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Review article

Entomopathogenic nematodes and their bacterial symbionts: The inside out of a mutualistic association

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Abstract

Entomopathogenic nematodes *Steinernema* and *Heterorhabditis* spp. (Nematoda: Steinernematidae, Heterorhabditidae) and their bacterial symbiont bacteria *Xenorhabdus* and *Photorhabdus* spp (Gram-negative Enterobacteriaceae) represent an emerging model of terrestrial animal-microbe symbiotic relationships. *Xenorhabdus* and *Photorhabdus* spp. are harbored as symbionts in the intestine of the only free-living stage of the nematodes, also known as the infective juvenile or 3rd stage infective juvenile. The bacterium-nematode pair is pathogenic for a wide range of insects and has successfully been implemented in biological control and integrated pest management programs worldwide. Moreover, realization of the practical use of these nematodes has spurred developments across a far broader scientific front. Recent years have seen an intensive worldwide search for fresh genetic materials resulting in an exponential growth of described new species and the discovery of thousands of new isolates worldwide. These nematodes and their bacterial symbionts are now considered a tractable model system that is amenable to study physiological, chemical, structural and developmental aspects of beneficial symbiotic associations. We herein provide an overview of the research done in relation to the study of the symbiotic interactions between *Steinernema* and *Heterorhabditis* nematodes and their bacterial symbionts.

Keywords: Insect pathogens, nematodes, bacteria, mutualists

1. Introduction

An emerging model of terrestrial animal-microbe symbiotic relationships is represented by the mutualistic partnership between the entomopathogenic bacteria *Xenorhabdus* and *Photorhabdus* spp. (Gram-negative Enterobacteriaceae) and their nematode hosts, *Steinernema* and *Heterorhabditis* spp. (Nematoda: Steinernematidae, Heterorhabditidae). Together, nematodes and their symbiotic partners form an insecticidal complex that is effective against a wide range of insect hosts (Gaugler and Kaya, 1990; Kaya and Gaugler, 1993). Interest in studying this nematode-bacteria entomopathogenic complex, mainly arose because of their potential application in the biological control of insects and other arthropod pests (Tanada and Kaya, 1993). Currently, numerous laboratories worldwide are implementing this nematode-bacteria complex in agriculture, medicine and industry. Progress in these areas coupled with the inherent experimental tractability of both nematode host and bacterial symbionts makes this mutualism an outstanding model for basic research in ecology, evolution, biochemistry, molecular genetics and symbiosis (Burnell and Stock, 2000; Goodrich-Blair and Clarke, 2007).

2. Life Cycle of a Mutualistic Partnership

The symbiotic bacteria are carried in the gut of a specialized free-living form of the nematode that is the third-stage infective juvenile (IJ). Steinernematid

