World J Microbiol Biotechnol (2016) 32:203
DOI 10.1007/s11274-016-2165-6

REVIEW

Rhizobacteria with nematicide aptitude: enzymes and compounds associated

C. Castaneda-Alvarez[1] · **E. Aballay**[1]

Received: 23 August 2016 / Accepted: 26 October 2016 / Published online: 1 November 2016

Abstract The use of rhizobacteria to control plant parasitic nematodes has been widely studied. Currently, the research focuses on bacteria-nematode interactions that can mitigate this complex microbiome in agriculture. Various enzymes, toxins and metabolic by-products from rhizobacteria antagonize plant parasitic nematodes, and many different modes of action have been proposed. Hydrolytic enzymes, primarily proteases, collagenases and chitinases, have been related to the nematicide effect in rhizobacteria, proving to be an important factor involved in the degradation of different chemical constituents of nematodes at distinct developmental stages. Exuded metabolites may also alter the nematode-plant recognition process or create a hostile environment for nematodes in the rhizosphere. Specific bacteria strains responsible for the production of toxins, such as Cry proteins, are one of the strategies used by rhizobacteria. Characterization of the rhizobacteria mode of action could strengthen the development of commercial products to control populations of plant parasitic nematodes. This review aims to provide an overview of different enzymes and compounds produced by rhizobacteria related to the process of antagonism to plant-parasitic nematodes.

Keywords Biological control · Enzymes · Metabolites · Plant-parasitic nematodes · Toxins

✉ C. Castaneda-Alvarez
carlos.castaneda@uchile.cl

[1] Department of Crop Protection, Faculty of Agronomical Sciences, University of Chile, P.O. Box 1004, Santiago, Chile

Introduction

Plant-parasitic nematodes (PPN) are primarily soil inhabitants that damage several crops. At present, nematode control is largely based on chemical pesticides that harm the environment and human health. The increasing demand for environmentally friendly products has encouraged the exploration of biological control agents for effective and sustainable alternatives that minimize the PPN effect. In the global market of pesticides, approximately 2.5% are nematicide products, which is a low proportion compared with the high amount of agricultural losses reported due to PPN. Losses due to PPN are estimated at USD130 billion without considering other losses indirectly by interactions with other pathogens (Becker 2014). Rhizobacteria have been demonstrated to be a sustainable and environmentally safe alternative to using chemicals.

Rhizobacteria promote the plant growth via different mechanisms, such as solubilization of minerals, hormones and other compounds (Santoro et al. 2011). Additionally, rhizobacteria help suppress pests and pathogens in the soil. Several studies describing how rhizobacteria reduce PPN populations have been reported over the last 20 years (Siddiqui 2000; Aballay et al. 2013; Noreen et al. 2015). Mainly, species of *Bacillus* (Padgham and Sikora 2007; Wei et al. 2014), *Pseudomonas* (Ali et al. 2002; Chen et al. 2015) and *Serratia* (Paiva et al. 2013; Almaghrabi et al. 2013) genera have been investigated the most for their ability to antagonize nematodes. Research on the distinct strategies used by rhizobacteria to control PPN activity became considerably important in the last decades, considering the physiological divergence of rhizobacteria, the structural and behavior difference between nematode species, their life cycle, and even the environment, plant and soil conditions. Moreover, these considerations must be

studied when seeking to enhance the use of rhizobacteria formulates on a commercial scale.

Different compounds of rhizobacteria have been associated with their nematicidal effect (Tian et al. 2007b; Padgham and Sikora 2007; Paiva et al. 2013; Castaneda-Alvarez et al. 2016). The biochemical composition of different nematodes' structures make PPN susceptible to a broad spectrum of bacteria and fungi antagonistic activities during various stages of its life cycle. Significant PPN structures include collagens and lipids during mobile stages, as well as chitin, proteins and lipids in sedentary stages.

The different rhizobacterial compounds involved in nematicidal activity can be divided in two groups: enzymes that affect the external structural components in one or more developmental stages of nematodes and metabolic by-products that may be lethal to nematode organs, may affect nematode behavior, or may modify the plant-parasite recognition process. Among enzymes, the most prominently studied enzymes are proteases, which are directly evaluated on infective stages of free living nematodes and cause significant damage to their cuticle (Huang et al. 2005; Niu et al. 2006, 2007). Collagenases and chitinases have both been reported to affect nematode cuticles (Millew and Sands 1977; Page et al. 2014) and eggs (Cronin et al. 1997b; Chen et al. 2015). In the second group of metabolic by-products include hydrogen cyanide (Siddiqui et al. 2003, 2006) and 2,4-diacetylphloroglucinol in the genus *Pseudomonas* (Cronin et al. 1997a; Siddiqui and Shaukat 2003; Meyer et al. 2009).

Despite advances in the recognition of many nematode antagonistic compounds associated with rhizobacteria, the specific modes of action for most of these compounds remains unclear. The objective of this review is to present how the primary rhizobacterial enzymes and compounds control PPN and provide a perspective on their possible use on a commercial scale.

