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Current Status of Nematode-bacteria Interaction: A Mini Review

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ABSTRACT

Nematodes are the most abundant metazoans on the planet can be found in almost all the ecosystems on earth. They may be free-living, marine, bacterivorous, or parasites of plants and animals, including human beings. Nematodes always interact with other organisms, including- viruses, fungi, protozoa, rickettsia, tardigrades and bacteria in their microbiome. Nematodes are particularly well suited for investigating host associations with bacteria because they have a long history of evolution. Nematode-bacteria interaction is now being used as a model for investigating animal-microbe interaction and can be positive (mutualistic) or negative (pathogenic/parasitic) and may be transient or stably maintained (symbiotic). In any disease complex, nematodes play important roles in association with bacteria to aggravate disease intensity. Bacteria is a potential food source for nematodes and pathogenic to nematodes. Bacterial antagonists are now getting importance as potential biocontrol agents in modern agriculture due to gradual phasing out of chemical nematicides. In addition to trophic and pathogenic interactions, bacteria can serve as mutualists by aiding nematodes in development, defense, reproduction and nutrient acquisition. Furthermore, since many mechanistic aspects of nematode-bacterium interactions are conserved, their study can provide broader insights into other types of associations, including those relevant to plant and human diseases.

Key words: Bacteria, Biocontrol agents, Disease complex, Interaction, Nematode.

Nematodes are triploblastic, bilaterally symmetrical, unsegmented, pseudocoelomic invertebrates can be found in all the ecosystems on earth. They may be free-living, bacterivorous, parasites of plants and animals and are the most abundant metazoans. They usually interact with their bacterial counterpart in their microhabitats. It may be of different types also (Table 1). Such natural interactions are useful to explore different biological relationships.

Different sorts of interactions can be found between nematodes and bacteria. It may be neutral if no one is harmed. In obligate mutualistic interactions, such as between entomopathogenic nematodes (EPNs) and their symbiotic bacteria, both parties gain. In case of a disease complex, protocooperation is noticed. Commensalism is uncommon, although it does occur. Both get injured during competition. Nematodes suffer when natural enemies attack them. WhIn case of obligate parasitic interaction, nematodes suffer during interacting (Sayre, 1988).

Synergistic interactions and disease complex

Nematodes play a critical role in the establishment of disease complexes and proliferate the disease symptoms in a variety of ways (Back *et al*., 2002). Nematode, being an obligatory parasite, rarely kills plants on their own to ensure their own existence.

Nematodes as a wounding agent

Here they act like an incitant by forming infection courts for plant pathogenic bacteria and by creating a direct passage for the entry of different soil bacteria.

Predisposition

Nematodes make conditions favourable for other organisms for the disease development in the healthy host tissues.

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Nematode-induced physiological changes to the host plant

Different sedentary endoparasites form galls (giant cells) or syncytia, *i.e*., nutrient-rich cells, which act as a substrate for bacterial colonization.

Aggravator

Infecting worms release metabolic byproducts capable of killing host cell directly and/or predispose unaffected cells to invasion by other organisms and/or stimulate the growth of harmful organisms.

Modifications within the rhizosphere

Plant-parasitic nematodes (PPNs) infected root exudates invite other microorganisms from the soil ecosystem by modifying their chemosensory behavior.

Nematodes as a vector

They transmit plant pathogenic bacteria from soil and other plant parts to meristematic tissues without involving themselves in the disease aetiology.

Reduction of host resistance

PPNs break the genetic defense of the plant by interacting with R genes and make one resistant cultivar susceptible. They usually break the mechanical barrier or alter the host physiological pathways.

Pathogen-induced changes to the host plant

Sometimes increase nematode activity in rhizosphere.

Nematode-bacteria disease complex

The Plant parasitic nematodes may predispose plants to bacterial disease through micro punctures. These may provide increased opportunities for bacteria to enter the roots directly. Nematodes carry bacterial cells externally on body surface or internally within their gut. Hunger reported for the first time in 1901 that tomato plants are readily attacked by *Pseudomonas solanacearum* in presence nematodes. The role of nematode in bacterial disease complex is to provide the bacteria with an infection court or easy access of bacteria into host through wounding. Various disease complexes are found to occur in natural or agroecosystem (Table 2).

Bacterivorous nematodes and soil health

Organic matter decomposition in the soil food web follows either a slower fungal or a faster bacterial channel (Fig 1). When bacterivorous worms graze on these bacteria and activate them, CO_2, NH_4^+ and other nitrogenous chemicals are released. In this way, they have a direct impact on the C:N ratio. They enhance mineralization by disseminating bacterial spores throughout the soil. Different metabolites released by nematodes affect bacterial growth directly or indirectly. Nematodes with a higher C: N ratio ingest more N_2 than they require, when they consume bacteria with a lower C: N ratio. They release this excess N_2 into the soil in form of excretory secretions, increasing soil N_2-content (Fig 2). The increased growing efficiency of nematodes also contributes to this. Overgrazing may have the reverse effect, since omnivores and predators at the top of the food chain consume bacterivores and bring the situation back to normal (Wang and McSorley, 2005).

