Bacillus and Biopesticides in Control of Phytonematodes

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Abstract

Currently the major challenge of humanity is focused on population growth/increase through agricultural production in order to meet the demand for food. Overtime, different pests have emerged, with some being of great importance. Among these pests, the nematodes are noted for attacking leguminous plants, grasses, citrus, and other fruits. The main pest species are of the genus *Heterodera*, *Meloidogyne*, *Pratylenchus,* and *Globodera*. These nematodes cause losses up to 100 %, preventing agriculture of certain areas. Financially, about $100 billion annual damage is caused by nematodes. These amount to 90 % of the yield of cotton, yams, beans, and soybeans, and in citrus damage is estimated at 14 % of production. Alternative methods of control are being studied, and in this context, a bacterium of the genus *Bacillus* has prominence and importance. Besides *Bacillus subtilis*, some by pesticides are already marketed for the control of nematodes, such as Bioarc® the basis of *Bacillus megaterium*, Bio Zeid® the basis of *Trichoderma album*, and also using the brown alga, *Ascophyllum nodosum* (Algaefol®), among others. The objective of this chapter is to report the use of different *Bacillus* species and some biopesticides used to control nematodes of agricultural importance.

Keywords

Biopesticides • Bacillaceae • Biological control • Nematoda

1.1 Introduction

The biggest challenge for agriculture worldwide is to supply the demand for food, due to the ever-growing population. Data from FAO, in 2012, shows 2.2 % reduction in the world production of grains compared to 2011. It is a significant loss (52 millions of tons) and is associated, besides

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the occurrence of agricultural pests, to the widespread and severe drought. Production of secondary grains such as soy, maize, and sorghum decreased 1.5 %. Wheat production decreased 2 % and rice, the only cereal with a lesser loss, decreased 0.2 %. The world demand for grains for 2012/2013 has been estimated in 2.3 millions of tons, a reduction of 2 %, which may result in the increase of the price for those products. Furthermore, FAO evaluates a demand of 2.4 millions of tons of those grains in 2012–2013.

In the animal kingdom, the phylum Nematoda represents about 80,000 described species, according to the Brazilian Society of Nematology. The nematodes are morphologically classified as elongated, cylindrical, or subcylindrical. They may be free-living, phytoparasites or zooparasites. The phytoparasites, also known as phytonematodes, are noted by their economic importance, attacking leguminous plants, grasses, citrus, and other fruits. The main pest species are of the genus *Heterodera*, *Meloidogyne*, *Pratylenchus,* and *Globodera*. These pests are considered the "hidden enemies" of the producers, for being hard to identify in the field. Symptoms on plant leaves may be mistaken as diseases, nutrient deficiency, drought, and soil compression. Some species may degrade plant tissues, while others may cause hormonal disorders, inducing the formation of galls, and some may release phenolic compounds. Furthermore, these parasites may transmit bacteria externally, on the plant surface, or internally, inside the alimentary canal (Moens and Perry 2009).

Phytonematodes survive over years in the soil or in vegetal remnants, being easily disseminated by agricultural implements, irrigation water, or floods, and adhered to animal feet or feces and vegetal material, such as seedlings and seeds (Oka et al. 2009). The losses may vary, from imperceptible damage to the death of a large number of plants, preventing agriculture of certain areas. Financially, nematodes cause approximately $100 billion in production losses annually (Oka et al. 2009). Researches in the biological control field aim the application of bacteria, fungi, and algae to control these pests. They are mainly focused in rhizosphere compounds with the capacity to modify this environment, affecting these parasites directly or indirectly (Machado et al. 2012). The bacteria of this genus are sporulating, are found in different habitats, and have the peculiarity of being, in many cases, entomopathogenic. Moreover, it may be deduced that *Bacillus*-based products may interfere in the nematode lifecycle, being used in the practice of biological control of these pests.

Based on this information, the general objective of this chapter is to explain the use of the *Bacillus* biopesticides and the practice of biological control of nematodes. Aspects of the biology of major nematodes will be discussed. Rrecent data of *Bacillus thuringiensis*, *Ba. subtilis*, *Ba. megaterium,* and *Ba. firmus*, especially the metabolites produced by these bacterial species, and other biopesticides based on algae and fungi for control of nematodes will be presented. Throughout the chapter, images shall be submitted illustrating the bacterial species and the phytonematodes.