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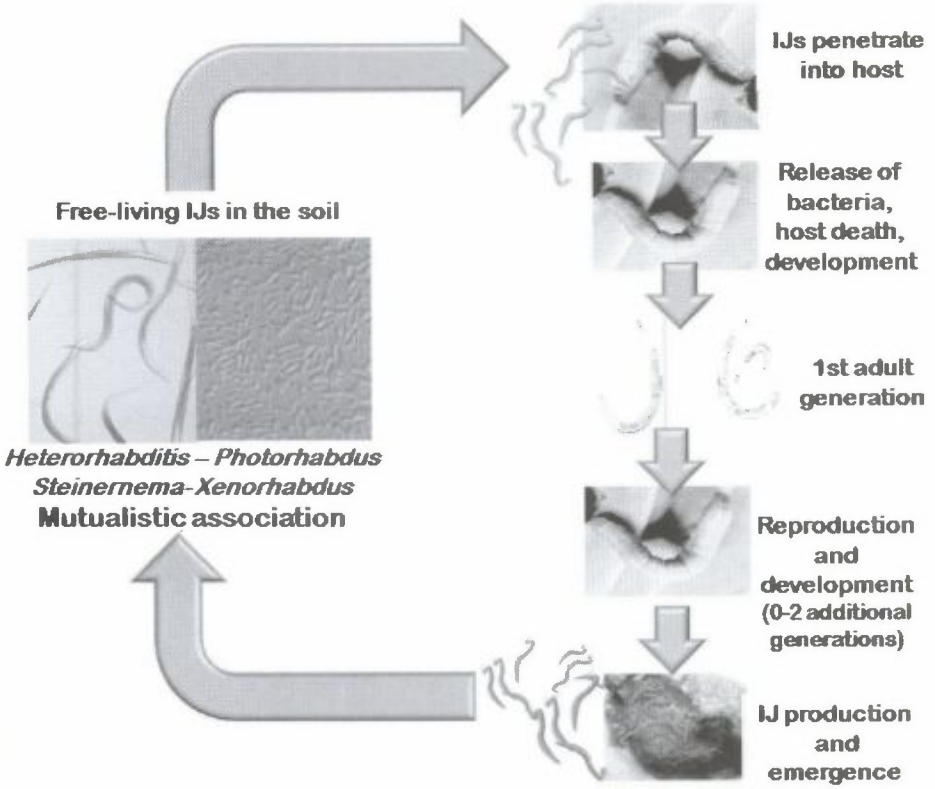

Figure 1. Generalized life cycle of entomopathogenic nematodes.

nematodes harbor symbiotic bacteria in their intestine and live in the soil until they invade a susceptible insect host. Once in the hemocoel, the IJ release their symbionts. At this point in their life cycles, bacteria and nematodes exist separately although in close proximity to one another. The released bacteria contribute to the killing of the insect host and grow to high density in the resulting cadaver. These specific bacteria are essential for nematode growth and development presumably both by serving as a direct food source and by supplying nutrients through degradation of the insect carcass. When nematode numbers become high and nutrients become limiting in the insect cadaver, nematode progeny re-associate with bacteria and differentiate into the colonized, non-feeding IJ form that emerges into the soil to forage for a new host. IJ formation and concomitant colonization with bacteria is a model of the process by which a specific and intimate association between a microbe and a eukaryotic host in a benign relationship develops (Fig. 1).

Entomopathogenic nematode isolates and species show substantial (and in certain cases surprising) variation in behavior, host range, infectivity, reproduction, and environmental tolerances. This biological variation has stimulated interest in more fully characterizing the genetic diversity of these nematodes because new strains and species may have different biological and/or ecological traits and may also prove more useful than those currently considered as biological control agents against agriculturally important pests (El-Borai et al., 2007; Oestergaard et al., 2006; Shapiro-Ilan et al., 2006; Vinciguerra and Clausi, 2006). Moreover, a number of recent technical advances have made this nematode-bacterium system amenable to study physiological, chemical, structural and developmental aspects of the symbiosis not previously possible (Flores-Lara et al., 2007; Hallem et al., 2007; Heungens et al., 2002; Joyce et al., 2006; Vivas and Goodrich-Blair, 2001; Volgyi et al., 2000; Forst and Boylan, 2002). All these aspects, together with the genetic tractability of this system, the development of diverse molecular tools and the ability to reconstitute in the laboratory the natural biological life cycle of both organisms, make this symbiotic partnership an ideal model system in which to study biological, ecological and evolutionary details of beneficial host-microbe interactions.

3. Nematode Hosts

Taxonomic status

Entomopathogenic nematodes currently comprise two families: Steinernematidae (Chitwood and Chitwood, 1937)

and Heterorhabditidae (Poinar, 1976). Steinernematidae comprise two genera: *Steinernema* (Travassos, 1927) with more than 50 species and *Neosteinernema* (Nguyen and Smart, 1994) with only one species *N. longicurvicauda*. Heterorhabditidae is a monotypic family, with only one genus, *Heterorhabditis* (Poinar, 1976) and more than a dozen species described (Table 1).

