br. 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 redivivus* 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. 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). 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 controls the switch from saprotrophy to parasitism in order to formulate effective commercial nematode control agents.

13.2.2 Antagonistic Soil Bacteria

Many species of soil bacteria are capable of decomposing plant and animal residues. A succession of these bacteria facilitates stepwise degradation of soil organic matter. The products released by the metabolic activity of the bacteria vary from complex to the simplest molecules. Some of these products accumulate in the soil and may be toxic, antibiotic, or inhibitory to plant-parasitic nematodes. During natural decomposition of plant residues, ammonifying bacteria apparently produce enough ammonia to influence nematodes.

Other compounds like hydrogen sulfate and ammonia produced by bacteria have also been found to have deleterious effects on *Hirschmanniella oryzae* in rice fields and root-knot nematodes (Jacq and Fortuner 1979; Zavalata 1985). Soil bacteria like *Bacillus thuringiensis* var. *thuringiensis* (Prasad and Tilak 1972) producing butyric acid, hydrogen sulfide, cyanide, and exotoxins, have been demonstrated to be antagonistic to nematodes. Ammonia produced by ammonifying bacteria during decomposition of nitrogenous organic materials can result in reduced nematode populations in soil (Rodriguez-Kabana 1986).

13.2.2.1 Cry-Protein-Forming Bacteria

Bacillus thuringiensis, a spore-forming aerobic, Gram-positive bacterium belonging to the genus *Bacillus*, is considered a potential biocontrol agent. More than 200 isolates of *B. thuringiensis* have been grouped into more than 12 stereotypes. The classification was done by combination of *Heterodera antigens* (stereotypes) and biotypes, particularly the esterase types (Norris 1964). *B. thuringiensis* occurs in the dead matter of insects, litter of sericulture form, and soils. Chahal and Chahal (1991) perhaps for the first time investigated *B. thuringiensis* toxic to eggs and larvae of *Meloidogyne* sp. Chahal and Chahal (1999) examined the effect of different strains of *B. thuringiensis* on wheat galls and on egg masses of *Meloidogyne incognita*. The result showed a drastic inhibition of egg masses and death of all J_2S of *M. incognita*. The gelatinous matrix of egg mass was disintegrated due to bacterial action which might be due to the ability of bacteria to produce enzyme chitinase (Chigaleichik 1976), an enzyme which hydrolyze chitin present in the egg shell and gelatinous matrix of egg masses (Spiegel and Cohn 1985), thereby affecting the permeability.

Bird and McClure (1976) and Ignoffo and Dropkin (1977) reported that a thermostable toxic of *B. thuringiensis* was found to be toxic to population *Meloidogyne*, *Panagrellus*, and *Aphelenchus* and prevented *M. incognita* juveniles from forming galls on tomato roots. *B. thuringiensis* (Bt) produces one or more parasporal crystal inclusions (Cry or d-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 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*. 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 *B. thuringiensis israelensis*, *B. thuringiensis kurstaki*, and another parasporal-crystal-forming bacterium, *B. 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 and Tinelli 1987; Meadows et al. 1989). The toxicities of these strains were inhibited by antibiotics and neither 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 and 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.

13.2.2.2 Rhizobacteria

Rhizospheric bacteria mainly fluorescent *Pseudomonas* (Oostendrop and Sikora 1989; Spiegel et al. 1991) and certain others like *B. subtilis* and *B. cereus* (Oka et al. 1993), *B. sphaericus* (Racke and Sikora 1992), *Anthrobacter* (Kloepper et al. 1988), *Scroratia* (Kloepper et al. 1988), and *Agrobacterium* (Racke and Sikora 1992) play an important role in biocontrol of plant-parasitic nematodes. The rhizobacteria usually comprise a complex assemblage of species with many different modes of action in the soil (Siddiqui and Mahmood 1999). Rhizobacteria reduce nematode populations mainly by regulating nematode behavior (Sikora and Hoffmann-Hergarten 1993), interfering with plant–nematode recognition (Oostendorp and

Sikora 1990), competing for essential nutrients (Oostendorp and Sikora 1990), promoting plant growth (El-Nagdi and Youssef 2004), inducing systemic resistance (Hasky-Gunther et al. 1998), or directly antagonizing by means of the production of toxins, enzymes, and other metabolic products (Siddiqui and 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 and Jasson 1984; Siddiqui and Mahmood 1999). There are two commercial bionematicidal agents based on *Bacillus* species. Through a PGPR research program of the ARS (Agricultural Research Service, USA), a commercial transplant mix (Bio Yield TM, Gustafson LLC) containing *Paenibacillus 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 and 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 and 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 bionematicide formulation have on the microbial community of the rhizosphere. Previous studies have shown that the addition of manure or other organic amendments stimulates the activity of the indigenous soil microbial community (Giannakou and Prophetou-Athanasiadou 2004).