1.2 Phytonematoda

The genus *Pratylenchus* (Pratylenchidae) has the broadest host range. Through research, this genus is recognized as a major pest of economically important crops. The *Pratylenchus*, or root lesion, is distributed in cool, tropical, and temperate environments and comprises 68 nominal species (Moens and Perry 2009). The root lesion causes internal browning in potato tubers and in the roots of corn, lettuce, peas, carrots, tomatoes, and brassicas (Guerena 2006). Therefore, the sedentary endoparasitic nematodes, which include the root-knot nematodes of genus *Meloidogyne* spp. and the cyst nematodes of genus *Heterodera* and *Globodera* spp., are the most economically important pests (Davies and Curtis 2011). The genus *Meloidogyne* has currently more than 80 species recognized (Karssen and Von Hoenselaar 1998) although most of those species are associated with particular plant species and have been little studied, in part because of their limited agricultural importance (Bird et al. 2009).

Root-knot nematodes form galls that block water and nutrient flow, stunting growth, impairing fruit production, causing foliage to yellow and wilt, and injuring plant tissue (Guerena 2006). The infective juvenile stage detects plant roots, in soil, using chemoreception and sensory perception, penetrates in the root, moves intercellularly in vascular cylinder, and forms feeding sites in the differentiation zone inducing nuclear division in host cells, giving multinucleated cells, termed giant cells (Guerena 2006; Atkinson et al. 2012). The female *Meloidogyne* lays up to 2,500 eggs, called egg mass, that hatch into larvae and develop into adults. Each adult age is characterized by phases of development of sexual and digestive systems (Dyakov and Zinovyeva 2007). The cyst nematodes, *Heterodera* spp. and *Globodera* spp., are the most important pests of soybean, potato, and sugar beet, respectively. Cyst nematodes have a small host range and their characteristic features allow regarding them as evolutionarily advanced parasites (Dyakov and Zinovyeva 2007). The genus *Heterodera* gives plants an unthrifty or malnourished appearance, foliage is liable to wilt and curl, while roots become thick and tough and take a red or brown coloring (Guerena 2006). Reproduction requires males; after fertilization, the eggs remain inside the female, gradually filling its entire body forming a bag of eggs, causing the death of the female (Williamson and Gleason 2003; Dyakov and Zinovyeva 2007). Table 1.1 shows the species of nematodes and their host plants.

Dyakov and Zinovyeva (2007) report that out of the 20,000 described species of phytonematodes, approximately 20 % or 4,000 species are connected with plants. The phytonematodes are microscopic organisms, their size varying from 300 μm to 8 mm (Fig. 1.1). The identification of the different species of nematodes may be associated to morphological identification techniques, considered as conventional techniques, and recently to the use of molecular tools. This is the case of a research of Shahina et al. (2012), which associated these techniques for the identification of *M. incognita* and *M. javanica*, in Pakistan. Furthermore, the authors associated the occurrence of these species in new cultivars, such as in olive (*Olea europaea* L.), spinach (*Basella rubra* L.), and sugarcane (*Canna indica* L.) in India, which were not previously registered.

1.3 *Bacillus thuringiensis*

The entomopathogen *Bacillus thuringiensis* (Berliner 1911), a Gram-positive bacterium, is naturally found in the soil (Höfte and Whiteley 1989). It is characterized by crystal production during sporulation (Fig. 1.2), containing Cry proteins, encoded by the *cry* genes, with a wide division of classes and subclasses according to their insecticide activity (Höfte and Whiteley 1989), and presently classified according to the percent identity between Cry protein sequences (Schnepf et al. 1998; Crickmore et al. 1998; Crickmore 2014).

These toxins present insecticidal activity to different insect orders, such as Lepidoptera, Coleoptera, Diptera, Hymenoptera, Hemiptera, Isoptera, Orthoptera, Siphonaptera, and Thisanoptera (Feitelson et al. 1992; Aranda et al. 1996; Cavados et al. 2001; Castilhos-Fortes et al. 2002; Pinto et al. 2003; De Maagd et al. 2001, 2003) in addition to nematodes (Marroquin et al. 2000; Jouzani et al. 2008). Currently, 735 Cry proteins, 38 Cyt proteins, and 122 Vip proteins have been described in the *B. thuringiensis* database (Crickmore 2014). Apart from those proteins, other virulence factors indentified in this bacterium comprise degrading enzymes such as phospholipase C, hemolysins, proteases, and cytotoxins (Vilas-Bôas et al. 2012).