Animal parasitic nematodes-bacteria interaction

Wolbachia is a widespread intracellular bacterial symbiont of arthropods and common in insects. One of their more exotic and unexpected hosts is the filarial nematodes, parasites responsible for several severe maladies, such as-onchocerciasis (river blindness), lymphatic filariasis (elephantiasis) and dirofilariasis (heartworm) (Taylor *et al.*, 2013). *Wolbachia* is only present in a subgroup of the filarial nematodes and does not extend to other groups of nematodes, either parasitic or free-living. *Wolbachia*, as a symbiont has evolved as an essential partner to key biological processes in the nematode's life, especially in the medically and veterinary important species to that extent where antibiotic elimination of the bacteria leads to a potent and effective anti-filarial drug treatment (Taylor *et al.*, 2013). The cellular and molecular basis of *Wolbachia* filarial interactions completely depends on the key processes, provided by the endosymbiont. This reliance is generally limited to lifecycle phases with high metabolic demands, such as larval growth and development and adult female embryogenesis. Furthermore, depletion of the symbiont, which has provided a safe and effective treatment to kill adult parasites with antibiotics for the first time, has impaired the persistence of filarial parasites (Taylor *et al.*, 2013).

It is a tri-partite symbiotic interaction where three different biological entities are involved. They are 1) The host reservoir (Humans and other mammals, like- dogs, cats and monkeys, *etc.*, having complex digestive system making

Table 1: Types of interactions between nematodes and bacteria.

Types	Effect on B and N				Results	Example
	Not interacting		Interacting			
	B	N	B	N		
Neutralism (Both independent)	0	0	0	0	No effect on each other	*Escherachia coli* and *Caenorhabditis elegans*
Mutualism (Both symbiotic)	-	-	+	+	Both benefitted (obligatory)	*Xenorhabdus* and *Steinernema*
Protocooperation (B and N are cooperators)	0	0	+	+	Both benefitted: facultative	*Corynebacterium tritici* and *Anguina tritici*
Commensalism (B commensal and N host)	-	0	+	?	Obligatory for B, not for N	*Arthromitus cristatus* and *Thelastoma attenuatum*
Competition (B and N competitors)	0	0	-	-	Mostly affected population got eliminated	*Rhizobium japonicum and Heterodera glycines*
Ammensalism (B inhibitor and N amensal)	0	0	0	-	B is not affected, N inhibited	*Pseudononas fluorescens and Meloidogyne spp.*
Parasitism (B parasite and N host)	-	0	+	-	Obligatory for B, N inhibited	*Pasteuria penetrans and Meloidogyne incognita*

B= Bacteria, N= Nematodes

Table 2: PPNs and associated bacteria involved in disease complex.

Crop	Disease	Nematode	Bacteria	Role in disease
Wheat	Tundu	*Anguina tritici*	*Corynebacterium tritici*	Essential
Tobacco	Vascular wilt	*Meloidogyneincognita*	*Pseudomonas solanacearum*	Assist
Tomato	Vascular wilt	*Meloidogyne hapla*	*Pseudomonas solanacearum*	Assist
	Vascular wilt	*Helicotylenchus nannus*	*Pseudomonas solanacearum*	Assist
	Canker	*Meloidogyne incognita*	*Corynebacterium michiganens*	Assist
Potato	Vascular wilt	*Meloidogyne incognita*	*Pseudomonas solanacearum*	Assist
Gladiolus	Scab	*Meloidogyne javanica*	*Pseudomonas marginata*	Assist
Carnation	Vascularwilt	*Meloidogyne incognita*	*Pseudomonas caryophylli*	Assist
Carnation	Wilt	*Meloidogyne*	*Pseudomonas caryophylli*	Assist
Lucerne	Vascular wilt	*Ditylenchus dipsaci*	*Corophium insidiosum*	Essential andAssist
Raspberry	Crown gall	*Meloidogyne hapla*	*Agrobacterium tumefacians*	Assist
Strawberry	Cauliflowerdisease	*Aphelenchodies ritzemobosi*	*Rhodococcus faciens*	Essential
Peach, Plum	Canker	*Criconemella xenoplex*	*Pseudomonas syringae*	Assist
Peach	Crown gall	*Meloidogyne javanica*	*Agrobacterium tumefacians*	Assist
Begonia	Leaf spot	*Aphelenchoides fragariae*	*Xanthomonas begoniae*	Assist
Rose	Hairy root	*Pratylenchus vulnus*	*Agrobacterium rhizogenes*	Assist

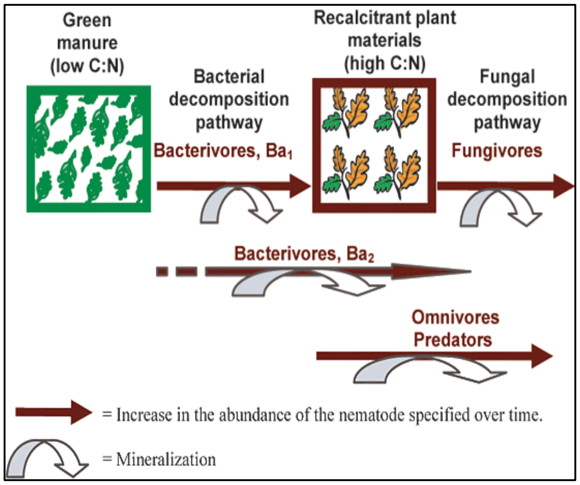

Fig 1: Nematode community succession in relation to C: N ratios of soil amendments (modified from Ingham *et al*., 1985).