Enzymes that affect the structural components of nematodes

Bacteria, similar to other soil living microorganisms, release enzymes that facilitate attacks toward a broad host range. Several types of enzymes were considered especially interesting due to their ability to quickly degrade the main biochemical constituents of the nematode cuticle and eggshell (Yang et al. 2013). A decline in PPN populations was measured using organic amended applications with high concentrations of the main substrate compounds for the enzyme to enhance the various enzymatic activities of bacteria and soil microorganisms (Galper et al. 1990; Akhtar and Malik 2000; Ahmad and Ismail 2016). Bacteria and enzymes, part of the strategies that generate PPN suppression effect, are summarized in Table 1, with the nematode targets and susceptible developmental stages.

Proteases

Proteases are enzymes with the ability to hydrolyze peptide bonds and are classified into four classes depending of their catalytic mechanism (serine, cysteine, aspartyl and metalloproteases), but serine and cysteine proteases are reported most in bacteria with nematicidal features. These enzymes have been extensively evaluated in decades of laboratory work on rhizobacteria involved in PPN control (Millew and Sands 1977; Siddiqui et al. 2005; Niu et al. 2006; Castaneda-Alvarez et al. 2016). Nematodes present different types of structural protein and their proportions change throughout their life cycles. For example, the egg stage is comprised of a chitin/protein complex (Wharton 1983), while the mobile stage primarily has an outermost cuticle layer comprised of glycoproteins and lipids, also as insoluble proteins called cuticlin in the cortical layer (Fujimoto and Kanayh 1973; Page et al. 2014). In studies of rhizobacteria for PPN control, *Bacillus* spp. proteases are reported most (Lian et al. 2007; Castaneda-Alvarez et al. 2016).

Protease effects have been verified in field trials with application of protein sources on eggs and larval stages of *Meloidogyne javanica* (Galper et al. 1990). Activity of this particular enzyme in cuticle degradation by a rhizobacteria extracted protease was described on mobile stages of *B. xylophilus* (Paiva et al. 2013), and other free-living nematodes (Huang et al. 2005; Niu et al. 2007; Tian et al. 2007a).

Chitinases

Chitinases are enzymes that hydrolyze *N*-acetyl-D-glucosamine polysaccharide chains present in chitin. This chitin polymer is composed of structures that provide mechanical resistance during the nematode life cycle (Gortari and Hours 2008). Studies have identified genes encoding chitin synthesis, with differences between free-living and plant parasites nematodes (Veronico et al. 2001). In PPN, the highest chitin content is observed during the egg stage (Bird and Bird 1991) and is considered the most resistant stage of the nematode life cycle (Curtis et al. 2011). The chitin layer found in the egg shell is not exposed; rather, it is embedded in a protein matrix (Wharton 1983). Chitin as a constituent of larval or mobile stages has not been found to date (Veronico et al. 2001). However, in vitro assays with chitinases achieved 100% control on mobile stages of *Tylenchorynchus dubius* after 48 h, but not on *Pratylenchus penetrans* (Millew and Sands

Table 1 Enzymes reported on the PPN control by Rhizobacteria

Enzyme	Bacteria/Source	PPN	Stage	Reference
Proteases	*Brevibacillus laterosporus* G4	*Bursaphelenchus xylophilus*	Mobile stages	(Huang et al. 2005)
	Bacillus. nematocida	*Bursaphelenchus xylophilus*	Mobile stages	(Niu et al. 2006)
	Bacillus megaterium	*Meloidogyne graminícola*	Juvenile	(Padgham and Sikora 2007)
	Lysobacter capsici YS1215	*Meloidogyne incognita*	Juvenile	(Lee et al. 2013)
Chitinases	*Lysobacter capsici* YS1215	*Meloidogyne* spp.	Eggs	(Jung et al. 2014)
	Lysobacter capsici YS1215	*Meloidogyne incognita*	Eggs	(Lee et al. 2014)
	Paenibacillus illinoisenis KJA-424	*Meloidogyne incognita*	Egg hatching	(Woo-Jin et al. 2002)
	Stenotrophomonas M1-12	*Globodera rostochiensis*	Egg hatching	(Cronin et al. 1997b)
	Serratia marcescens	*Meloidogyne hapla*	Eggs, juvenile	(Mercer et al. 1992)
Lipases	*Rahnella aquatilis*	*Bursaphelenchus xylophilus*	Juvenile	(Paiva et al. 2013)
	Exogenous	*Tylenchorhynchus dubius*	Mobile stages	(Millew and Sands 1977)
Collagenases	*Bacillus cereus*	*Meloidogyne javanica*	Juvenile	(Sela et al. 1998)
	Bacillus thuringiensis FB833T	*Xiphinema index*	Juvenile, adults	(Castaneda-Alvarez et al. 2016)

1977). Reports with chitinases extracted from *Lysobacter capsici* demonstrate decreased hatching and cause damage to egg shells of *Meloidogyne* spp. (Jung et al. 2014). A synergistic antagonic effect has been found when chitinase of *Pseudomonas aeruginosa* combined with *Bacillus thuringiensis* Cry proteins was able to in vitro degrade the cuticle, shell eggs and intestines of *Caenorhabditis elegans* free-living nematode (Chen et al. 2015). Additionally, chitinases and proteases work better to prevent hatching and causes damage in egg shells of *M. javanica* (Khan et al. 2004).