Delimitation of species has mainly been based on comparison of morphological and/or morphometric data and cross-breeding tests, with the biological species concept forming the conceptual framework for their identification (Poinar, 1990; Hominick et al., 1997; Nguyen and Smart, 1997; Kaya and Stock, 1997). Cross breeding tests are labor- and time-intensive, and interpretation of morphological features for species identification of *Steinernema* requires substantial expertise to ensure accuracy. Diagnostic methods have been developed based on polymerase chain reaction methods (PCR), including PCR-RFLP and Random Amplified Polymorphic DNA (RAPDs) that facilitate species diagnosis by non-experts (Reid and Hominick, 1993; Joyce et al., 1994; Liu and Berry, 1995; Grenier et al., 1996; Stock et al., 1998). Molecular characters have also been used to examine if previously described species and new isolates of *Steinernema* are characterized by sequence differences (Szalanski et al., 2000; Spiridonov et al., 2004) or evidence of lineage independence (Nguyen et al., 2001; Stock et al., 2001; Spiridonov et al., 2004). The latter is required for species delimitation in Nematoda based on evolutionary species concepts (Adams et al., 1998; Nadler, 2002). The application of nucleotide sequence data in conjunction with evolutionary (phylogenetic) species concepts represents a relatively new approach to finding and describing new species of nematodes (Adams et al., 1998; Nadler, 2002; Baldwin et al., 2001) including members of the Steinernematidae and Heterohabditidae (Stock and Reid, 2003; Stock et al., 2004; Stock and Hunt, 2005).

Origins and evolutionary relationships

Steinernematidae and Heterorhabditidae are two nematode families that are not closely related from an evolutionary standpoint (Blaxter et al., 1998). However, it has been speculated they share life histories and morphological and ecological similarities throughout convergent evolution. These observations were first postulated by Poinar (1993) who speculated that in the mid-Paleozoic (approximately 350 million years ago) ancestors of the Heterorhabditidae and Steinernematidae began to independently explore mutualistic relationships with Gram-negative enteric bacteria (Enterobacteriaceae), the respective lineages of which would evolve to comprise *Photorhabdus* and *Xenorhabdus* (Poinar, 1993).

Blaxter et al.'s (1998) molecular phylogenetic framework for the Nematoda depicted the Heterorhabditidae as being most closely related to the Strongylida, a group of parasites of vertebrates that shares a most recent common ancestor with *Pellioiditis*, a free-living bacterivore. The same hypothesis depicted the Steinernematidae as being most closely related to the Panagrolaimoidea (free-living and insect associates) and Strongyloididae (vertebrate parasites), and as a member of a larger clade that includes free-living, fungal-feeding and plant parasitic taxa. This phylogenetic study supports Poinar's hypothesis (1993) that the heterorhabditids probably arose from a free-living bacterivorous ancestor, while for steinernematids, reconstruction of the trophic habits of the ancestors remains vague (Blaxter et al., 1998). The Nematoda new classification scheme by De Ley and Blaxter (2002) identified Steinernematidae within the suborder Tylenchina, which also includes other insect parasites such as allantonematids and neotylenchids. In this classification, Heterorhabditidae were placed within the suborder Rhabditina which includes, among others animal parasitic Strongylidae.

Explicit evolutionary hypotheses for *Steinernema* spp. have mainly been based on molecular data from nuclear ribosomal DNA (rDNA) (Spiridonov et al., 2004; Stock et al., 2001), although mitochondrial DNA sequences have been used to investigate 5 species (Szalanski et al., 2000). The most recent evolutionary framework for steinernematids considered a multigene approach in combination with morphological traits (Nadler et al., 2006). In this study sequence analysis of one nuclear gene (28S rDNA) and two mitochondrial (*cox*I and 12S) genes were taken into consideration. Combined phylogenetic analysis of the 3-gene dataset by parsimony and Bayesian methods yielded well-resolved and highly similar trees. Parsimony analysis of the morphological dataset yielded a poorly resolved tree, whereas total evidence analysis (molecular plus morphological data) yielded a phylogenetic hypothesis consistent with, but less resolved than trees inferred from combined molecular data. Parsimony mapping of morphological characters on the 3-gene trees showed that most structural features of steinernematids are highly homoplastic (Nadler et al., 2006).