Jouzani and coworkers (2008) identified the *cry*5, *cry*6, *cry*12 *cry*13, *cry*14, and *cry*21 genes, which presented activity against nematodes of the genus *Meloidogyne*. Beyond these genes, Höss et al. (2008) have already indicated the utilization of strains containing genes from the *cry*1 family, showing promising results against the nematode *Caenorhabditis elegans*. A recent study from Khalil et al. (2012) used the product DiPel 2x® containing 32,000 iu/mg of bacterium *B. thuringiensis* var. *kurstaki* (Valent, Canada) against *M. incognita*, in tomato plants, showing the reduction of 80 % in the nematode population. Furthermore, DiPel 2x® reduced in 60 %

Table 1.1 Important species of genus *Pratylenchus*, *Globodera*, *Heterodera*, *Meloidogyne,* and their host plants

Species	Culture	Citation
Pratylenchus coffeae	Coffee, citrus, yam, potato	Moens and Perry (2009), Pionner (2012)
Pratylenchus goodeyi	Banana and plantain	Moens and Perry (2009), Pionner (2012)
Pratylenchus brachyurus	Cassava, peanut, pineapple, potato, rice, tea	Moens and Perry (2009), Pionner (2012)
Pratylenchus zeae	Rice, sugarcane	Moens and Perry (2009), Pionner (2012)
Pratylenchus thornei	Broad beans, cereals, tobacco	Moens and Perry (2009), Pionner (2012)
Pratylenchus penetrans	More than 350 hosts including woody plants (e.g., fruit trees, roses) and herbaceous plants (e.g., potato, vegetables)	Moens and Perry (2009), Pionner (2012)
Pratylenchus vulnus	Woody plants – approximately 80 hosts	Moens and Perry (2009), Pionner (2012)
Globodera rostochiensis	Potato	Qin et al. (2000)
Globodera pallid	Potato	Reitz et al. (2000)
Meloidogyne incognita	Vegetables, tomato, eggplant, melon, cotton, maize, soybean, beans	Pionner (2012) Hashem and Abo-Elyousr (2011), Collange et al. (2011)
Meloidogyne hapla	Eggplant, tomato, melon, lettuce, onion, carrot, potato, bean	Chen et al. (2000)
Meloidogyne javanica	Eggplant, tomato, melon, soybean	Pionner (2012), Collange et al. (2011)
Meloidogyne exigua	Coffee, tomato, rubber trees	Rocha et al. (2010)
Meloidogyne arenaria	Tomato, lettuce	Terefe et al. (2009)
Meloidogyne hispanica	*Ficus* sp., squash plants, grapevine, sugarcane, snapdragon	Maleita et al. (2012)
Meloidogyne enterolobii	*Psidium* spp.	Miranda et al. (2012)
Heterodera glycine	Soybean	Pionner (2012)
Heterodera schachtii	Sugar beet, potato	Madani et al. (2005)

Fig. 1.1 *Meloidogyne javanica* in differential interference contrast microscopy. (**a**) Immature eggs. (**b**) Juvenile stage (J2) (Source: authors)

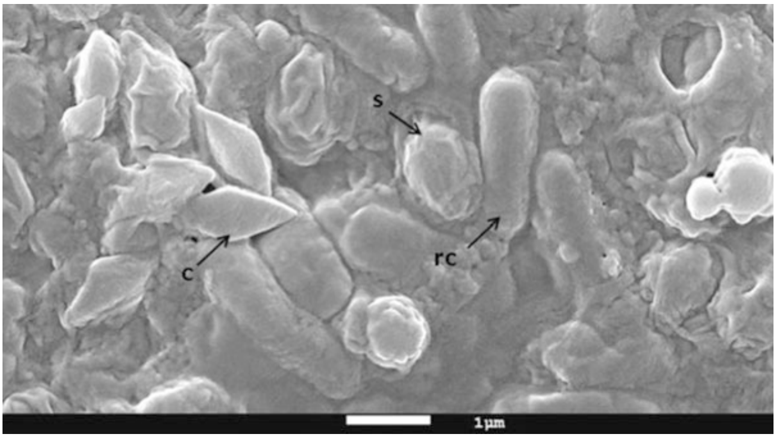

Fig. 1.2 *Bacillus thuringiensis* in scanning electron microscopy. *s* spore, *c* protein crystal, *rc* rod cell (Source: authors)

the amount of galls/5 g of roots and in 74 % the egg mass/5 g of roots. In terms of plant development, the product significantly increased plants' length, dry weight, and fresh weight. This bacterial subspecies is known to contain a family of *cry*1 genes.