Applications of organic amendments with high levels of chitin (Ahmad and Ismail 2016), and chitosan (Tian et al. 2000; Mota and dos Santos 2016) can decrease plant parasitic nematode populations. Specifically, *Heterodera glycines* (Tian et al. 2000), *Pratylenchus* spp. (Westerdahl et al. 1992; Cretoiu et al. 2013) and *Meloidogyne* spp (Westerdahl et al. 1992) populations were suppressed using above specified amendments.

Collagenases

The cuticle is the outer layer structure of nematodes and is the one with the highest collagen content. This structural protein is excreted by the hypodermal cells and is continuously renewed throughout the nematode life cycle, especially of migratory species. However, the proportions and types of collagen, are distinct during nematode life cycles and, furthermore, across different genera (Ray and Hussey 1995). Many enzymes are involved in cuticle formation (Page et al. 2014) and more than 150 genes in the *C. elegans* genome dedicated to collagens (Ray and Hussey 1995). Specialized enzymes that directly affect the cuticle have been detected in natural enemies of nematodes, leading to nematode death or increasing their susceptibility to opportunistic infections.

In vitro studies have demonstrated *Bacillus cereus* can damage the previously extracted cuticle during the second stage of the *M. javanica* juvenile (Sela et al. 1998). Likewise, ground collagen application determined a 50% reduction in *M. javanica* root galls and 90% reduction in the number of eggs per plant. Therefore, there is a stronger collagenase antagonistic effect on nematodes compared to other proteases (Galper et al. 1990). Collagenases seem to have a high PPN control potential, but there are few studies reporting its use in nematode management.

Lipases

Lipases are enzymes that hydrolyze the glycerol esters, preferably of long-chain fatty acids. The lipid content, (including the reserves) of free-living and PPN is ranging between 11 and 67% dry weight, which is higher than that of animal parasitic nematodes (Wright and Perry 2006). The egg stages of *Heterodera glycines* and *H. schachtii* possess lipid layers (Perry and Trett 1986); also, low amounts of lipids are found in the cuticle structures of the mobile stages (Page et al. 2014).

Few studies have investigated the antagonistic effect of lipases on nematodes, but one study on the activity of lipases observed that *Tylenchorhynchus dubius* populations were reduced up to 100% (Millew and Sands 1977). *In vitro* studies on *X. index* showed nematode control reported of *B. thuringiensis*, *B. megaterium* and *B. amyloliquefaciens* (Castaneda-Alvarez et al. 2016). Additional lipases from bacterial species that belonging to Microbacteriaceae,

Xanthomonadaceae, Enterobacteriaceae, Burkholderiaceae and Pseudomonadaceae families also control *Bursaphelencus xylophilus* populations (Paiva et al. 2013).

Other enzymes

Ample spectra of rhizobacteria enzymatic activities may be involved in the control efficacy on PPN. Glucanases, cellulases and pectinases from *Pseudomonas* genus have been reported to control *M. incognita* (Krechel et al. 2002). Additionally, gelatinases (metalloprotease proteins) extracted from *L. capsici* seem to be effective against *M. incognita* juveniles (J2) (Lee et al. 2013, 2014). Nevertheless, the mode of action and PPN spectrum control remains unknown.

Metabolites that affect the PPN behavior or host recognition

Secondary metabolites are important in the antagonistic action of rhizobacteria in PPN control. Although the metabolite's precise mode of action has not been elucidated, several successful examples in PPN control are discussed below and briefly presented in Table 2.

Hydrogen cyanide (HCN)

HCN is a gaseous organic compound produced by some species of *Pseudomonas* as a result of the oxidative decarboxylation of glycine (Blumer and Haas 2000). This toxic compound for the aerobic organisms, may provide an ecological advantage to bacteria in certain niches (Vining 1990). Production of this metabolite has been documented in *M. javanica* control studies using *P. fluorescens* CHA0 under lab conditions. The effect of HCN is related to inhibition of mitochondrial cytochrome oxidase (Blumer and Haas 2000; Gallagher and Manoil 2001) and coincides with low oxygen availability during in vitro assays on juvenile and eggs hatching (Siddiqui et al. 2006).