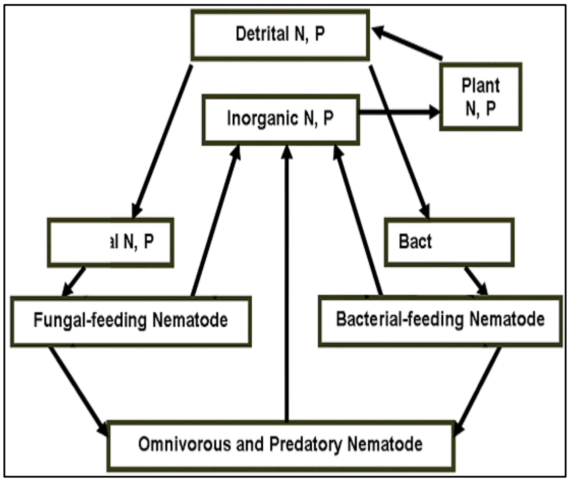

Fig 2: Roles of nematodes in organic matter decomposition (modified from Ingham *et al*., 1985).

them ideal to host parasites and endosymbionts), 2) Parasite (Filarial nematodes: thread-like adults reside in lymphatic/ subcutaneous tissues. Microfilariae circulate in the blood or migrate through tissues), 3) Endosymbiont (*Wolbachia*: bacteria which infects arthropods and some nematodes. In some cases, the host cannot reproduce, or even survive, without it).

Wolbachia is obligate, *Rickettsia*-like, endosymbiotic, maternally inherited, intracellular α-proteobacteria, round with a diameter of 0.5µm, enclosed in a double trilaminate membrane with a cytoplasm containing ribosomal-like granules (Werren *et al*., 1995, Stouthamer *et al*., 1999, Peixoto *et al*., 2001). They are found to be concentrated in intracytoplasmic vacuoles within the lateral hypodermal cords, ovaries, oocytes, developing embryos in all developmental stages of nematodes (Kozek, 1977, Taylor *et al*., 1999). Evidence of horizontal gene transfer (HGT) from *Wolbachia* has been found in *Wolbachia*-infected filariae. LGT events have either added essential *Wolbachia* genes to the nematode genome or are genetic “ghosts” of a parasitic relationship (Dunning Hotopp *et al*., 2007, McNulty *et al*., 2010, Slatko *et al*., 2010).

Wolbachia in Filarial Biology and Development of Pathology

Wolbachia is responsible for embryogenesis, normal development, fertility, survival and potent inflammatory responses from macrophages. Endosymbiont surface proteins (WSP family) are the most likely to be engaged in symbiotic interactions. The *Wolbachia* genome contains proteins (wBm0100, wBm0284 and wBm0432) that are related to the outer membrane protein (OMP) family of bacteria that are known to be involved in symbiosis (Melnikow *et al*., 2013). WSP family proteins are involved in optimizing *B. malayi*’s energy production pathway and attaching the endosymbiont to the host’s cytoskeleton

(Melnikow *et al*., 2011). *Wolbachia*'s main contribution towards nematodes and this symbiotic relationship is its ability to provide riboflavin, flavin adenine dinucleotide (FAD), heme and nucleotides.

The host nematode, on the other hand, provides *Wolbachia* with the amino acids it needs to develop. During female development, *Wolbachia* genes were mostly found in the chaperone function, energy production, nucleotide biosynthesis, DNA replication and anti-oxidative defence categories. A crucial aspect of the endosymbiotic association may be the ability to perform aerobic respiration and metabolize iron while responding to oxidative stress. At the advent of rapid bacterial growth and expansion coincided with the activation of autophagy, indicating that, despite their mutualistic association, the nematode's immune system recognizes *Wolbachia* as a 'pathogen.' The genetic and chemical activation or suppression of autophagy resulted in a corresponding decrease or increase in bacterial populations. Indeed, activating autophagy produced anti-bacterial activity comparable to antibiotic elimination, which could be used as a novel target for bactericidal drugs. *Wolbachia* must evade or avert autophagic destruction in order to maintain its population, which may be accomplished through a balance between the rate of bacterial growth and the rate of autophagic elimination, probably through modification or imitation of autophagy pathway components. (Voronin *et al*., 2012).

Wolbachia as a target against filariasis

By lowering bacteria-induced inflammation, antibiotic medicines against *Wolbachia* currently provide a viable way of therapeutic control. A strategy for breaking the parasitic cycle (*e.g*., reducing microfilarial production and preventing their development in their vectors) as well as a method for eliminating parasites that have already established themselves (*i.e*., by a macrofilaricidal effect). Anti-*Wolbachia* drugs (tetracycline) have been demonstrated to sterilize adult worms in human clinical studies. After six weeks of daily doxycycline regime application, there was a sustained reduction in microfilaremia (Taylor *et al*., 2010).

Conclusions and prospects: Animal parasitic nematodes-bacteria interaction

In a nutshell, *Wolbachia* is vital in the biology of clinically significant filarial nematodes, including a variety of cellular and molecular interactions. The essentiality of the mutualism is mostly linked with periods of rapid growth and development with high metabolic demands. But it also extends to other biological processes including vector transmission and longevity of adult worm. This symbiotic relationship provides potential for filariasis control and does not rely on a single mechanism or pathway, but rather on more complicated interactions that presumably vary over the parasite's life cycle. In embryos and microfilariae, depletion of the endosymbiont causes death of both germinal line and somatic cells.