Evolutionary relationships among *Heterorhabditis* spp. have been inferred using sequences of the ITS-1 region of the tandem repeat unit of rDNA (Adams et al., 1998). In this study, relationships between closely related 'species' (i.e. *H. indica* and *H. hawaiiensis*; *H. bacteriophora* and *H. argentinensis*) were well established. However, relationships among more distantly related species, i.e. *H. zealandica* in relation to *H. megidis* and *H. marelata*, could not be resolved. A more extensive study at the population level might contribute to a better resolution and/or interpretation of the relatedness between these species. More recently, the tropical and subtropical *Heterorhabditis* species, *H. amazonensis, H. indica*,

Table 1. Taxonomic summary of the entomopathogenic nematode and symbionts species.

Steinernematidae	Bacterial symbiont
***Steinernema* Travassos, 1927**	
S. abbasi Elawad, Ahmad and Reid, 1997	*X. indica*
S. aciari Qui, Yan, Zhou, Nguyen and Pang, 2005	Unknown
S. affine (Bovien, 1937) Wouts, Mrácek, Gerdin and Bedding, 1982	*X. bovienii*
S. akhursti Qui, Hu, Zhou, Mei, Nguyen and Pang, 2005	Unknown
S. anatoliense Hazir, Stock and Keskin, 2003	*X. bovienii*
S. apuliae Triggiani, Mrácek and Reid, 2004	Unknown
S. arenarium (Artyukhovsky, 1967) Wouts, Mrácek, Gerdin and Bedding, 1982	*X. kozodoii*
S. ashiunense Phan, Takemoto and Futai, 2006	Unknown
S. bedding Qui, Hu, Zhou, Pang and Nguyen, 2005	Unknown
S. asiaticum Anis, Shahina, Reid and Rowe, 2002	Unknown
S. bicornutum Tallosi, Peters and Ehlers, 1995	*X. budapestensis*
S. carpocapsae (Weiser, 1955) Wouts, Mrácek, Gerdin and Bedding, 1982	*X. nematophila*
S. ceratophorum Jian, Reid and Hunt, 1997	
S. costaricense Stock, Uribe-Lorio and Mora, 2007	*X. szentirmaii*
S. cubanum Mrácek, Hernandez and Boemare, 1994	*X. poinarii*
S. diaprepesi Nguyen and Duncan, 2002	*X. doucetiae*
S. feltiae (Filipjev, 1934) Wouts, Mrácek, Gerdin and Bedding, 1982	*X. bovienii*
S. glaseri (Steiner, 1929) Wouts, Mrácek, Gerdin and Bedding, 1982	*X. poinarii*
S. hermaphroditum Stock, Griffin and Chaenari, 2004	*X. griffiniae*
S. intermedium (Poinar, 1985) Mamiya, 1988	*X. bovienii*
S. jollieti Spriridonov, Krasomil-Osterfeld and Moens, 2004	*X. bovienii*
S. karii Waturu, Hunt and Reid, 1997	*X. hominickii*
S. kraussei (Steiner, 1923) Travassos, 1927	*X. bovienii*
S. kushidai Mamiya, 1988	*X. japonica*
S. leizhouense Nguyen, Qui, Zhou and Pang, 2006	Unknown
S. litorale Yoshida, 2004	*X. bovienii*
S. loci Phan, Nguyen and Moens, 2001	Unknown
S. longicaudum Shen and Wang, 1992	Unknown
S. monticolum Stock, Choo and Kaya, 1997	*X. hominickii*
S. neocurtillae Nguyen and Smart, 1992	Unknown
S. oregonense Liu and Berry, 1996	*X. bovienii*
S. pakistanense Shahina, Anis, Reid, Rowe and Maqbool, 2001	Unknown
S. puertoricense Román and Figueroa, 1994	*X. romanii*
S. puntauvense Stock, Uribe-Lorio and Mora, 2007	*X. bovienii*
S. rarum (de Doucet, 1986) Mamiya, 1988	*X. szentirmaii*
S. riobrave Cabanillas, Poinar and Raulston, 1994	*X. cabanillasii*
S. ritteri de Doucet and Doucet, 1990	Unknown
S. robustispiculum Phan, Subbotin, Waeyenberge and Moens, 2004	Unknown
S. sangi Phan, Nguyen and Moens, 2001	Unknown
S. sasonense Phan, Spiridonov, Subbotin and Moens, 2006	Unknown
S. serratum Li, 1992**	*X. ehlersii*
S. scapterisci Nguyen and Smart, 1990	*X. innexi*
S. scarabaei Stock and Koppenhöfer, 2003	*X. koppenhoeferii*
S. siamkayai Stock, Somsook and Kaya, 1998	*X. stockiae*
S. sichuanense Mrácek, Nguyen, Tailliez, Boemare and Chen, 2006	Unknown
S. silvaticum Sturhan, Spiridonov and Mrácek, 2005	*X. bovienii*
S. tami Luc, Nguyen, Spiridonov and Reid, 2000	Unknown
S. thanhi Phan, Nguyen and Moens, 2001	Unknown
S. websteri Cutler and Stock, 2003	*X. nematophila*
S. weiseri Mrácek, Sturhan and Reid, 2003	*X. bovienii*
S. thermophilum Ganguly and Singh, 2000	*X. indica*
S. yirgalemense Nguyen, Tesfamariam, Gozel, Gaugler and Adams, 2004	Unknown
***Neosteinernema* Nguyen and Smart, 1994**	
Neosteinernema longicurvicauda Nguyen and Smart, 1994	Unknown
Heterorhabditidae	
***Heterorhabdtis* Poinar, 1976**	
H. amazonensis Andaló, Nguyen and Moino, 2006	Unknown