13.2.2.3 *Pseudomonas fluorescens*

In many crop–pathogen systems, the primary mechanism of biocontrol by fluorescent pseudomonads is production of HCN and antibiotics such as 2,4-diacetylphloroglucinol (2,4-DAPG), pyoluteorin, pyrrolnitrin, and phenazines, playing an important role in biocontrol of pathogens (Défago et al. 1990). It is not clear exactly how the plant-growth-promoting properties of *P. fluorescens* are achieved; theories include that the bacteria might induce systemic resistance in the host plant, so it can better resist attack by a true pathogen; the bacteria might outcompete other (pathogenic) soil microbes, e.g., by siderophores giving a competitive advantage at scavenging for iron; and the bacteria might produce compounds antagonistic to other soil microbes, such as phenazine-type antibiotics or hydrogen cyanide.

P. fluorescens produces some siderophores (iron-chelating substances) which act as growth factors and disease-suppressive siderophores like pseudoactin which can

presumably deliver iron to plants they benefit; otherwise, these plants would develop iron chlorosis and become susceptible to pathogens (Leong 1986). 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 and Kluepfel 1993; Cronin et al. 1997a; Jayakumar et al. 2002; Siddiqui and Shaukat 2002, 2003a, b; Andreogloua et al. 2003; Siddiqui and Singh 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 and Shaukat 2002, 2003a, b).

P. fluorescens controlled cyst nematode juveniles by producing several secondary metabolites such as 2,4-diacetylphloroglucinol (DAPG) which reduces juvenile mobility (Cronin et al. 1997a; Siddiqui and Shaukat 2003a, b). Additionally, mortality of root-knot and cyst nematode juveniles in culture filtrates of *P. fluorescens* has also been observed (Gokta and Swarup 1988). Mena and Pimentel (2002) reported that *Corynebacterium paurometabolum* inhibited nematode egg hatching by producing hydrogen sulfide and chitinase. Some other rhizobacteria reduce deleterious organisms and create an environment more favorable for plant growth by producing compounds such as antibiotics or hydrogen cyanide (Zuckerman and 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 defense 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 and 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).

The mechanism involved in resistance development seems to be directly related to nematode recognition and penetration of the root (Reitz et al. 2001; Mahdy et al. 2001). However, Siddiqui and 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. Ganeshan and Kumar (2005) used *Pseudomonas fluorescens* as a potential biopesticide for augmentative biological control of many diseases of agricultural and horticultural importance. Biological control by plant-growth-promoting fluorescent pseudomonad*s* protects the plant from pathogens by activating defense

genes encoding chitinase, 1,3 glucanase, and peroxidase (Chen et al. 2000). *P. fluorescens* strain PF-1 was toxic to *R. reniformis*, with all tested concentration exhibiting toxic effects (Jayakumar et al. 2002).

Plant growth promotion by rhizobacteria can effect directly (Glick 1995; Presello-Cartieaux 2003) by fixation of nitrogen, solubilization of minerals, production of siderophores that solubilize and sequester iron, or production of plant growth regulators (auxin, cytokinin, gibberellins, ethylene, or abscisic acid) that enhance plant growth at various stages of development, whereas indirect growth promotions occur when PGPR promotes plant growth by improving growth restricting conditions (Glick et al. 1995). Shanti et al. (1998) reported suppression in nematode multiplication (root-knot) in grapevine root even after 8 months (second-generation crop) with application of *P. fluorescens*. Fluorescent pseudomonad*s* have received much attention as biocontrol agents because they generally act through direct antagonism to pathogens, through antibiotic production, through competition with pathogen, or more directly through plant growth promotion (Gamlial and Katan 1993).