Such research will improve our ability to exploit this reliance and could lead to new lymphatic filariasis treatments. Recent breakthroughs in 'omics technology have aided in the refinement of the target and/or techniques utilized against the bacteria. The discovery of endosymbiotic bacteria infecting the most of pathogenic filarial nematode species has opened up new avenues for research in filarial pathophysiology and immunology.

Endosymbionts and PPNs

Endosymbiotic bacteria have major effects on virulence and biology of associated PPNs. *Cardinium*, *Wolbachia*, *Xiphinematobacter* and *Burkholderia* are four major endosymbionts who arise from quite divergent phyla (Bacteroidetes, Verrucomicrobia, Alphaproteobacteria and Betaproteobacteria) and found to be associated with PPNs. Wang and Wu in 2017 explained simultaneous evolution of PPNs with their associated endosymbiont. Early plant feeding nematodes are mainly ectoparasites and have limited efficiency in feeding and inducing parasitism. But sometimes, they may acquire endosymbionts from interacting soil eukaryotes that make them fit to digest plant cells or synthesize essential nutrients which have limited supply in roots, *e.g*., *Xiphinematobacter* (endosymbiont) may synthesize essential amino acids to supplement *Xiphinema americanum* (nematode)'s diet of root phloem. Abundant carbon assimilation from newly accessible plant matter or bacteria-supplied nutrients (*e.g*., heme, lipoate and amino acids) increase the efficiency of nematodes. Nematodes achieve cell wall degrading capacity (*e.g*., cellulases, glucanases and pectinases) by acquiring additional endosymbionts, like- *Wolbachia*-like Rickettsiales ancestor. They also keep evolving with symbionts until the HGT occurs because after that these enzymes will be no longer needed from symbionts' end. Nematodes with ability to support symbionts are more rapidly colonized by new endosymbionts. It may be called as symbiont facilitation or replacement, possibly in case of *Cardinium*- or *Burkholderia*-like symbionts.

Cardinium: A Discontinuous Symbiont

This rod-shaped, gm (-) ve bacteroidetes is of major interest as its capacity to modify host reproduction through parthenogenesis induction and cytoplasmic incompatibility (CI). It has a very ancient association with PPN (~270 MYA). Earliest record of *Cardinium* from PPN was by Shepherd *et al*., 1973 in *Globodera rostochiensis* as a "rickettsia-like organism". They are abundant in the cells of ovary, wall of oviduct and oocytes suggesting vertical transmission. They colonize pseudocoelom and intestine (juveniles, males and females), hypodermis (juveniles and males), sperm and they are found in close association with mitochondria and sperm nucleus (Walsh *et al*., 1983).

Males of *G. rostochiensis* had the highest infection densities in the esophageal glands but there was no effect on the survival between infected and uninfected populations

(Walsh *et al*., 1983). Subsequent 16S rRNA and gyrB gene sequencing designated the organism as genus *Cardinium* [formerly *Paenicardinium* (Noel and Atibalentja, 2006)], under the family Amoebophilaceae and phylum Bacteroidetes (Santos-Garcia *et al*., 2014). The degree of infection (*i.e*., number of bacteria/host) increased after hatching and infected J2s died little earlier than uninfected ones. It may be due to depletion of the stored lipid. Even when the ovaries and oviduct were highly infected, tissues containing *Cardinium* cells seemed to be unaffected and fertilized eggs appeared normal, suggesting a commensal or facultative mutualist role.

Localization of *Candidatus*

Electron micrographs confirmed the presence of '*Candidatus Paenicardinium endonii*' in *H. glycines*. Bacteria can be observed in the intestine, pseudocoelom and spermatozoa within a testis but cannot found in somatic muscles, hypodermis or the cuticle. In female, bacteria are found to be localized in ovary, oocytes and pseudocoelom (Noel and Atibalentja, 2006).

They are closely associated with host nuclei or free in cytoplasm of muscle cells, marginal cells and cells adjacent to the dorsal esophageal glands. They are found encircled by a dense matrix of RER, in parallel or concentric arrays adjacent to secretion granules (Endo, 1979). They can be treated as commensal or facultative mutualist (Walsh *et al*., 1983).

Host	Location	References
Globodera rostochiensis	Bolivia	Shepherd *et al*., 1973
Heterodera avenae	China	Yang *et al*., 2017
Heterodera goettingiana	UK	Shepherd *et al*., 1973
Heterodera glycines	US	Noel and Atibalentja, 2006
Pratylenchus penetrans	US	Denver *et al*., 2016

Metabolic and genomic features

First *Cardinium* genome strain *cEper1*, described from *Encarsia pergendiella* lacks major biosynthetic pathways. So, it needs metabolites and ATP from host. It has many transporter genes which aid in uptake of oligopeptides and amino acids. Biotin and lipoate biosynthesis genes supplement host nutrition. Lipoate is a conserved S-containing cofactor in oxidative reactions, associated with pathogen virulence (Penz *et al*., 2012).