Table 1. Continued.

Steinernematidae	Bacterial symbiont
H. bacteriophora Poinar, 1976	*P. luminescens* subsp. *luminescens* (for Brecon isolate) *P. luninescens* subsp. *laumondii* (for HP88 strain) *P. temperata* (for NC1 strain)
H. baujardi Phan, Subbotin, Nguyen and Moens, 2003	Unknown
H. brevicaudis Liu, 1994**	Unknown
H. downesi Stock, Burnell and Griffin, 2002	*P. temperata*
H. floridensis Nguyen, Gozel, Koppenhofer and Adams, 2006	Unknown
H. indica Poinar, Karunakar and David, 1992	*P. luminescens* subsp. *akhurstii*
H. marelata Liu and Berry, 1996	
H. megidis Poinar, Jackson and Klein, 1987	*P. temperata*
H. mexicana Nguyen, Shapiro-Ilan, Stuart, McCoy, James and Adams, 2004	Unknown
H. poinari Kakulia and Mikaia, 1997**	Unknown
H. taysearae Shamseldean, Abou El-Sooud, Abd-Elgawad and Saleh, 1996	Unknown
H. zealandica Poinar, 1990	*P. temperata*

** Nomina dubia.

H. floridensis and H. baujardi were shown to form one clade separated from known species mainly from temperate regions (Phan et al., 2003; Nguyen et al., 2006).

Mitochondrial genes have also been explored to study the evolutionary history of *Heterorhabditis* spp. (Liu et al., 1999), the results broadly agreeing with those of Adams et al. (1998). Although not studying all species (*H. zealandica* and *H. downesi* were not included) their study also indicated poor support for nodes involving *H. megidis* and *H. marelata*.