13.2.2.4 *Pseudomonas aeruginosa*

Siddiqui and Shaukat (2003a, b) reported on biocontrol agents *Pseudomonas aeruginosa* IE-6 and IE-6S^{+} (previously shown to suppress several soil-borne plant pathogens) on soil microfungi and plant-parasitic nematodes as well as on the root-knot development and growth of tomato (*Lycopersicon esculentum*). The biocontrol agents significantly reduced root-knot development and enhanced shoot growth of tomato over the controls.

Gulnaz et al. (2008) used *P. aeruginosa* and *B. japonicum* alone or with mineral fertilizers significantly reduced infection of tomato roots by the root-rotting fungi *Macrophomina phaseolina*, *Rhizoctonia solani*, and *Fusarium solani*. Use of *P. aeruginosa* or *B. japonicum* alone or with mineral fertilizers suppressed the root-knot nematode *M. javanica* by reducing numbers of galls on roots, nematode establishment in roots, and nematode populations in soil. The tallest plants and maximum shoot fresh weight occurred due to treatment with *P. aeruginosa*. Siddiqui and Akhtar (2007) found that *P. aeruginosa* reduced galling and nematode multiplication the most followed by *A. awamori and G. intraradices*. Combined inoculation of these microorganisms caused the greatest increase in plant growth and reduced the root-rot index more than individual inoculations. Pathogens adversely effected root colonization by *G. intraradices*. However, root colonization and root nodulation were increased when co-inoculated with *P. aeruginosa* and *A. awamori* whether in the presence or absence of pathogens.

13.2.2.5 *Bacillus subtilis*

Numerous *Bacillus* strains can suppress pests and pathogens of plants and promote plant growth. Some species are pathogens of nematodes (Gokta and Swarup 1988;

Li et al. 2005). The most thoroughly studied is probably *B. subtilis* (Krebs et al. 1998; Siddiqui and Mahmood 1999; Siddiqui and Shaukat 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 and Swarup 1988; Kloepper et al. 1992; Madamba et al. 1999; Siddiqui and Mahmood 1999; Insunza et al. 2002; Kokalis-Burelle et al. 2002; Meyer 2003; Giannakou and Prophetou-Athanasiadou 2004; Li et al. 2005).

B. subtilis improved plant growth by inhibiting nonparasitizing root pathogens, producing biologically active substances or by transforming unavailable minerals and organic compounds into forms available to plants (Broadbent et al. 1997). El-Hassan and Gowen (2006) found that the formulation of *B. subtilis* decreased the severity by reducing colonization of plants by pathogen, promoting their growth, and increasing the dry weight of lentil pea. *B. subtilis* is not a nematode parasite, but it has a high degree of larvicidal property (Siddiqui and Mahmood 1995a), and it produces many biological active substances. Gokta and Swarup (1988) also reported that isolates of *B. subtilis* and *B. pumilus* were found most effective against *M. incognita*, *H. cajani*, *H. zeae*, and *H. avenae*.

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* (Tables 13.1 and 13.2; Fig. 13.1) (Jacq and Fortuner 1979; Kloepper et al. 1992; Racke and Sikora 1992; Guo et al. 1996; Cronin et al. 1997b; Duponnois et al. 1999; Neipp and Becker 1999; Siddiqui and Mahmood 1999, 2001; Meyer et al. 2001; Mahdy et al. 2001; Hallmann et al. 2001; Insunza et al. 2002; Khan et al. 2002; Mena and Pimentel 2002; Meyer 2003).

13.2.2.6 *Azotobacter*

Another bacterium, *Azotobacter*, is an aerobic, nonsymbiotic Gram-negative nitrogen-fixing bacteria, which occurs in most of the cultivated soil, is gaining importance in controlling phytoparasitic nematodes. Verma and Bansal (1996) showed the inhibitory effect of *A. chroococcum* on hatching of *M. javanica*. Racke and Sikora (1992) found that out of 179 bacterial isolates isolated from roots and cysts, only six caused a significant reduction (>25 %) in *Globodera pallida* penetration of potato roots. Six of these isolates caused significant reductions in repeated greenhouse tests. The antagonistic activity was shown to be directly correlated with the number of colony-forming units (cfu) present on the tuber. The isolates *Agrobacterium radiobacter* and *Bacillus sphaericus* at densities of 9.7×10^8 and 3.16×10^9 cfu ml^{-1}, respectively, caused significant reductions in root infection of 24–41 % in repeated experiments.