Biotin is an essential carboxyl carrier involved in fatty acid metabolism and amino acid catabolism and can't be synthesized by most of the animals. Vitamin B is thought to be synthesized by obligate symbionts. Bacteria-like biotin synthase gene (*HgBioB*) has found to be encoded in the genome of *H. glycines.* It appears to be associated with virulence factor and suspected to be the result of HGT (Bekal *et al*., 2015). *Cardinium* genome in a strain *cBtQ1* from *Bemisia tabaci* is found to be devoid of pyridoxal (precursor of vitamin B6) synthesis (*pdxT*) and biotin synthesis genes (*bioB, bioF*) (Santos-Garcia *et al*., 2014). The reasons behind the loss of these Vitamin biosynthesis genes may be from the relaxed needs for those vitamins in whiteflies due to presence of an array of 1° and 2° symbionts which can participate in vitamin biosynthesis. The genome also contains gliding motility genes which aid in migration within host and transmission through plant tissues (Gonella *et al*., 2015). Some potent toxin genes (*rtxBDE*) are also acquired through HGT from *Vibrio spp*. and some are similar to *Wolbachia* to provide protection against parasitoids. Abundant ubiquitin-modifying enzymes and conserved antifeeding prophage (AFP)-like protein secretion system interfere with eukaryotic cell regulation and CI (Penz *et al*., 2012). Transfers of vitamin B synthesis genes and plant cell digesting enzymes are the signs of evolution of plant parasitism.

Xiphinematobacter: A Nutritional Mutualist

It was first recorded in 1963 by Adams and Eichenmuller but incorrectly classified as *Pseudomonas denitrificans.* They are widespread in *Xiphinema americanum* group (clade I), occurring in 27 nominal species within this complex. They can be observed in oocytes, ovaries (always in amphidelphic ovaries), undifferentiated and embryonated eggs containing J1. In eggs, clustering is occurred at the anterior pole, specifically in the endodermal founder cell, from where they spread developmentally to all endodermal daughter cells and adult intestinal epithelia *i.e*., they are maternally inherited.

Localization of *Xiphinematobacter*

Palomares-rius *et al*., 2016 detected bacterial endosymbionts of *Xiphinema pachtaicum* in FISH experiments using the Burkho_1 probe and analyzed by confocal laser scanning microscopy. It showed the presence of bacterial endosymbionts inside the ovaries and intestine, which occupies mostly the entire female nematode body.

Host	Location	References
Pratylenchus penetrans	US	Brown *et al*., 2016
Radopholus arabocoffeae	Vietnam	Haegeman *et al*., 2009
Radopholus similis	Uganda, Indonesia	Haegeman *et al*., 2009

Xiphinematobacter are found to be occupied the intestinal wall of females and larvae, especially the anterior intestine. In males, the gut epithelium is the main site of infection which proves the possible role in nutrition (Vandekerckhove *et al*., 2002). Endosymbionts supplement the phloem feeding and it is considered as a convergent evolution like hemipteran insects (Brown *et al*., 2015). They have a possible role in inducing parthenogenesis.

Metabolic and genomic features

Xiphinematobacter lacks the major biosynthetic genes (required for free-living life style) for amino acids, vitamins and coenzymes/cofactors for TCA cycle as well as plant cell digesting enzymes. They supplement non-essential amino acids to host with conserved lipoate and heme synthesis genes. Burkholderiaceae (Betaproteobacteria) is associated with members of *X. americanum* clade II and colonize the gut lumen, intestinal walls and ovaries with abridged amino acid pathways.

Coevolution of host and endosymbiont

Initial loss of *Xiphinematobacter* as an endosymbiont in some nematode population leads to subsequent colonization by *Burkholderia*. Coevolution is completely related to phylogenetic relationship. For some species, phylogenetic position is difficult to explain with unique introduction event theory. *e.g.*: *Xiphinema vallense* and *X. madeirense*.

Wolbachia: An early endosymbiont

It is an obligate, gm (-) ve, Rickettsia-like, maternally inherited, intracellular á-proteobacteria, round, enclosed in a double trilaminate membrane with a cytoplasm containing ribosomal-like granules (Taylor *et al*., 2013). It has a very old association with PPN of almost ~462 million years. They are found to be concentrated in intra cytoplasmic vacuoles within the lateral hypodermal cords, ovaries, oocytes, developing embryos in all developmental stages. They supplement riboflavin and other vitamin B genes, flavin adenine dinucleotide (FAD), heme and nucleotides and act as a facultative mutualist or secondary symbiont (Moriyama *et al*., 2015).

There are two types of *Wolbachia* supergroups. First type (A and B): They impact host reproduction through CI and second type (C, D and F): They are essential for nematode survival and therefore they are targeted for antibiotic treatment. They have a potential role in reproductive behaviour and sex ratio manipulation. They mainly induce reproductive abnormalities in 4 ways: CI, male killing, genetic male feminization and parthenogenesis induction. They remain densely packed in the anterior intestine of host. Presence of *wPpe* and *wRad* strains of *Wolbachia* in PPN (respectively *Pratylenchus penetrans* and *Radopholus similis*) proves that they are long diverged nearest sisters in phylogeny.

Phylogenetic position and sex ratio distortion

Phylogenetic trees are showing basal place of *wPpe* which indicates that invaded nematodes evolved at least 2 times (Wasala *et al*., 2019, Brown *et al*., 2016).