2, 4-diacetylphloroglucinol (DAPG)

DAPG is a phenolic compound principally found in *P. fluorescens*. The optimal production temperature of DAPG is 12 °C and may be influenced by the presence of sucrose, fructose and mannitol in the soil (Shanahan et al. 1992). Its specific effect on nematodes has not been elucidated, but DAPG acts as a repellent compound, stressor and nematicide (Neidig et al. 2011). DAPG has nematicidal activity on *M. javanica* (Siddiqui and Shaukat 2003), *Globodera rostochiensis* (Cronin et al. 1997a). Additionally, DAPG was associated to the effect of rhizobacteria filtrate on *Xiphinema americanum*, *M. incognita* and *Helicotylenchus indicus* (Khan et al. 2012). Studies of DAPG antagonistic activity on PPN and free-living nematodes determined its possible selective features, lethal on *M. incognita* and *X. americanum* larvae, as well as an egg hatching stimulant on *C. elegans* (Meyer et al. 2009).

Table 2 Compounds reported on the PPN control by Rhizobacteria

Compounds	Bacteria/Source	PPN	Stage	Reference
HCN	*Pseudomonas chlororaphis* O6	*Meloidogyne hapla*	Juvenile	(Lee et al. 2011)
	Pseudomonas aeruginosa	*Meloidogyne javanica*	Juvenile	(Siddiqui et al. 2003)
	Pseudomonas fluorescens CHA0	*Meloidogyne javanica*	Juvenile	(Siddiqui et al.2006)
	Pseudomonas fluorescens	*Heterodera cajani*	Juvenile	(Kumar et al. 2005)
H_2S	Exogenous	*Tylenchorhynchus martini*; *Radopholus oryzae*	Juvenile	(Rodriguez-Kabana et al. 1965)
	B. amyloliquefaciens	*Xiphinema index*	Juvenile, adults	(Castaneda-Alvarez et al. 2016)
DAPG	*Pseudomonas fluorescens* CHA0	*Meloidogyne javanica*	Juvenile	(Siddiqui and Shaukat 2003)
	Exogenous	*Meloidogyne incognita*	Egg hatch	(Meyer et al. 2009)
		Xiphinema americanun	Adults	
	Pseudomonas fluorescens F113	*Globodera rostochiensis*	Egg hatch, juvenile	(Cronin et al. 1997a)
9*H*-purine	*Bacillus cereus and B. subtilis*	*Meloidogyne exigua*	Juvenile	(Oliveira et al. 2014)
Dihydrouracil	*Bacillus cereus and B. subtilis*	*Meloidogyne exigua*	Juvenile	(Oliveira et al. 2014)
Pyoluteorin	*Pseudomonas fluorescens* CHA0	*Meloidogyne javanica*	Juvenile	(Siddiqui and Shaukat 2003)
Cry protein	*Bacillus thuringiensis*	*Meloidogyne incognita*	Juvenile	(Salehi et al. 2008)
	Bacillus thuringiensis YBT-1518	*Meloidogyne hapla*	Juvenile	(Guo et al. 2008)

Hydrogen sulfide (H_2S)

Hydrogen sulfide is a gaseous metabolite produced in animal cells and several bacterial species. This metabolite's toxic effect on PPN was demonstrated in application from exogenous sources (Rodriguez-Kabana et al. 1965). In addition, it has been reported to work under controlled conditions in bacterial strains already with other nematicidal features (Castaneda-Alvarez et al. 2016). Contrarily, positives effects were observed using lower amounts of H_2S on *C. elegans*, which resulted in an increased lifespan and higher temperature tolerance (Miller and Roth 2007).

Cry proteins

Cry proteins are produced by some *Bacillus* species and classified into families Cry1 to Cry54 on the basis of their amino acid sequence homology. Among these 54 families, Cry5, Cry6, Cry12, Cry13, Cry14, Cry21 (Guo et al. 2008), and Cry55 (Frankenhuyzen 2009) have nematicidal activity. Cry proteins cause intestinal damage of free-living nematodes and PPN (Salehi et al. 2008; Iatsenko et al. 2014b). Likewise, it appears to display a synergistic effect between some Cry proteins groups in their nematicidal action (Iatsenko et al. 2014a).

Other metabolites

Predominantly using bacterial filtrates of *Pseudomonas* and *Bacillus* genera, some other metabolites with possible nematicidal effects have been identified. Dihydrouracil, uracil, 9H-purine, were reported in the *M. exigua* mortality without a clear mode of action (Oliveira et al. 2014). Prodigiosin, the red pigment of *Serratia marcescens* efficiently controls *M. javanica* and *Radopholus similis* (Rahul et al. 2014). Furthermore, the volatile metabolites phenol, octanol, benzaldehyde, benzene acetaldehyde, decanal, 2-nonanone, 2-decanone, cyclohexene and dimethyl disulfide all are toxic on *Panagrellus redivivus* and *B. xylophilus* (Gu et al. 2007). Similarly, volatile compounds have been identified with high percentages of control on *M. incognita*, such as 2-nonanone, 2-undecanone from *B. megaterium* YFM3.25 (Huang et al. 2010).