Table 13.1 Antagonistic rhizosphere for the control of phytonematodes

Biotic agent	Nematode sp.	Crop	Reference
Bacillus licheniformis	*M. incognita*	–	Siddiqui and Hussain (1991)
Pseudomonas mendocina	*M. incognita*	–	Siddiqui and Hussain (1991)
Bacillus subtilis	*M. incognita*	–	Gokta and Swarup (1988)
B. pumilus, *B. cereus* *Pseudomonas* sp.	*H. cajani*, *H. zeae*, *H. avenae*		
P. fluorescens	*H. avenae*	Wheat	Kamra and Dhawan (1997)
P. fluorescens	*M. incognita*	Tomato	Verma et al. (1999)
P. stutzeri	*M. incognita*	Tomato	Khan and Tarannum (1999)
B. subtilis, *P. fluorescens*	*M. incognita*	Tomato	Santhi and Sivakumar (1995)
P. fluorescens	*M. incognita*	Black pepper	Eapen et al. (1997)
P. fluorescens	*H. cajani*	Pigeon pea	Siddiqui and Mahmood (1995a, b)
B. subtilis, *Bradyrhizobium japonicum*	*H. cajani*	Pigeon pea	Siddiqui and Mahmood (1995a)
P. fluorescens	*Hirschmanniella gracilis*	Paddy	Ramakrishnan and Sivakumar (1999)
B. subtilis	*M. incognita*	Chickpea	Siddiqui and Mahmood (1993)
P. fluorescens	*H. cajani*	Black gram	Latha and Shivakumar (1998)
P. fluorescens	*Tylenchulus semipenetrans*	Sweet orange and lime	Santhi et al. (1999)
P. fluorescens	*Globodera* sp.	Potato	Mani et al. (1998)

Ali (1996) found that the population density of nematode species was reduced by application of five bacterial isolates (*Arthrobacterium* spp., *Bacillus* spp., *Corynebacterium* spp., *Serratia* spp., and *Streptomyces* spp.). Reductions of nematode populations were ranged between 46 % and 100 %. Youssef et al. (1998) studied the potential of *A. chroococcum*, *Bacillus megaterium*, and *Rhizobium lupine* for the control of *M. incognita* infecting cowpea and tomato plants. They noticed a number of both root galls and egg masses of *M. incognita* were decreased in soil treated with *B. megatherium* and *A. chroococcum* except *R. lupine*-treated soil. El-Sherif et al. (1995) studied the effect of culture filtrates of 5 isolates for their nematotoxic effect against plant-parasitic nematode (*Bacillus* spp., *Corynebacterium* spp., *Serratia* spp., *Arthrobacterium* spp., and *Streptomyces* spp.). The authors determined the culture filtrate concentration as 0.1 % to inhibit the hatching of the eggs and 0.6 % to be highly toxic to the juveniles. The toxic effect of the filtrate varied with the different nematode species.

Siddiqui and Futai (2009) studied the effects of antagonistic fungi (*Aspergillus niger* v. Teigh, *Paecilomyces lilacinus* (Thom) Samson, and *Penicillium chrysogenum*

Table 13.2 Mode of action of various bacterial groups

Nematophagous bacterial group	Genus and species	Target nematodes	Pathogenic effects on nematodes	Action mode
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
Opportunistic parasitic bacteria	*Bacillus nematocida* (Bacillus sp. B16), *Brevibacillus laterosporus*, *Bacillus* sp. RH219, etc.	*Panagrellus redivivus* and *Bursaphelenchus xylophilus*	*Br. laterosporus* strain G4 could penetrate the nematode cuticles and eventually digest the target organism in the laboratory	Parasitism, production of enzymes and toxin
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
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
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
Symbiotic bacteria of entomopathogenic nematodes	Two genera: *Xenorhabdus* and *Photorhabdus*	*Bursaphelenchus xylophilus*, *M. incognita*, and their eggs	Toxic to juveniles of root-knot and pinewood nematodes and inhibit egg hatch	Toxin production (ammonia, indole, and stilbene derivatives)