Another study shows the utilization of ten different isolates of *B. thuringiensis*, which purified Cry proteins and the supernatant, containing vegetative proteins, was applied to *M. incognita*, in laboratory and in greenhouse (Mohammed et al. 2008). The results showed that four isolates, *Bt*7, *Bt*7N, *Bt*Den, and *Bt*soto, presented 90 and 100 % mortality when utilizing Cry proteins and vegetative protein fractions, respectively. In greenhouse, these isolates were efficient, with *Bt*7N showing the best result in reducing the number of galls, the egg mass, and the number of eggs when compared to the other treatments and the control.

Soil samples from okra, eggplant, tomato, cotton, cabbage, onion, and watermelon crops were collected in Pakistan and used to isolate new strains of *B. thuringiensis* to tests against *M. javanica* (Khan et al. 2010). The authors selected five isolates to studies in greenhouse using okra plants and all the isolates reduced the infection caused by the nematodes. Different mechanisms of action may be related to the toxicity of *B. thuringiensis* against the phytonematodes, since the mode of action of the Cry proteins described by different authors is the ingestion of toxic fragments. These mechanisms may be related with the production of secondary metabolites, which reduce the attraction of the nematodes to the roots or degrade specific exudates of the roots that control the behavior of these species (Khan et al. 2010).

The review of Vachon et al. (2012) shows these theories, developed by many researchers from this field of study, about the mode of action of the nematicide and insecticide Cry proteins. The general conclusion of these authors reveals that the model mostly accepted is still the classic model described in the 1980s and 1990s, and these are protein digestion, solubilization, pore formation in the membrane, and the binding of the toxic fragments. Thereafter, occurs the cellular lysis, the intestinal paralysis and the target pest ceases its feeding and dies.

Data from Berlitz et al. (2012) show the toxicity of *B. thuringiensis* isolates of soil samples from Rio Grande do Sul state, Brazil, which presented corrected mortality of 91 % and 93 % to the isolates *Bt* 3434-2P and *Bt* 2974-11P, against second stage (J2) *M. incognita*. These results were published on the 45th Annual Meeting of Society Invertebrate Pathology, in Buenos Aires (2012), an event that gathers worldwide researchers, with specific conferences in the nematology field. These results corroborate with the results from Li et al. (2012), which isolated 3,800 bacterial strains from soil samples, with 438 belonging to the Bacilli group. One strain was identified as *B. thuringiensis* and was tested, showing 100 % mortality against juveniles of second stage (J2) *M. incognita*. The simultaneous utilization of *B. thuringiensis* and nematicides also occurs, being tested by Khan et al. (2011a). The results of the combination of *B. thuringiensis* and the product Fertinemakil® showed an increase in the plant growth between 300 and 400 %. This product is an organic nematicide, whose main compound is neem, and its function is to increase the nutrient content of soil, providing better physical and chemical conditions to the plant.

Bacillus thuringiensis jordanica, serotype H71, was tested against *Meloidogyne* on concentration of 10^7 viable spores/ml^{-1} using the product Vydate® as control. Based on the results presented above, this subspecies showed 52 % reduction in the number of nematodes in the roots of tomato, thereby decreasing the number of galls per root system (Khyami-Horani and Al-Banna 2006). Researchers from Korea identified a relevant nematicidal action of *B. thuringiensis* through a screening of 114 isolates, where two of them showed a high toxicity when compared to the product Carbofuran®. After 30 days of treatment in greenhouse, Carbofuran® showed 45 % of mortality and BM2x (*B. thuringiensis*) showed 22 %. However, after 60 days, the treatments showed, respectively, 60 and 52 % of mortality (Seo et al. 2012).

The eggplant crop is also the target of attack by nematodes, mainly of the genus *Meloidogyne*. The authors Ashoub and Amara (2010) used different microorganisms in vitro assays and in a greenhouse. Among these microorganisms, three isolates of *B. thuringiensis* identified were Biovar 1, Biovar 2, and Biovar 3. Second stage juveniles of each isolate were tested separately, with a mortality rate of 70 %, 85 %, and 90 %, respectively. However the mortality was higher (92 %) when the three isolates were used simultaneously after a 24 h exposure. In greenhouse tests, the authors applied a mixture of microorganisms, and the best treatments were *B. thuringiensis*, *Mycobacterium*, and *Rhizobium,* causing total nematode mortality after 72 h of treatment application.

Bacillus thuringiensis has also been used in conjunction with other microorganisms with nematicidal activity, such as *Paecilomyces marquandii* and *Streptomyces costaricanus*, in the research of Chen et al. (2000) for controlling *M. hapla* in lettuce, also showing positive results in relation to reducing the number of juveniles. A more complete understanding of the relationship between the proteins of *B. thuringiensis* and the free-living nematodes can elucidate how these potential hosts and the pathogen may have co-evolved. The extent to which bacterial toxins typically affect nematodes also has environmental implications of the impact of its widespread use in programs to reduce insect. Some proteins from *B. thuringiensis* can intoxicate multiple nematode species, while other proteins cannot (Wei et al. 2003).