4. Bacterial Symbionts

Taxonomy and evolutionary relationships

Xenorhabdus and *Photorhabdus* are assigned to the family Enterobacteriaceae, which belongs to the gamma subdivision of the Proteobacteria. Members of these two families are Gram-negative rods, facultatively anaerobic, negative for oxidase, non-spore-forming, chemoorganic heterotrophs with respiratory and fermentative metabolisms and with the exception of a few, are motile by peritrichous flagella. *Xenorhabdus* and *Photorhabdus* are atypical of the Enterobacteriaceae, and no other member of the family is phenotypically similar (Holt et al., 1994). Numerous substances and phage have been found associated with species of both genera of entomopathogenic nematodes (Webster et al., 1998). For example, bacteriocins have been found attacking those bacteria species closely related to these symbiotic species. In addition to this, several antimicrobial agents that kill fungi and/or bacteria have also been identified. Xenorhabdins (dithiolopyrrolones), xenocoumacins (benzopyranone derivatives) and a range of indole derivatives have been isolated from various strains and species of *Xenorhabdus* and they all have antibiotic activity. Similarly, *Photorhabdus* produces hydroxystilbenes that have antibiotic activity. Moreover, these metabolites exhibit other bioactive properties, such as nematicidal and insecticidal activities (Webster et al., 1998).

The classical approach of comparing biochemical and cultural characteristics has been widely applied to diagnose *Xenorhabdus* and *Photorhabdus* spp. (Akhurst and Boemare, 1988; Lengyel et al., 2005; Nishimura et al., 1994; Somvanshi et al., 2006). Molecular methods such as DNA/DNA hybridization and total protein and isozyme profiles have been contemplated for distinguishing species and subspecific groups (Akhurst and Boemare, 1990). More recently, 16S rRNA sequences have been considered in combination with conventional phenotypic tests for characterization and description of new *Xenorhabdus* spp. (He et al., 2000; Lengyel et al., 2005). Until now, only 20 *Xenorhabdus* spp. and three *Photorhabdus* spp. have been described (Bonifassi et al., 1999; Brunel et al., 1997; Fischer-Le Saux et al., 1998; Tailliez et al., 2006) (Table 1). The limited number of currently described species clearly indicates that several taxonomic issues have yet to be resolved, and that proper and comprehensive species descriptions, in the context of a sound phylogenetic framework, remain major aspects to achieve. According to Stackebrandt et al. (2002), all species descriptions of bacterial symbionts should now include the 16S rDNA sequence, and phenotype, including chemotaxonomic characters.

Until now, only phylogenies based on sequence analysis of 16S rDNA gene have been published for interpreting evolutionary relationships of *Xenorhabdus* and *Photorhabdus* (Liu et al., 2001; Marokhazi et al., 2003; Fischer-Le Saux et al., 1999; Lengyel et al., 2005; Hazir et

al., 2004; Liu et al., 1997; Rainey et al., 1995; Suzuki et al., 1996; Szallas et al., 1997; Tailliez et al., 2006). However, consideration of 16S rDNA sequences for inferring phylogenetic relationships of bacterial groups has been considered not suitable due to the poor level of resolution that it is obtained in the analyses. This factor together with the consideration that nuclear genes can undergo lateral gene transfer across taxonomic groups or can be recombined, providing false evolutionary data (Yap et al., 1999) calls for consideration of other genes to explore evolutionary relationships of members of these taxa.

Thorough knowledge of the phylogenetic relationships of entomopathogenic nematodes and bacteria has clear implications in the understanding of how mutually beneficial relationships evolve and how specificity within associations is achieved. Furthermore, a cohesive picture of if and how these nematode-bacterium associations have co-evolved will provide a framework in which to interpret the rapidly expanding data regarding the molecular mechanisms underlying them. Over the past decade significant advances have been made in understanding such mechanisms in both *Heterorhabditis-Photorhabdus* and *Steinerema-Xenorhabdus* associations, and these advances have revealed striking differences in the molecular underpinnings of the two types of entomopathogenic associations (Goodrich-Blair and Clarke, 2007). The remainder of this manuscript will focus primarily on describing the symbiosis between *Xenorhabdus* and *Steinernema*.