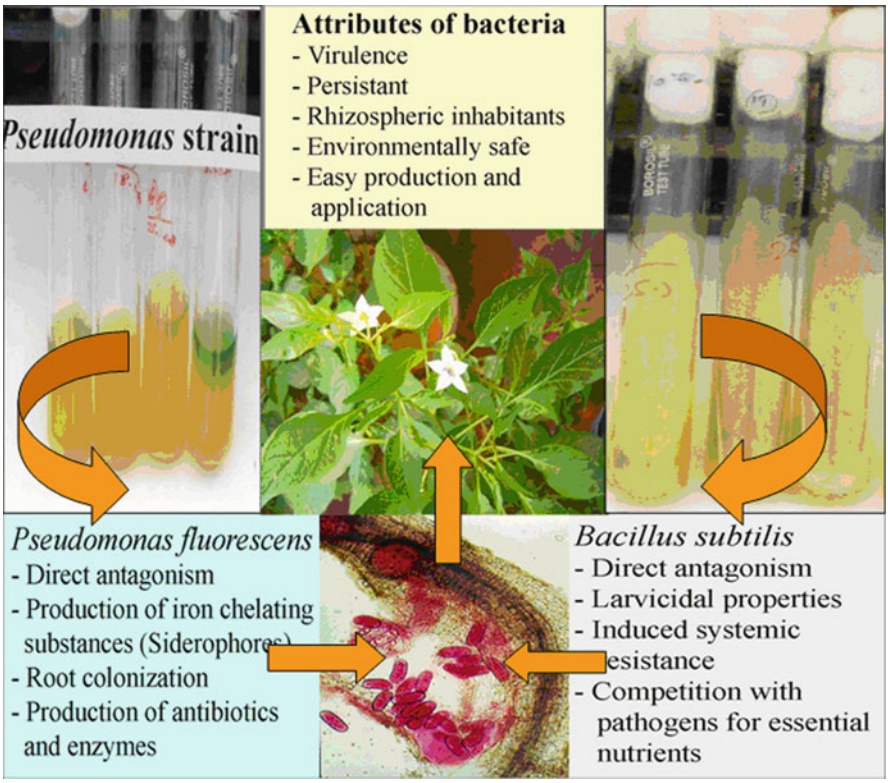

Fig. 13.1 Mechanism of biocontrol by *Pseudomonas fluorescens* and *Bacillus subtilis*

Thom), and plant-growth-promoting rhizobacteria (PGPR) (*A. chroococcum* Beijer, *B. subtilis* (Ehrenberg) Cohn, and *Pseudomonas putida* (Trev.) Mig.) were assessed with cattle manure on the growth of tomato and on the reproduction of *M. incognita* (Kof. and White) Chitwood. Application of antagonistic fungi and PGPR alone and in combination with cattle manure resulted in a significant increase in the growth of nematode-inoculated plants. Siddiqui (2004) conducted glasshouse experiments to assess the influence of *P. fluorescens*, *A. chroococcum*, and *Azospirillum brasilense* and composted organic fertilizers (cow dung, horse dung, goat dung, and poultry manure) alone and in combination on the multiplication of *M. incognita* and growth of tomato.

13.2.3 Other Soil Bacteria: Rhizobia

Nodulation is a complex symbiotic process between host plant and *Rhizobia*. For successful nodulation, the *Rhizobia* must multiply to a sufficient population level

and colonize the rhizosphere before making contact with the legume roots. Subsequently, the bacteria attach themselves to roots and penetrate root hairs and stimulate formation of nodules. This process can be disrupted by biotic stresses on either host plant or *Rhizobia*. Survival and colonization of *Rhizobia* in the rhizosphere are greatly influenced by root exudation of host plants (Bhagwat and Thomos 1982). Carbohydrates, amino acids, and a variety of nutrients by soybean roots as root exudates. Among amino acids, a variety of nutrients are released by soybean roots as root exudates. Among amino acids, tryptophan is easily converted by *Rhizobium* to indoleacetic acid that stimulates the formation and elongation of root hairs. This facilitates the bacteria to enter soybean roots via epidermal cells of root hairs and initiate the bacterial nodulation (Barker and Hussey 1976). However, plant-parasitic nematodes have been shown to alter quality and quantity of root exudates of infected plants (Wang and Bergeson 1974). These changes have an impact on the efficacy of tryptophan in formation and elongation of root hairs.