The biotechnological tools are also being used for the control of major species of nematodes in crops of agricultural importance. This is the case of tomato (*Lycopersicon esculentum* var. Rutgers select), which has been transformed with the *cry*6A gene to be expresses in the roots of these plants (Li et al. 2007). The authors choose the protein expressed by the *cry*6A gene for being toxic to the nematode *Acrobeloides* spp., which is phylogenetically closely related to plant parasitic nematodes. Another reason is the size of the protein Cry6A which is the smallest of the nematicidal proteins, making it easier to synthesize and to troubleshoot gene expression. The data of these authors demonstrated that *M. incognita* was capable of ingesting Cry6A protein of 54 kDa and this protein caused toxicity to the phytoparasitic nematode, indicating a decrease in the production of descendants up to four times. These results indicate that the Cry protein of *B. thuringiensis* may confer to a plant the resistance to phytoparasitic nematodes and the Cry proteins have the potential to control nematodes in transgenic plants. *Bacillus thuringiensis* serovar *roskildiensis* was used by Sato and Asano (2004) for cloning and sequencing of a new gene, *cry*21Ba1. The authors identified 67 % similarity in amino acid sequence with the gene *cry*21Aa1, which also has nematicidal activity. These data are important because they can be used as a basis for the use in plant transformation, conferring to them resistance to attack by nematodes.

1.4 *Bacillus subtilis*

Bacillus subtilis, common in nature, has been widely studied as a potential biological agent against various plant diseases worldwide (Yang et al. 2009), is nontoxic and harmless to humans and other animals, and nonpathogenic to plants (Acea et al. 1988). The bacterium produces antimicrobial compounds in vitro, including the antibiotics zwittermicin A and kanosamine (Leifert et al. 1995), lipopeptides (Pal-Bais et al. 2004), and antifungal protein bacisubin (Liu et al. 2007). The bacteria produce endotoxins and a variety of polypeptide antibiotics of the bacillomycin group, iturin, fungistatin, mycobacillin, and mycosubtilin (Kudryashova et al. 2005). On a worldwide scale, this stimulates the isolation and selection of new *B. subtilis* strains which exhibit an even broader spectrum of activity against plant pathogens. Moreover, the strains to produce hydrolytic enzymes such as proteases, lipases, b-glucanases, and cellulases (Cazorla et al. 2007) were recorded. The mechanisms of control of this bacterium involve competition, parasitism or predation, production of metabolites, and induction of structural abnormalities caused by the diffusion of volatile compounds from the bacteria (Chaurasia et al. 2005). In soil, this species interferes in the reproductive cycle of the nematodes, acting on the larvae orientation to the host plant (Sharma and Gomes 1996). On the other hand, *B. subtilis* can also act as a plant growth promoter and helps in the control of phytopathogenic fungi, such as *Rhizoctonia solani* and *Colletotrichum truncatum*, in soybean (Araújo et al. 2005).

A research from Xia et al. (2011) utilized five *B. subtilis* strains to control *M. javanica*: 69, OKB105, M1, M1-1, and M1-2, with the three last mutants from OKB105 through genetic construction. The authors tested the cultures supernatant with different dilutions and diluents. The isolates OKB105 and 69 were the most efficient, showing 100 and 89 % mortality to the nematodes, respectively. The nematicide ingredients from the supernatant of OKB105 were evaluated in SDS-PAGE showing a protein fragment of 1,000 DA, which presented high stability in in vitro tests. The authors also identified the *pur*L gene, which regulates the synthesis of intermediary metabolites of purine and may be related to the nematicide activity of *B. subtilis* OKB105. The product Stanes Sting®, containing 1 × 10^9cell/mL of *B. subtilis* (Ehrenberg) Cohn (Stanes Company, India), was tested against *M. incognita*, in tomato plants, and reduced in 82 % the population of this nematode species, in addition to reducing the number of galls in 54 % and the egg mass/root in 72 % (Khalil et al. 2012).