Generally, in uninfected field population, male and female ratio of nematodes remains uniform and statistically significant *i.e*., 1:1 but infected population shows female biasness. The same phenomenon ca also be observed in case of soft bodied insects and lepidopterans.

Localization of *Wolbachia*

Localization of *Wolbachia wPpe* in *P. penetrans* was detected by fluorescence in situ hybridization (FISH) using confocal microscopy. Anterior region of adult female nematode was found to be densely packed with *Wolbachia*. Ovaries containing developing oocysts also contains *Wolbachia* cells (Brown *et al*., 2016).

Metabolic and genomic features

Typical endosymbiont characteristics of *WPpe* are reduced size of genome and G+C content, loss of metabolic capacity. They have evolved from bottlenecks, mutational bias (towards deletions and C to T transitions) and relaxed purifying selection on host-supplied metabolic processes. They possess highly conserved iron metabolism genes across the strains including *WPpe* as iron is limited in roots. Insect dominating clades share amino acid coding genes (*metK, argD* and *aspC*), vitamin B synthesis genes (*pdxJ* and *fgs*), a terpenoid gene (*ispA*) and a biotin transporter gene (*bioY*).

The obligate mutualistic clades share amino acid genes (*gltA, proP, iscS, dapA, gltB* and *adiC*) and vitamin/cofactor genes (*coaE, coaD* and *hemE*). These genes are mostly related to *wDim* and *wLs*. Only one (*ribB*) out of six riboflavin synthesis genes retained in *Wolbachia* from *P. penetrans* as per riboflavin provisioning hypothesis with a complex and nonvertical evolutionary history (Moriyama *et al*., 2015). Absence of biotin and thiamine biosynthesis genes supports the HGT in *wCle* from an ancestor of the bedbug. Placing of PPN *Wolbachia* strains basally at the root of the clade supports an ancient association of this endosymbiont with PPNs. Subsequent invasions of the phylum Nematoda (in clades F and C+D) which fits the pattern of host-use. C+D groups require intermediate blood-feeding arthropod hosts to transmit filarial nematode parasites to vertebrates. Close evolutionary relationship with *wPni* and *wBry* suggests early emergence as a plant specialist.

EPN-microbe symbiosis

Roles of nematode counterpart

Nematodes carry the symbiotic bacteria inside the insect body or hemocoel and protect those symbionts from external environment and also inhibit the antibacterial protein of host.

Roles of bacterial counterpart

Bacteria kill the insect host by causing septicemia and create a suitable environment for nematode development. They prevent secondary infection by microbes and protect the nematodes from antibiotics produced by other bacteria. Nematodes fetch their nutrition from digested host tissue, upon act of bacteria.

Heterorhabditis-photorhabdus system

Photorhabdus is a gram-negative gamma-proteobacteria belonging to the Morganellaceae family that lives in symbiosis with *Heterorhabditis*. They invade the proximal part of IJs' guts and are closely associated with the gut epithelia. *Photorhabdus* contributes to IJ recovery, nematode growth and development and colonization of the newly produced generation of IJs (transmission) (Clarke, 2020).

Phases of symbionts

Phase I (primary phase) promotes nematode growth, produces antibiotics, forms cottony blue colony and easily revert to 2nd phase, whereas, phase II (secondary phase) is a laboratory phenomenon, no antibiotic is produced, not easily revert to 1st phase and forms red colony.

Different forms of symbionts

Photorhabdus has two different forms: one is pathogenic (P) and the other is mutualistic (M). A reversible switch known as the 'mad switch' controls the phase variation. P-form is essential for insect pathogenicity and supports nematode growth and development, whereas M-form is mainly involved in transmission. The orientation of the mad switch regulates phase variation. With the increasing age of culture, the number of M-form cells rises in a population (Somvanshi *et al*., 2012).

HexA, a LysR-type transcriptional regulator (LTTR) originally represses mutualism. HexA inhibits numerous activities secondary metabolic activities, including bioluminescence and formation of IPS (Isopropyl stilbene) and AQ (Anthroquinone) (Engel *et al*., 2017, Kontnik *et al*., 2010). Mutualism-related actions are suppressed by HexA. It's important for pathogenicity and symbiosis (Joyce and Clarke, 2003).

Hfq is an RNA chaperone that facilitates base pairing between small regulatory RNA and their target mRNA transcripts (Hör et al., 2018, Woodson et al., 2018). Hfq mutant *P. laumondii TTO1* does not produce IPS or AQ, but their virulence remains same. They do not support nematode growth and development, hence, no mutualism. Here hexA was overexpressed. Hfq and hexA double mutant restored mutualism, suggesting hfq-mediated post-transcriptional repression of hexA production (Tobias *et al*., 2017). This regulatory network plays a crucial role in the transition from pathogen to mutualist. Where amino acid is deficit, ppGpp regulates gene expression and supports transition from P to M form.

During pathogenic activity, PhoPQ, a bicomponent pathway, confers CAMP resistance. Its mutation shows an avirulent reaction. Through T3SS, inhibition of phagocytosis occurs by insect hemocytes. LPS modifies AMPs in different ways, including L-aminoarabinose's ligation to lipidA to shield negative charges associated with PO_4^{3-} in the moiety. This modification is catalyzed by proteins encoded by genes in the PhoPQ-regulated pbgPE operon, which serves virulence and AMP resistance in bacteria (Bennett and Clarke, 2005, Derzelle *et al*., 2004). pbgPE operon is required for transmission and colonization.