Plant lectins are the specific carbohydrate-binding proteins, constituting approximately 10 % of the extractable nitrogen in the seeds of leguminous plants and have been extensively used in the study of cell surface architecture. Earlier work on lectin distribution in plant tissues as well as lectin-mediated cell–cell interactions provides strong evidence for their involvement in the defense of plants against infection and also in *Rhizobium*–legume symbiosis. During the symbiotic biological nitrogen fixation, the bacteria of the genus *Rhizobium* living in the rhizospheric region of the leguminous plants adhere to the legume roots and are subsequently internalized to form nitrogen-fixing nodules. The *Rhizobium*–legume interactions are specific, and the specificity is achieved through the action of plant lectins.

It has been demonstrated that the lectin in beans extract could help bind the specific bacteria to the roots of *Phaseolus vulgaris*. Systematic studies in this direction were subsequently made in soybean–*Rhizobium japonicum* and clover–*Rhizobium trifolii* systems (Musarrat and Akhtar 2000). Plant lectins extruded by soybean roots are proteins capable of binding sugar or sugar containing proteins. Several studies suggest that *Rhizobia* bind to soybean roots via soybean lectins on the root surface (Bohlool and Schimt 1974). Soybean cyst nematode *Heterodera glycines* may affect the bacterial binding sites on the root to limit bacterial establishment for nodulation. An interaction between root surface lectins and surface carbohydrates of the nematode may be prerequisite for the nematode penetration (Zuckerman and Jasson 1984). *Rhizobium japonicum* cells also bind with soybean lectins (Balasubramanium 1971). Hence, there is a competition between *Meloidogyne* spp. and *R. japonicum* for binding to soybean root surface lectin. It also causes reduction of bacterial nodulation. Few studies have assessed the effect of *Meloidogyne* infection on bacterial nodulation of legume crops, and these studies have shown that *Meloidogyne* spp. have retarded the development of root system and the bacterial nodulation of legume crops (Balasubramanium 1971; Huang et al. 1984).

The presence of sugars such as *N*-acetylglucosamine, galactose, *N*-acetylgalactosamine, and mannose and/or glucose on the cuticle surface of plant-parasitic nematodes may play an important role in the interaction between

nematodes and their hosts. It has been demonstrated that the binding of *Rhizobia* to nematode-free roots was inhibited only after pretreatment with certain sugars. Studies on the interference of nematodes with soybean lectin metabolism showed the reduced binding of *Rhizobia* to *H. glycines*-infected soybean roots, suppressing the nodule formation. Furthermore, the root-knot nematodes *M. incognita* infecting mungbean, chickpea, cowpea, wandopea, and green gram; *M. hapla* infecting white clover; and *Meloidogyne* spp. infecting horsebean, lupin, clover, and pea have been reported to inhibit nodulation. Interrelationship between *M. incognita*, *Heterodera cajani*, and *Rhizobium* sp. on cowpea (*Vigna sinensis*) has been investigated. Hussaini and Seshadri (1975) reported that *M. incognita* and *H. cajani*, singly or in concomitant inoculum, significantly reduce the growth of cowpea; *M. incognita* reduced N-content to a greater extent than *H. cajani*. Similarly, Hussaini and Seshadri (1975) reported that *M. incognita* inoculated before and after or simultaneously with *Rhizobium* caused significant decrease in plant height, fresh and dry weight of shoot and root, number of nodules on root, and nitrogen content of root when compared to nematode-free plants. Presumably, the common sugars on the cuticle surface of nematodes compete for the plant lectins, resulting in reduced rhizobial binding sites (Musarrat and Akhtar 2000).

The association of rhizobia with legume hosts has a beneficial effect on plant nutrition and growth. In contrast, the plant–nematode relationship has adverse effects on plant growth. The role of plant-parasitic nematodes on rhizobial nodulation and nitrogen fixation of host plants has been reviewed by a number of workers (Huang 1987; Khan 1993). As a result of nematode infection, the nodulation and nitrogen fixation has been reported to be suppressed (Hussaini and Seshadri 1975), or stimulated (Hussey and Barker 1976), or remain unaffected (Taha and Raski 1969). The role of rhizobia in the control of plant diseases of various leguminous crops has already been discussed (Sawada 1982), and biological control of plant diseases is now increasingly capturing the imagination of plant pathologists (Papavizas and Lumsden 1980). Some of the possible reasons for the reduced nematode reproduction caused by root-nodule bacteria are physiological and biochemical changes, change in host nutrition, and histopathological numbers.