Araujo and Marchesi (Araújo and Marchesi 2009) tested the PRBS-1 strain of *B. subtilis* isolated from soil under soybean, against the gall-forming nematode, and compared it to the chemical carbofuran. The results showed that *B. subtilis* increases the aboveground biomass of the plants and reduces nematode infestation. Some formulation compounds were tested by Khan et al. (2011b) and obtained as a result the formulation composed by 1 part stock (sawdust–soil molasses mixture) and 20 parts of carrier (fly ash–soil–molasses mixture) and the *B. subtilis* cells, naming it Biocure-X®. Thereafter, the authors applied this formulate in chickpea and pigeon pea seeds to control *M. incognita*, which reduced in 39 and 43 % the incidence of nematodes in these plants, respectively. Despite these recent studies with *B. subtilis*, many studies are still necessary to correctly elucidate the mode of action of this bacterium against the phytonematoids, since the data obtained in the studies abovementioned showed efficiency of this species as nematicide.

1.5 *Bacillus firmus*

Bacillus firmus is a bacterial species that has been a subject of different studies in relation to its action against nematodes. Isolates of this species secrete some toxins that are associated to an ovicidal activity in nematodes; in other words, these toxins damage the external egg pellicle of the gall-forming nematodes, inhibiting the hatching, in addition to acting against their juvenile stages (Terefe et al. 2009). The authors tested the product BioNem® WP, based on empiric informations about its nematicidal action. This formula, based on *B. firmus*, was produced in Israel and tested in tomato plants, in greenhouses. The authors demonstrated that, in vitro, BioNem® inhibits egg hatching of *M. incognita*, reducing in 91 % the gall formation in the plants roots when compared to the control. Important data of this study also showed the low concentration of the product used: concentrations of 0.5 %, 1 %, and 2 % reduced between 98 % and 100 % the egg hatch, 24 h after treatment application, in vitro. When utilized against juvenile stages (J2), the concentrations of 2.5 % and 3 % showed 100 % nematode mortality after 24 h. Despite the laboratory assays, these data show importance and promise in regarding the nematicide activity of BioNem®, requiring field studies to evaluate its efficiency.

According to Anastasiadis et al. (2007), the formulate BioNem® contains 3 % of lyophilized bacterial spores and 97 % of nontoxic active ingredients, such as plant and animal extracts. The authors also tested the efficiency of this product against nematodes, and on dilution of the 4 kg/ha^{-1}, the product presented efficiency to control the egg hatch, which is related to the production of nematotoxic substances mentioned above. Besides the mode of action of *B. firmus* elucidated by Terefe et al. (2009), it is poorly understood and few active compounds have been characterized. Another hypothesis, of the nematicidal action, is the production of toxic metabolites during bacterial fermentation, which acts on nematode survival in the juvenile stage, or J2 (Mendoza et al. 2008). A third hypothesis is focused on the competition between the bacteria and the nematodes for habitat or space on the roots, acting directly on the juveniles' death.

1.6 *Bacillus megaterium*

According to Huang et al. (2010), *B. megaterium* can promote the growth of plants because it increases the availability of phosphorus in soil. Some isolates of *B. megaterium* also produce volatile compounds, which are lethal to nematodes and can strongly inhibit hatching. The data of these authors show that after 6 days of incubation, the eggs hatched were completely inhibited, whereas the control showed 83 % hatching. Strains of *B. megaterium* may also produce antibiotic compounds (Vary 1992). In 2007, researchers from Germany isolated endophytic bacteria from rice roots, *Oryza sativa* (L.), and from seeds of five rice strains most cultivated in Bangladesh (Padgham and Sikora 2007). The authors performed a screening with 31 isolates, in which *B. megaterium* (isolate Ni5SO11) was the most efficient on nematode suppression. The methodology utilized was differenced because the rice plants of 3 weeks old were embedded in a bacterial solution of 6.10^{6} CFU/ml^{-1} and then transplanted. After 1 week, 900 J2 *M. graminicola* nematodes were applied around the roots and the evaluations were performed after 20 days of the nematode application. The results obtained showed that *B. megaterium* reduced the egg hatch in 4 % and in 14 % after 4 and 11 days, respectively.

Neipp and Becker (1999) reported that various isolates of *B. megaterium* were effective against *H. schachtii*, reducing between 38 and 59 % the penetration of J2 nematodes in eggplants. Another study also demonstrated that *B. megaterium* reduced in 50 % the penetration of both *M. chitwoodi* and *P. penetrans* in potato (Al-Rehiayani et al. 1999). These studies suggest that the condition created by the oxygen limitation in soil helps in the biological control of rice nematodes. The reduction in *M. graminicola* migration to the rice roots suggests a deficiency in the nematodes' ability to colonize the roots once *B. megaterium* may interfere in the chemical factors of perception that conduct the nematodes to the target plants.