Mad pilli are the major bacterial determinants involved in adherence and invasion (Somvanshi *et al*., 2010). Invertible DNA (mad switch) is used to code them. Mad pili is formed when it is turned on. The bacteria develop small colony variants that don't produce stilbene antibiotics, light, don't support nematode growth and become avirulent, but nevertheless play a role in transmission. Mad pili are essential for pathogenicity, growth and development and are not synthesized when it is turned off (Somvanshi *et al*., 2012). During colonization and pathogenicity, genes associated in the LPS production pathway play a key role. In the adult nematode gut, HdfR plays a critical role in modulating the temporal course of bacterial infection.

Xenorhabdus

It is a motile, Gram-negative, gammaproteobacteria under the family Morganellaceae in a symbiotic association with *Steinernema*. They colonize in a pocket (vesicle) distinct from the intestine, within a vesicle in the anterior region (Bird and Akhurst, 1983). *X. nematophila* adheres to anucleate clusters of spheres, collectively known as intravesicular structures (IVS).

Molecular basis of interaction

PhoPQ also controls *Xenorhabdus* colonization and pathogenicity. FlhDC controls flagellar regulation and secretion, which is further regulated by three transcription factors. Transcription of FlhDC is negatively influenced by bicomponent regulatory pair Envz/OmpR (Kim *et al*., 2003; Park and Forst, 2006) and positively regulated by global regulator Lrp (Cowles *et al*., 2007) and this LysR type transcription factor. ompR mutants are fully virulent, whereas Lrp or LrhA mutants are defective in virulence (Forst and Boylan, 2002; Cowles *et al*., 2007). NilR expression causes reduced colonization of bacteria. This global regulator synergistically acts with Lrp, NilA, B and C to permit colonization (Goodrich-Blair and Clarke, 2007). Thus, Lrp is required for normal nematode development and colonization.

Nematode and its bacterial antagonists

Pasteuria

Pasteuria is a gram-positive, dichotomously branched, endospore-forming bacterium (*Bacillus-Clostridium* clade) with septate mycelium hyper-parasitizes nematodes. They are parasitic on Nematoda and Anomopoda, two different groups of invertebrates. *P. ramosa* has been discovered to be parasitic on *Daffnia* for the first time. *P. penetrans* is parasitic on *M. incognita, P. nishizawae* on cysts and *P. thornei* on *Pratylenchus brachyurus*. The actual endospore of *Pasteuria* is a central, multilayered core surrounded by layers of epicortical parasporal fibres and outside episporic coatings (exosporium). They have a distinctive rounded, cup-like 'aerodynamic' shape that allows them to withstand the stresses exerted on their surface by the moving host.

Life cycle

Bacterial growth begins inside the nematode with the formation of the germ tube (peg) from the endospore, that gives rise to a vegetative microcolony 8-10 days after penetration. Following that, the "cauliflower-like" mycelial microcolony matures and fragments. The fragmentation continues through the formation of quartets, triplets, doublets and the subsequent production of immature endospores at 25-28 days after inoculation. The pre-endospores then mature and ultimately turn into ellipsoidal cup-shaped when fully developed. Almost after 6 weeks of inoculation, millions of endospores come out from the nematode body. The

Pasteuria infected females exhibit no trace of genital development as compared to the normal ones, become sterile and do not produce any egg mass (Phani and Rao, 2018). Different life stages of *Pasteuria penetrans* infecting *Meloidogyne incognita* as redescribed by Phani and Rao in 2018: A) Endospore encumbered J2, B) Endospore germination, C) Cauliflower-like microcolony, D) Fragmented microcolony, E) Star-shaped stage, F) Quartet, G) Triplet, H) Doublet, I) Endospore development, J) Mature endospores.

Infection pathology

A fibrous nap surrounds and covers the central body of the skirt-like endospore. Davies proposed this velcro-like model in 2009. It is made up of collagen and fibronectin, which help to attach on the juvenile cuticle (Davies and Danks, 1993). Surface coat characteristics are influenced by genetics and play a role in bacterial adhesion. Host-specificity is modulated by the variety of microfibrils on spores. Many cuticular genes involved in bacterial adhesion also have a role in glycosylation. Glycosylation takes place at the Golgi bodies or the ER, where proteins can be sugar-decorated, a process that demands the use of glycosyltransferases (Darby *et al*., 2007). Nucleotide sugar transporters and glycosyltransferases are involved with bacterial adherence encoding genes. Mucins (highly glycosylated polypeptides) contain a lot of serine and threonine, along with a lot of O-glycosylation sites (Hicks *et al*., 2000).