13.2.3.1 Physiological and Biochemical Changes

The root-nodule bacteria which fix atmospheric nitrogen are reported to produce toxic metabolites inhibitory to many plant pathogens (Haque and Gaffar 1993). *Rhizobium japonicum* secretes rhizobitoxine, which is inhibitory to charcoal root fungus *Macrophomina phaseolina* (Chakraborty and Purkayastha 1984). Chakraborty and Chakraborty (1989) reported an increased level of phytoalexin (4-hydroxy-2,3,9-trimethoxypterocarpan) when pea seeds were bacterized with *R. leguminosarum* prior to inoculation with *Fusarium solani* f. sp. *pisi*. This phytoalexin may have an important role in cross-protection against many pathogens. Siddiqui and Mahmood (1994) observed higher activity of peroxidase, nitrate reductase, and catalase in pigeon pea plants inoculated with *Bradyrhizobium*

japonicum than in plants without *B. japonicum*. An increase in peroxidase activity due to *B. japonicum* inoculation indicates increasing resistance of the plant because it catalyzes the polymerization of phenolic compounds and forms cross-links between extensin, lignin, and feruloylated polysaccharides (Siddiqui and Mahmood 1994). An increase in nitrate reductase and catalase may be correlated with the rate of protein synthesis and resistance of the plant to pathogens, respectively (Siddiqui and Mahmood 1994). Roslycky (1967) reported production of an antibiotic bacteriocin by rhizobia bacteria. Some properties of antibiotics of rhizobia bacteria have also been reported by others (Drapeau et al. 1973; Schwinghamer and Belkengren 1968; Tu 1978, 1988). Antibiotics and phytoalexin produced by rhizobia bacteria probably reduce damage from nematodes and other pathogens.

13.2.3.2 Change in Host Nutrition

Damage to plant growth by nematodes can be lessened by the application of nitrogen fertilizer (NH_4, NO_3) (Ross 1969), indicating that combined nitrogen can improve growth of diseased plants. Combined nitrogen, such as nitrate, at a high level is a powerful inhibitor of nodulation (Dart 1977) and also has an adverse effect on the development of nematodes (Barker et al. 1972). Barker and Huisingh (1970) observed necrosis in nodular tissues following invasion by nematodes; this may in part account for reduced nematode development. All this suggests that application of rhizobia bacteria which increase nitrogen content and plant growth can also reduce nematode populations.

13.2.3.3 Histopathological Changes

Endo (1964) indicated that nematodes, especially males, often caused plant necrosis and degeneration of syncytia as the nematodes matured. Endo (1965) found that nematodes induced much necrosis in resistant plants. Some reactions that he observed were very similar to those of nodular tissues where the surrounding tissues, as well as the nematode, died. This type of reaction may partially explain the reduced number of nematodes obtained when nematodes and *Rhizobium* were added simultaneously to soybean (Barker et al. 1971). Sharma and Sethi (1976) reported that both the nematodes, namely, *M. incognita* and *H. cajani*, either singly or in combination, significantly reduced the growth of cowpea and addition of *Rhizobia* tended to reduce this damage to some extent.

Mishra et al. (1994) reported improved plant growth in *R. leguminosarum*-inoculated *Phaseolus aureus* L. plant as compared to reniform-nematode-infected plant. Datal and Bhatti (2002) studied the interaction between *H. cajani* and *Rhizobium* in different combinations and revealed that alone or prior addition of *Rhizobium* enhanced nodulation but reduced multiplication on mungbean and

cluster bean. Sharma and Sethi (1976) and Khan and Hussain (1990) reported that the addition of rhizobia tends to reduce the damage caused to the host plant in combined inoculation of phtyoparasitic nematodes.

Siddiqui and Singh (2005) conducted glasshouse experiments to assess the ash amendments (0, 20, and 40 % with soil), a phosphate-solubilizing microorganism *Pseudomonas striata* and a root-nodule bacterium *Rhizobium* species on the reproduction of root-knot nematode *M. incognita* along with the growth and transpiration of pea. Amendments of fly ash with soil had no effect on transpiration. However, *M. incognita* reduced the rate of transpiration from first week onward after inoculation, while inoculation of *Rhizobium* sp and *P. striata* increased transpiration from first week onward after their inoculation both in nematode-inoculated and nematode uninoculated plants. *Rhizobium* sp. had greater adverse effect on galling and nematode multiplication than *P. striata*. Use of both organisms together had greater adverse effect on galling and nematode multiplication than caused by either of them alone. Highest reduction in galling and nematode multiplication was observed when both organisms were used in 40 % fly-ash-amended soil.