Outlook

Rhizobacteria use has been proposed for PPN control and also for controlling other crop pests. Bacteria products currently hold 74% of the world market (Thakore 2006). The development of products based on a mix of bacterial strains with nematicide potential has been encouraged and proven to be a good choice because of their diversity of strategies (Burkett-Cadena et al. 2008). On the other hand, knowledge about the rhizobacteria action mechanisms enables their use as a powerful tool to control PPN populations (e.g. transgenic bacteria or plants, novel biochemical products). In this sense, different promising transgenic organisms were engineered to incite nematode susceptibility or to combine different antagonist characters. Chitinases genes isolated from *Paecilomyces lilacinus* nematophagous fungus were successfully inserted into the tomato plants genome, to control *M. incognita* damage (Chan et al. 2010). Likewise, tomato plants engineered to incorporate *B. thuringiensis* Cry genes were evaluated and found to control *M. incognita* attack (Li et al. 2008). Nevertheless, due to the diverse structure of nematodes, the new control strategies must involve distinct mechanisms to improve their activity. Besides, it is important to consider that the application of synthetic products decreases the levels of antagonistic soil microorganisms associated with nematode control, and lowers hydrolytic enzyme activity in suppressive soils (Bao et al. 2011). Therefore, the use of synthetic pesticides should be reevaluated in PPN management approaches that include rhizobacteria. In summary, rhizobacteria is a valuable tool to explore the biological control field, being a sustainable option against the indiscriminate use of pesticides.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

References

Aballay E, Ordenes P, Mårtensson A, Persson P (2013) Effects of rhizobacteria on parasitism by *Meloidogyne ethiopica* on grapevines. Eur J Plant Pathol 135:137–145. doi:10.1007/s10658-012-0073-7

Ahmad K, Ismail SI (2016) Utilization of Biomaterials as Soil Amendments and Crop Protection Agents in Integrated Nematode Management. In: Hakeem KR, Akhtar MS, Abdullah SNA (eds) Plant, Soil and Microbes. Springer, Cham, pp 225–251

Akhtar M, Malik A (2000) Roles of organic soil amendments and soil organisms in the biological control of plant-parasitic nematodes: a review. Bioresour Technol 74:35–47. doi:10.1016/S0960-8524(99)00154-6

Ali NI, Siddiqui IA, Shahid Shaukat S, Zaki M (2002) Nematicidal activity of some strains of *Pseudomonas* spp. Soil Biol Biochem 34:1051–1058. doi:10.1016/S0038-0717(02)00029-9

Almaghrabi OA, Massoud SI, Abdelmoneim TS (2013) Influence of inoculation with plant growth promoting rhizobacteria (PGPR) on tomato plant growth and nematode reproduction under greenhouse conditions. Saudi J Biol Sci 20:57–61. doi:10.1016/j.sjbs.2012.10.004

Bao Y, Chen S, Neher D (2011) Effect of soil disturbance and biocides on nematode communities and extracellular enzyme