Radwan et al. (2012) used the commercial product BioArc®, based on *B. megaterium* against *M. incognita* in tomato plants. The results obtained by the authors showed inhibition of 88 % in the number of galls and 98 % in the occurrence of J2 on plant roots, when the product is used at low concentration (5 g/kg soil). *Bacillus megaterium* PSB2 was isolated and

Table 1.2 Microorganisms of the genus *Bacillus* and their main characteristics, important to the formulation of biopesticides

Species	Characteristics	Citation
Bacillus thuringiensis	Toxic crystal production during sporulation; genes *cry*5, *cry*6, *cry*12 *cry*13, *cry*14, and *cry*21 which presented activity against nematodes; production of secondary metabolites, which reduce the attraction of the nematodes to the roots or degrade specific exudates of the roots that control the behavior of these species; phospholipase C, hemolysins, proteases, and cytotoxins	Höfte and Whiteley (1989), Jouzani et al. (2008), Vilas-Bôas et al. (2012), Khan et al. (2010)
Bacillus subtilis	Produces antibiotics zwittermicin A and kanosamine, lipopeptides, and antifungal protein bacisubin; endotoxins and a variety of polypeptide antibiotics of the bacillomycin, iturin, fungistatin, mycobacillin, and mycosubtilin groups; in soil, interferes in the reproductive cycle of the nematodes, acting on the larvae orientation to the host plant	Leifert et al. (1995), Sharma and Gomes (1996), Pal-Bais et al. (2004), Kudryashova et al. (2005), Liu et al. (2007)
Bacillus firmus	Toxin's ovicidal activity in nematodes; damages the external egg pellicle, inhibiting the hatching; acts against their juvenile stages	Anastasiadis et al. (2007), Mendoza et al. (2008), Huang et al. (2010)
Bacillus megaterium	Increases the availability of phosphorus in soil, produces volatile compounds, produces antibiotic compounds, inhibits hatching of the nematodes	Vary (1992), Terefe et al. (2009)

tested by El-Hadad et al. (2010) showing high capacity in inhibiting the colonization of *M. incognita* and showed 100 % mortality against J2 of this species. These authors identified a high enzyme activity via the production of proteases, chitinases, and gelatinases by such isolate, and high phosphate solubilization (80 ppm). The discussion of these results is focused on the improvement of soil quality and the consequent growth of plant roots by *B. megaterium* and may be indicated for biofertilization and control of nematodes. Table 1.2 shows the data from these major Bacillus species discussed above.

1.7 Biopesticides

Bacilli are considered excellent candidates for formulations applied to biological pest control. The main reason is the longevity of the species belonging to the genus *Bacillus*; due to the presence of spores, structures that are formed under unfavorable physiological conditions can remain idle for many years and are able to germinate in active cells during more favorable conditions. This ability to survive through spores is a desirable feature, as it prolongs the shelf life of commercial products. Besides the pesticides mentioned above, many bacilli species, as well as algae and other microorganisms, are already used in commercial formulations and are suitable for the control of nematodes. Table 1.3 shows these formulations marketed in different countries.

Another perspective is the joint use of biological control agents, as in the case of *B. firmus* and *P. lilacinus*. These two biopesticides were used for Anastasiadis et al. (2007), where *P. lilacinus* and *B. firmus* obtained a suppression of J2 *M. incognita* of 58 % and 66 % after 7 and 14 days, respectively, compared with the control. The blue-green alga *Microcoleus vaginatus* was utilized by Khan et al. (2005) against the nematode *M. incognita* on tomato plants. The beneficial effect on plant roots increased

Table 1.3 Other biopesticides indicated for phytonematodes

Biopesticide	Species phytonematode	Citation
Bio Zeid® (*Trichoderma album*)	*M. incognita* in tomato	Radwan et al. (2012)
Plant Gard® (*Trichoderma harzianum*)	*M. incognita* in tomato	Radwan et al. (2012)
Algaefol® (*Ascophyllum nodosum*)	*M. incognita* in tomato	Radwan et al. (2012)
Bio-Cure-B® (*Pseudomonas fluorescens*)	*M. incognita* in tomato	Khalil et al. (2012)
Bio-Nematon® (*Paecilomyces lilacinus*)	*M. incognita* in tomato	Khalil et al. (2012)
Bionem-X® (*Pochonia chlamydosporia*)	*Meloidogyne* and other nematodes	Khan et al. (2011b)
Biocomp-X® (*Pseudomonas fluorescens*)	*Meloidogyne* and other nematodes	Khan et al. (2011b)

with the increase in seaweed filtrate concentration. The nematode populations were reduced by 66 % and 97 % when used in higher concentrations. These data indicate a wide range of potential factors that may be exploited in different fields and with different technologies and products available in the market.