13.2.4 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 and 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 and Matheson 1996; Hallmann et al. 1999; Azevedo et al. 2000; Munif et al. 2000; Shaukat et al. 2002; Sturz and 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, *Microbacterium esteraomaticum* and *Kocuria 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 and 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 from symbiotic bacteria have been identified as nematicidal agent: ammonia, indole, and stilbene derivatives (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 pinewood (Bursaphelenchus xylophilus) and inhibited egg hatching of *M. incognita* (Hu et al. 1999).

13.3 Some Important 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 and Holden 1996; Aballav and Ausube 2002; Tan 2002; Barker 2003). 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. Mutational analysis, 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. Comparative genomics, this technique can identify pathogenic genes by comparing genomic sequences of pathogenic and nonpathogenic strains or other sequences from strains of interest of the same genus.

13.4 Conclusion and Future Perspectives

Over the past 20 years, a large number of studies have been undertaken to investigate the use of microorganisms as biocontrol agents against nematode pests. All these groups of bacteria have undoubtedly generated a lot of interest in acting as natural enemies and for their role in biological control of phytoparasitic nematodes. However, the major constraints in the development of effective biocontrol agents have been the mass production, storage and distribution of fresh materials, and effect of abiotic factors like pH, moisture, and soil types which influence the activities of these microbial biopesticides, host range, and virulence of the inoculum. For instance, the major attributes which favor *Pas. penetrans* as a successful biocontrol agent are long viability of spores, resistance to heat and desiccation, persistence in soil, compatibility with chemical nematicides, nontoxicity to plants and other soil biota, and easy storage, but major hurdles are the following: lack of a technique enabling culture of the bacterium in vitro on any of standard biological media; neither vegetative cells nor spores of the organism can be harvested in sufficient numbers to test extensively in laboratory conditions or to infest soil in large-scale field tests to determine its influence; and with the currently available methods of mass multiplication, its commercial use may be limited to glasshouse crops or horticultural crops only, and more research is required to be conducted in order to exploit important aspects of bacterium–nematode interaction with particular emphasis on the mechanism of action for the control of plant pathogens and nematodes.

Only a few commercial biocontrol products from the bacteria with nematicidal potentials have been developed and used in the agriculture system (Whipps and 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.

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 and 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 signaling pathways that induce the host-plant defense mechanism, and the

infection process. Such information should provide novel approaches to improve the efficacy of nematophagous bacteria for biological control applications, to increasing the expression of toxins or enzymes from the microorganisms, and for formulation of 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.

Microorganisms as biocontrol agents have a relatively narrow spectrum of activity compared with synthetic pesticides (Barker 1991; Janisiewicz 1996) and often exhibit inconsistent performance in practical agriculture. Application of a mixture of inoculated biocontrol agents would more closely mimic the natural situation and might broaden the spectrum of biocontrol activity. A good colonization capacity and compatibility of inoculated microorganisms constitutes an important prerequisite for successful development of biocontrol (Barker 1990). Phosphate-solubilizing microorganisms improved the growth of plants possibly through an inhibitory effect on nematode development as reported by Becker et al. (1988), Kloepper et al. (1992) and Hasseb et al. (2005). Pseudomonads may improve plant growth by suppressing parasitic and nonparasitic root pathogens (Oostendorp and Sikora 1990) by the production of biologically active substances (Gamlial and Katan 1993) or by converting unavailable minerals and organic compounds into forms available to plants (Broadbent et al. 1997; Siddiqui and Mahmood 1999). *Bacillus* and *Pseudomonas* are known to suppress diseases by inhibition of pathogens by competition of Fe (III), inhibition of pathogen by diffusible or volatile products, induction of resistance in plants, and aggressive root colonization and stimulation of plant growth (Kloepper et al. 1988; Weller 1988; Siddiqui and Mahmood 1999). Similarly, the presence of rhizobia in the rhizosphere presumably protects the host roots from pathogens, besides fixing atmospheric nitrogen. The use of these symbionts will reduce the damage without use of chemical pesticides, which are costly and have health hazards. Therefore, using a consortium of rhizobia and other phosphate-solubilizing microorganisms such as fluorescent *Pseudomonas* and *Bacillus* species could provide a better solution against phytonematodes.

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