activity in soybean cyst nematode suppressive soil. Nematology 13:687–699. doi:10.1163/138855410X541230
Becker JO (2014) Plant Health Management: Crop Protection with Nematicides. Encyclopedia of Agriculture and Food Systems. Elsevier, London, pp 400–407
Bird AF, Bird J (1991) The Egg. The Structure of Nematodes, 2nd edn. Elsevier, London, pp 7–43
Blumer C, Haas D (2000) Mechanism, regulation, and ecological role of bacterial cyanide biosynthesis. Arch Microbiol 173:170–177. doi:10.1007/s002039900127
Burkett-Cadena M, Kokalis-Burelle N, Lawrence KS et al (2008) Suppressiveness of root-knot nematodes mediated by rhizobacteria. Biol Control 47:55–59. doi:10.1016/j.biocontrol.2008.07.008
Castaneda-Alvarez C, Prodan S, Rosales IM, Aballay E (2016) Exoenzymes and metabolites related to the nematicidal effect of rhizobacteria on *Xiphinema index* Thorne & Allen. J Appl Microbiol 120:413–424. doi:10.1111/jam.12987
Chan YL, Cai D, Taylor PWJ et al (2010) Adverse effect of the chitinolytic enzyme PjCHI-1 in transgenic tomato on egg mass production and embryonic development of *Meloidogyne incognita*. Plant Pathol 59:922–930. doi:10.1111/j.1365-3059.2010.02314.x
Chen L, Jiang H, Cheng Q et al (2015) Enhanced nematicidal potential of the chitinase *pachi* from *Pseudomonas aeruginosa* in association with Cry21Aa. Sci Rep 5:14395. doi:10.1038/srep14395
Cretoiu MS, Korthals GW, Visser JHM, Van Elsas D (2013) Chitin amendment increases soil suppressiveness toward plant pathogens and modulates the actinobacterial and oxalobacteraceal communities in an experimental. Agric Field 79:5291–5301. doi:10.1128/AEM.01361-13
Cronin D, Fenton A, Dunne C et al (1997a) Role of 2,4-diacetylphloroglucinol in the interactions of the biocontrol pseudomonad strain F113 with the potato cyst nematode *Globodera rostochiensis*. Appl Environ Microbiol 63:1357–1361
Cronin D, Moënne-Loccoz Y, Dunne C, O'Gara F (1997b) Inhibition of egg hatch of the potato cyst nematode *Globodera rostochiensis* by chitinase-producing bacteria. Eur J Plant Pathol 103:433–440. doi:10.1023/A:1008662729757
Curtis RHC, Jones JT, Davies KG et al (2011) Biological Control of Plant-Parasitic Nematodes. In: Davies K, Spiegel Y (eds) Biological Control of Plant-Parasitic Nematodes: Building Coherence between Microbial Ecology and Molecular Mechanisms, Progress in Biological Control. Springer, Dordrecht, p 311
Fujimoto D, Kanayh S (1973) Cuticlin: a noncollagen structural protein from *Ascaris cuticle*. Arch Biochem Biophys 157:1–6
Gallagher LA, Manoil C (2001) *Pseudomonas aeruginosa* PAO1 kills *Caenorhabditis elegans* by cyanide poisoning. J Bacteriol 183:6207–6214. doi:10.1128/JB.183.21.6207
Galper S, Cohn E, Chet I (1990) Nematicidal effect of collagen-amended soil and the influence of protease and collagenase. Rev Nematol 13:67–71
Gortari MC, Hours RA (2008) Fungal chitinases and their biological role in the antagonism onto nematode eggs. A review. Mycol Prog 7:221–238. doi:10.1007/s11557-008-0571-3
Gu YQ, Mo MH, Zhou JP et al (2007) Evaluation and identification of potential organic nematicidal volatiles from soil bacteria. Soil Biol Biochem 39:2567–2575. doi:10.1016/j.soilbio.2007.05.011
Guo S, Liu M, Peng D et al (2008) New strategy for isolating novel nematicidal crystal protein genes from *Bacillus thuringiensis* strain YBT-1518. Appl Environ Microbiol 74:6997–7001. doi:10.1128/AEM.01346-08
Huang X, Tian B, Niu Q et al (2005) An extracellular protease from *Brevibacillus laterosporus* G4 without parasporal crystals can serve as a pathogenic factor in infection of nematodes. Res Microbiol 156:719–727. doi:10.1016/j.resmic.2005.02.006
Huang Y, Xu C, Ma L et al (2010) Characterisation of volatiles produced from *Bacillus megaterium* YFM3.25 and their nematicidal activity against *Meloidogyne incognita*. Eur J Plant Pathol 126:417–422. doi:10.1007/s10658-009-9550-z
Iatsenko I, Boichenko I, Sommer RJ (2014a) *Bacillus thuringiensis* DB27 produces two novel protoxins, Cry21Fa1 and Cry21Ha1, which act synergistically against nematodes. Appl Environ Microbiol 80:3266–3275. doi:10.1128/AEM.00464-14
Iatsenko I, Nikolov A, Sommer R (2014b) Identification of Distinct *Bacillus thuringiensis* 4A4 nematicidal factors using the model nematodes *Pristionchus pacificus* and *Caenorhabditis elegans*. Toxins (Basel) 6:2050–2063. doi:10.3390/toxins6072050
Jung WJ, Kim KY, Park YS et al (2014) Purification and properties of a *Meloidogyne*-antagonistic chitinase from *Lysobacter capsici* YS1215. Nematology 16:63–72. doi:10.1163/15685411-00002745
Khan A, Williams KL, Nevalainen HKM (2004) Effects of *Paecilomyces lilacinus* protease and chitinase on the eggshell structures and hatching of *Meloidogyne javanica* juveniles. Biol Control 31:346–352. doi:10.1016/j.biocontrol.2004.07.011
Khan A, Shaukat SS, Islam S, Khan A (2012) Evaluation of fluorescent pseudomonad isolates for their activity against some plant-parasitic nematodes. Am J Agric Environ Sci 12:1496–1506. doi:10.5829/idosi.aejaes.2012.12.11.1841
Krechel A, Faupel A, Hallmann J et al (2002) Potato-associated bacteria and their antagonistic potential towards plant-pathogenic fungi and the plant-parasitic nematode *Meloidogyne incognita* (Kofoid & White) Chitwood. Can J Microbiol 48:772–786. doi:10.1139/w02-071
Kumar T, Kang SC, Maheshwari DK (2005) Nematicidal activity of some fluorescent pseudomonads on cyst forming nematode, *Heterodera cajani* and growth of *Sesamum indicum* var. RT1. Agric Chem Biotechnol 48:161–166
Lee JH, Ma KC, Ko SJ et al (2011) Nematicidal activity of a nonpathogenic biocontrol bacterium, *Pseudomonas chlororaphis* O6. Curr Microbiol 62:746–751. doi:10.1007/s00284-010-9779-y
Lee YS, Park YS, Anees M et al (2013) Nematicidal activity of *Lysobacter capsici* YS1215 and the role of gelatinolytic proteins against root-knot nematodes. Biocontrol Sci Technol 23:1427–1441. doi:10.1080/09583157.2013.840359
Lee YS, Nguyen XH, Naing KW et al (2014) Role of lytic enzymes secreted by *Lysobacter capsici* YS1215 in the control of root-knot nematode of tomato plants. Indian J Microbiol 55:74–80. doi:10.1007/s12088-014-0499-z
Li XQ, Tan A, Voegtline M et al (2008) Expression of Cry5B protein from *Bacillus thuringiensis* in plant roots confers resistance to root-knot nematode. Biol Control 47:97–102. doi:10.1016/j.biocontrol.2008.06.007
Lian LH, Tian BY, Xiong R et al (2007) Proteases from *Bacillus*: a new insight into the mechanism of action for rhizobacterial suppression of nematode populations. Lett Appl Microbiol 45:262–269. doi:10.1111/j.1472-765X.2007.02184.x
Mercer CF, Greenwood DR, Grant JL (1992) Effect of plant and microbial chitinases on the eggs and juveniles of *Meloidogyne hapla* Chitwood (Nematoda: Tylenchida). Nematologica 38:227–236. doi:10.1163/187529292X00199
Meyer SLF, Halbrendt JM, Carta LK et al (2009) Toxicity of 2,4-diacetylphloroglucinol (DAPG) to plant-parasitic and bacterial-feeding nematodes. J Nematol 41:274–280
Miller DL, Roth MB (2007) Hydrogen sulfide increases thermotolerance and lifespan in *Caenorhabditis elegans*. Proc Natl Acad Sci USA 104:20618–20622. doi:10.1073/pnas.0710191104
Millew PM, Sands DC (1977) Effects of hydrolytic enzymes on plant-parasitic nematodes. J Nematol 9:192–197