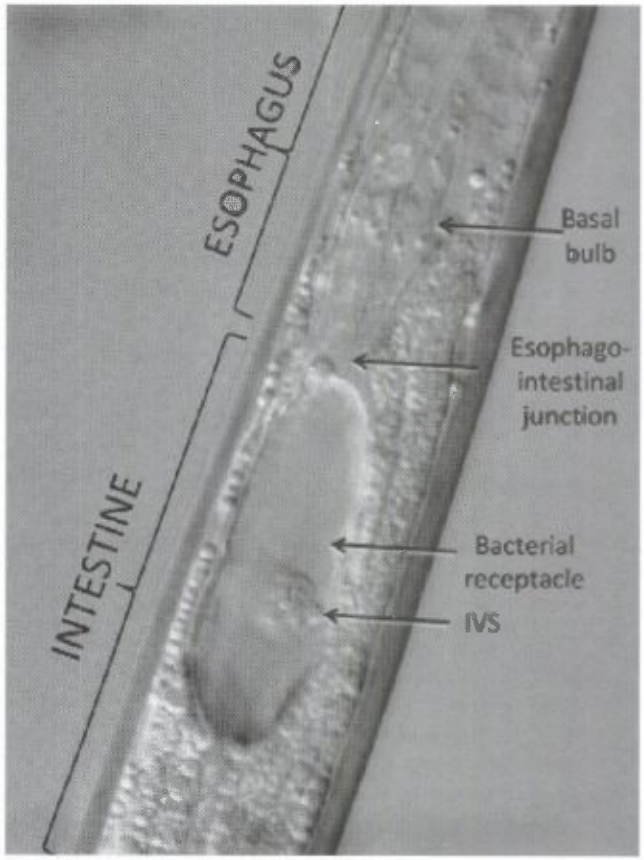

Figure 2. Uncolonized bacterial receptacle of *Steinernema carpocapsae* infective juvenile. Photo courtesy of S.K. Kim.

5. *Xenorhabdus-Steinernema* Interactions

Xenorhabdus bacteria and their *Steinernema* hosts have an intimate and dependent relationship with each other (Forst and Clarke, 2002). In natural settings, *Xenorhabdus* bacteria are thought to be unable to live outside the insect or nematode environments. However, further research on this aspect should be conducted to confirm these assumptions. The bacteria require their nematode hosts for dissemination from one insect to another (Akhurst and Boemare, 1990; Boemare, 2002). On the other side, *Steinernema* nematodes depend on the production of bacterial nutrients and secondary metabolites as a nourishment source that allows them to mature and reproduce (Forst and Clarke, 2002; Goodrich-Blair, 2007; Sicard et al., 2004b; Sicard et al., 2006). Several studies suggest each *Steinernema* species has an apparent specific natural association with only one *Xenorhabdus* species, though a single *Xenorhabdus* bacterial species may be associated with more than one nematode species (Boemare, 2002; Fischer-Le Saux et al., 1999) (see Table 1).

Questions regarding the mutualism between *Steinernema* and *Xenorhabdus* surround issues of how this association is maintained and transmitted through generations, what beneficial functions each partner provides to the other, and how partner specificity is maintained? Progress toward answering these questions has been made in part by examining the structure and physiology of the colonization site as well as the events occurring during colonization initiation and persistence. Much of this work has been conducted in the *S. carpocapsae* and *X. nematophila* symbiotic pair and is discussed below.