Polyclonal antibodies produced on the cuticle's surface impair the spore binding ability by blocking receptors on the surface (Davies and Lander, 1992). The spore attachment is improved by the use of a monoclonal antibody (HC/145) (Sharma and Davies, 1996). Spore attachment is affected by fibronectin, heparin and the gelatin binding domain (Mohan *et al*., 2001). The carbohydrate-protein interaction (lectin) is important for the specificity of carbohydrate moieties adhesion and interaction (Bucior and Burger, 2004). Antigenic mimicry (molecular camouflage) is a type of antigenic mimicry. Fucosyl-bearing glycoprotein is a monoclonal antibody produced particularly against nematode cuticle. The fucosyl-bearing epitope (antigen) is a signalling molecule that derives from nematodes. FAR (Fatty acid and retinol-binding protein) and SeBP (Selenium binding protein) protect the worm body from spore attachment (Phani *et al*., 2017; Phani *et al*., 2018).

Epiphytic nematicidal bacteria

Antibiotics, metabolites, volatile organic compounds (VOCs) and excretory enzymes produced by Rhizobacteria or PGPR (Plant growth-promoting rhizobacteria) are toxic to nematodes (Table 3). Upon breakdown, protein and nitrogenous compounds generate NH_3 and NO_3, which are directly harmful to soil nematodes. Cuticles are degraded by excretory enzymes, which subsequently kill nematodes. They are not host-specific and endoparasites are rarely affected by them. They use repellence, chemotactic modulation, nematicidal and plant growth promotion as methods of suppression. Plant growth is influenced by PGPR through indirect mechanisms such as nematode suppression, bacterial and fungal pathogen competition, antibiotic and siderophore-mediated suppression, HCN formation and induced systemic resistance (ISR) (Table 4).

Table 3: Bacterial groups with nematicidal activity.

Bacterial group	Members	Target nematodes	Pathogenic effects	Mode of action
Parasitic bacteria	*Pasteuria. penetrans,* *Candidatus P. thornei,* *P. nishizawae, P. usgae*	323 nematodes species of 116 genera	Major economic important PPNs have been observed to be parasitized	Parasitism
Opportunistic parasitic bacteria	*Bacillus nematocida* (*Bacillus sp.* B16); *Brevibacillus laterosporus*. *Bacillus sp*. RH219	*Panagrellus redivius* and *Bursaphelenchus xylophilus*	Penetrate the nematode cuticle and eventually digest the target organism	Parasitism, production ofenzymes and toxin
Parasporal crystal-formingbacteria	*Bacillus thuringiensis* (Cry5, Cry6, Cry12, Cry13, Cry14, Cry21)	*Trichostrongylus Colubriformis, C. elegans*	Cry proteins are toxic to larval stages of free-living and parasitic nematodes	Cry proteins damage to nematode intestine
Rhizobacteria	*Bacillus* (> 15 species) and *Pseudomonas* (> 11 species) are the most dominantpopulations	Reduce nematode populations in soil	Show different degrees of suppression onnematodes in various conditions	Interfere with recognition, toxin production, competition for food, plant-growthpromotion, induction of systemic resistance
Symbiotic bacteria of EPN	Two genera: *Xenorhabdus* and *Photorhabdus*	*B. xylophilus, M. incognita* and their eggs	Toxic to juveniles of these nematodes, inhibit egg hatch	Toxin production (NH_3,indole and stilbene derivative
Endophyticbacteria	Majority of rhizobacteria act as endophytes	Root-knot androot-lesion nematode *etc*.	Suppress nematode population	Same mechanisms as Rhizobacteria

Table 4: PGPRs in suppressing PPNs.

Species	Metabolites	Target	Host	References
B. pumilus	Protease Chitinase	*Meloidogyne arenaria*	Tomato	Lee and Kim, 2016
B. cereus	Sphingosine Zwittermycin A Kanosamine	*Heterodera avenae* *M. incognita* *M. javanica*	Wheat	Ahmed *et al.,* 2019
B. megaterium	Decanal Benzeneacetaldehyde Dimethyl disulphide	*H. glycines* *M. incognita* *M. graminicola*	Tomato	Huang *et al.*, 2010
B. firmus	Serine protease Sep 1	*Ditylenchus dipsaci*	Tomato	Terefe *et al.,* 2012
		Heterodera spp. *M. incognita*	Soybean	Xiong *et al.,* 2015
		Pratylenchus spp. *Radopholus similis*		Geng *et al.,* 2016
B. subtilis	Lipopeptide antibiotics Gelatinase	*Helicotylenchus multicinctus*	Tomato	Basyony and Abo-Zaid, 2018
	Protease Chitinase	*M. graminicola,* *M. incognita,* *M. javanica,* *R. reniformis*	Sugarcane	Mazzuchelli *et al.,* 2020
Pseudomonas chlororaphis	HCN	*M. hapla*	Tomato	Kang *et al.*, 2018
P. fluorescens	2,4-DAPG and HCN	*M. javanica*	Tomato	Siddiqui and Shaukat, 2003

CONCLUSION AND LEADS

- Understanding the basic biology of interaction can help in improving the biocontrol traits of bacterial bioagents by exploiting their basic parasitic nature, metabolical demands for sustenance, ecological needs, intra- and interspecific variations and the factors underlying specificity *etc*.
- Maneuvering the underlying gene expression profile can surely interfere with processes like response of biocontrol agents towards the chemical cues from PPNs to drag it in favor of nematode control.
- Interference with nematode attack can reduce the disease severity of lethal bacterial diseases of plants and animals.
- Symbiotic interactions between nematode and bacteria can be exploited to understand bacterial interaction with higher organisms.

Conflict of interest: None.

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