Mota LCBM, dos Santos MA (2016) Chitin and chitosan on *Meloidogyne javanica* management and on chitinase activity in tomato plants. Trop Plant Pathol 41:84–90. doi:10.1007/s40858-016-0072-x

Neidig N, Paul RJ, Scheu S, Jousset A (2011) Secondary metabolites of *Pseudomonas fluorescens* CHA0 drive complex non-trophic interactions with bacterivorous nematodes. Microb Ecol 61:853–859. doi:10.1007/s00248-011-9821-z

Niu Q, Huang X, Zhang L et al (2006) A neutral protease from *Bacillus nematocida*, another potential virulence factor in the infection against nematodes. Arch Microbiol 185:439–448. doi:10.1007/s00203-006-0112-x

Niu Q, Huang X, Zhang L et al (2007) Functional identification of the gene *bace*16 from nematophagous bacterium *Bacillus nematocida*. Appl Microbiol Biotechnol 75:141–148. doi:10.1007/s00253-006-0794-7

Noreen R, Ali SA, Hasan KA et al (2015) Evaluation of biocontrol potential of fluorescent *Pseudomonas* associated with root nodules of mungbean. Crop Prot 75:18–24. doi:10.1016/j.cropro.2015.04.018

Oliveira DF, Santos Junior HM, Dos Nunes AS et al (2014) Purification and identification of metabolites produced by *Bacillus cereus* and *B. subtilis* active against *Meloidogyne exigua*, and their in silico interaction with a putative phosphoribosyltransferase from *M. incognita*. An Acad Bras Cienc 86:525–538. doi:10.1590/0001-3765201402412

Padgham JL, Sikora RA (2007) Biological control potential and modes of action of *Bacillus megaterium* against *Meloidogyne graminicola* on rice. Crop Prot 26:971–977. doi:10.1016/j.cropro.2006.09.004

Page AP, Stepek G, Winter AD, Pertab D (2014) Enzymology of the nematode cuticle: a potential drug target? Int J Parasitol Drugs Drug Resist 4:133–141. doi:10.1016/j.ijpddr.2014.05.003

Paiva G, Proença DN, Francisco R et al (2013) Nematicidal bacteria associated to pinewood nematode produce extracellular proteases. PLoS ONE 8:e79705. doi:10.1371/journal.pone.0079705

Perry RN, Trett MW (1986) Ultrastructure of the eggshell of *Heterodera schachtii* and *H. glycines* (Nematoda: Tylenchida). Rev Nématologie 9:399–403

Rahul S, Chandrashekhar P, Hemant B et al (2014) Nematicidal activity of microbial pigment from *Serratia marcescens*. Nat Prod Res 28:1399–1404. doi:10.1080/14786419.2014.904310

Ray C, Hussey RS (1995) Evidence for proteolytic processing of a cuticle collagen in a plant-parasitic nematode. Mol Biochem Parasitol 72:243–246

Rodriguez-Kabana R, Jordan JW, Hollis JP (1965) Nematodes: biological Control in Rice Fields: Role of Hydrogen Sulfide. Science(80-) 148:524–526. doi:10.1126/science.148.3669.524

Salehi JG, Seifinejad A, Saeedizadeh A et al (2008) Molecular detection of nematicidal crystalliferous *Bacillus thuringiensis* strains of Iran and evaluation of their toxicity on free-living and plant-parasitic nematodes. Can J Microbiol 54:812–822. doi:10.1139/w08-074