1.8 Considerations

Biological evolution brings along with it various changes in cropping systems, and the producer, for best crops, must adapt to these changes. An example of these changes are pests that previously had no great importance became increasingly detrimental to agricultural crops, such as nematodes, which come with a wide variety of crop species as hosts. Fumigant and non-fumigant nematicides are not used against parasitic nematodes since 1950, especially methyl bromide (Giannakou et al. 2004). Its biocidal effect can cause biological or soil imbalances. This chemical molecule was banned in 2005 (Oka 2010) and consumer demand for safe food has forced farmers to reduce the use of chemical pesticides. According to Oka (2010), only a small number of nematicides of the organophosphate and carbamate classes and some soil fumigants are available for nematode control in most countries. These pesticides are highly toxic for humans and soil application also causes subterranean water contamination and some of them are also absorbed by plants.

According to this author, the possible mechanisms involved in the suppression of nematodes are as follows: (i) the release of preexisting nematicidal compounds in the soil, (ii) the production of nematicidal compounds, such as ammonia and acid during the degradation process soil, (iii) the increase and/or introduction of antagonist microorganisms into the soil, (iv) the tolerance and resistance of the host plant, and (v) changes in the soil physiology that are unsuitable for development and behavior of the nematode. Combinations of these mechanisms, at once, may reduce the infestation of nematodes in agricultural soils. The presented perspectives show that the biological control can be used to protect the plant roots during brief periods, such as the establishment of the seedling or directly after the transplantation because when the root system is reestablished, it becomes vulnerable again to the nematode attack. In the different cropping systems, antagonist bacteria of nematodes may be coated on the seeds, including pre-germinated seeds or the roots, during the transplant.

Another factor is the production of toxic substances by microorganisms, which may limit the damage caused by nematodes, for example, through the production of antibiotics, siderophores, and a variety of enzymes. These microorganisms can also act as competitors for nematode colonization sites and nutrient utilization (El-Hadad et al. 2010). The mode of action studies cited in this work showed that root endophytes are capable of achieving multiple points of vulnerability in the nematode life cycle by inhibiting root penetration, thereby reducing the reproductive capacity and mobility of nematode and inhibiting the larvae hatching. Crop rotation and the use of resistant cultivars remains the primary management strategy to regulate the populations of these pests, but the success of this technique is limited by the size of the growing area and the occurrence of mixed populations of nematodes.

The crop rotation system may be associated with the use of resistant cultivars or low reproduction factor of nematodes. In the case of the use of resistant cultivars, Pionner (2012) launched a technical communication that indicates different soybean and maize cultivars chosen according to the reproduction factor of every kind of nematode, from the most harmful to each crop. To the crop rotation, Pionner indicates the use of nonhost species, such as *Crotalaria* spp. According to Tian et al. (2007), a better understanding of the molecular basis of several pathogenic bacterial mechanisms to nematodes will assist in management decisions and could also lead to the development of new strategies for biological control. One example cited by the authors is the recognized factors of chemical attraction between the bacteria and their hosts. Knowledge of these mechanisms could be used to target or attract nematicidal bacteria to the nematodes or to regulate the populations of nematodes by these factors.

In addition to understanding the mechanisms of control of their effectiveness and environmental impacts caused by changes in organic soil, in-depth knowledge of phytochemistry, microbiology, soil chemistry, ecology, and nematology is important. In other words, a multidisciplinary research approach, involving a collaboration of the different aspects mentioned, is the best prospect in achieving the goal of research and helps in developing methods of nematode control, not only for organic farming systems or sustainable agriculture, but also for conventional agriculture (Oka 2010).

1.9 Future Recommendations

The demand for safe and "biologically" healthy foods has stimulated an increase in researches on biological control of pests and plant diseases. The pest control through these microorganisms, naturally found in soil, is an important ecosystem service that maintains the stability of agricultural systems and has the potential to mitigate costs for control of pests. In agroecosystems, bacteria and fungi are fairly abundant and may play a role in the regulation of pest populations. Biopesticides covered in this chapter have high application in programs of Integrated Pest Management (IPM). The relevance of such use, in an economic point of view in the agribusiness sector, refers to the reduction of production costs and a possible added value to the product. Furthermore, genetically modified plants with genes from *B. thuringiensis* have great value to be explored in the interaction with the control methods discussed in this chapter. Its importance also stands by applying cutting-edge technology, clean technology, which benefits the ecosystem by decreasing the applications of agrochemicals, which consequently reduces the toxic waste in food.

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