Santoro MV, Zygadlo J, Giordano W, Banchio E (2011) Volatile organic compounds from rhizobacteria increase biosynthesis of essential oils and growth parameters in peppermint (*Mentha piperita*). Plant Physiol Biochem 49:1177–1182. doi:10.1016/j.plaphy.2011.07.016

Sela S, Schickler H, Chet I, Spiegel Y (1998) Purification and characterization of a *Bacillus cereus* collagenolytic/proteolytic enzyme and its effect on *Meloidogyne javanica* cuticular proteins. Eur J Plant Pathol 104:59–67

Shanahan P, Sullivan DJO, Simpson P, Jeremy D (1992) Production Isolation of 2, 4-diacetylphloroglucinol from a fluorescent pseudomonad and investigation of physiological parameters influencing its production. Appl Environ Microbiol 28:353–358

Siddiqui IA (2000) Effects of cell suspension and cell-free culture filtrate of *Pseudomonas aeruginosa* in the control of root rot-root knot disease complex of tomato (*Lycopersicon esculentum* Mill.). Acta Agrobot 53:47–55

Siddiqui IA, Shaukat S (2003) Suppression of root-knot disease by *Pseudomonas fluorescens* CHA0 in tomato: importance of bacterial secondary metabolite, 2,4-diacetylpholoroglucinol. Soil Biol Biochem 35:1615–1623. doi:10.1016/j.soilbio.2003.08.006

Siddiqui IA, Shaukat SS, Khan GH, Ali NI (2003) Suppression of *Meloidogyne javanica* by *Pseudomonas aeruginosa* IE-6S+ in tomato: the influence of NaCl, oxygen and iron levels. Soil Biol Biochem 35:1625–1634. doi:10.1016/j.soilbio.2003.08.007

Siddiqui IA, Haas D, Heeb S (2005) Extracellular protease of *Pseudomonas fluorescens* CHA0, a biocontrol factor with activity against the root-knot nematode *Meloidogyne incognita*. Appl Environ Microbiol 71:5646–5649. doi:10.1128/AEM.71.9.5646-5649.2005

Siddiqui IA, Shaukat SS, Sheikh IH, Khan A (2006) Role of cyanide production by *Pseudomonas fluorescens* CHA0 in the suppression of root-knot nematode, *Meloidogyne javanica* in tomato. World J Microbiol Biotechnol 22:641–650. doi:10.1007/s11274-005-9084-2

Thakore Y (2006) The biopesticide market for global agricultural use. Ind Biotechnol 2:194–208. doi:10.1089/ind.2006.2.194

Tian H, Riggs RD, Crippen DL (2000) Control of soybean cyst nematode by chitinolytic bacteria with chitin substrate. J Nematol 32:370–376

Tian B, Yang J, Lian L et al (2007a) Role of an extracellular neutral protease in infection against nematodes by *Brevibacillus laterosporus* strain G4. Appl Microbiol Biotechnol 74:372–380. doi:10.1007/s00253-006-0690-1

Tian B, Yang J, Zhang K (2007b) Bacteria used in the biological control of plant-parasitic nematodes: populations, mechanisms of action, and future prospects. FEMS Microbiol Ecol 61:197–213. doi:10.1111/j.1574-6941.2007.00349.x

van Frankenhuyzen K (2009) Insecticidal activity of *Bacillus thuringiensis* crystal proteins. J Invertebr Pathol 101:1–16. doi:10.1016/j.jip.2009.02.009

Veronico P, Gray LJ, Jones JT et al (2001) Nematode chitin synthases: gene structure, expression and function in *Caenorhabditis elegans* and the plant parasitic nematode *Meloidogyne artiellia*. Mol Genet Genomics 266:28–34. doi:10.1007/s004380100513

Vining LC (1990) Functions of secondary metabolites. Annu Rev Microbiol 44:395–427. doi:10.1146/annurev.mi.44.100190.002143

Wei L, Shao Y, Wan J et al (2014) Isolation and characterization of a rhizobacterial antagonist of root-knot nematodes. PLOS ONE 9:e85988. doi:10.1371/journal.pone.0085988

Westerdahl BB, Carlson HL, Grant J et al (1992) Management of plant-parasitic nematodes with a chitin-urea soil amendment and other materials. J Nematol 24:669–680

Wharton DA (1983) The production and functional morphology of helminth egg-shells. Parasitology 86:85–97

Woo-Jin J, Jung S-J, Park R-D et al (2002) Effect of chitinase produced form *Paneibacillus illinoisensis* on egg hatching of root-knot nematode, *Meloidogyne* spp. J Microbiol Biotechnol Biotechnol 12:865–871

Wright DJ, Perry RN (2006) Reproduction, physiology and biochemistry. In: Perry RN, Moens M (eds) Plant Nematology, 2nd edn. CABI, Wallingford, p 447

Yang J, Liang L, Li J, Zhang K-Q (2013) Nematicidal enzymes from microorganisms and their applications. Appl Microbiol Biotechnol 97:7081–7095. doi:10.1007/s00253-013-5045-0