6. The Nature of the Infective Juvenile Nematode Bacterial Receptacle in *Steinernema* Nematodes

Unlike *Heterorhabditis* spp., in which the bacterial symbionts are distributed along a broad stretch of the anterior portion of the nematode intestine, in *Steinernema* nematodes the bacterial symbionts are harbored in a specialized structure known as the 'bacterial receptacle'. This receptacle is a modification of the ventricular intestine that lies immediately beneath the esophago-intestinal valve in the nematode IJ (Bird and Akhurst, 1983; Endo and Nickle, 1995; Snyder et al., 2007). Its formation does not

rely on the presence of bacteria (Bird and Akhurst, 1983; Martens and Goodrich-Blair, 2005). In *S. carpocapsae* the receptacle is a defined space between two cells that are morphologically distinct from the cells that comprise the intestine (Martens and Goodrich-Blair, 2005) (Fig. 2). Receptacle cells are stretched and have a 'lucid' appearance and unlike other intestinal cells, lack lipid granules in their cytoplasm (Kim et al., unpublished; Martens and Goodrich-Blair, 2005). Bacteria are spatially restricted in the receptacle, based on the finding that IJs derived from mixed lawns of red and green fluorescent *X. nematophila* always exhibit separation of the two strains (Martens et al., 2003). Within the receptacle is a cluster of spheres termed the intra-vesicular structure (IVS) (Fig. 2) (Martens and Goodrich-Blair, 2005). The IVS is present in IJs grown in the absence of bacteria and in colonized vesicles of all *Steinernema* spp. analyzed to date (Martens and Goodrich-Blair, 2005; Kim et al., unpublished). The IVS of *S. carpocapsae* is associated with a material that is reactive with wheat germ agglutinin lectin, suggesting it contains glycan residues (*N*-acetyl-glucosamine or -neuramic acid) (Martens and Goodrich-Blair, 2005).

Although the receptacle (with an IVS) forms in the absence of bacteria, the symbionts affect its morphology. For example, in uncolonized *S. bibionis* (=S. feltiae) IJs the vesicle wall is thicker than in colonized IJs (Bird and Akhurst, 1983). The receptacle of *S. carpocapsae* IJs varies in size and shape depending on IJ age and bacterial load (Goetsch et al., 2006; Flores-Lara et al., 2007).

7. The Bacterial Receptacle and Its Role in *Xenorhabdus* Nutrition

Approximately 200 colony forming units of *X. nematophila* bacteria can colonize the receptacle of a non-feeding IJ, but this population is founded by only 1–2 individual bacterial cells (Martens et al., 2003). The growth of *X. nematophila* in the receptacle was monitored through time in a population of IJs. Periods of exponential increase in population size were punctuated by periods of population decline. These data were interpreted to mean that *X. nematophila* bacteria grow (and are also periodically killed) in the receptacle of non-feeding *S. carpocapsae* IJs, suggesting they have a food source (Martens et al., 2003). Indeed, studies by Mitani et al. (2004) suggested that IJs may contribute energy reserves to feed the bacteria, thereby ensuring future reproductive success within an insect host.

The type of nutrients within the receptacle was explored by assessing the colonization phenotype of metabolic mutants of *X. nematophila* with defects in nutrient salvaging, or amino acid/vitamin biosynthetic pathways (Martens et al., 2005; Heungens et al., 2002). *X. nematophila* colonization proficiency of a mutant defective in the biosynthesis of a particular amino acid is taken as evidence that the relevant amino acid is present in the receptacle in sufficient quantities to support growth, while a colonization defect of such a mutant would suggest the nutrient is not sufficiently available. Using such reasoning, Martens et al. (2005) discovered that several amino acids (serine, branched chain amino acids, and histidine) and vitamins (nicotinate and pantothenate) are available to support the growth of *X. nematophila* mutants unable to synthesize these products. However, several metabolic mutants displayed colonization defects, leading to the conclusion that methionine and threonine are not sufficiently abundant to support growth (Flores-Lara et al., 2007; Martens et al., 2005). These data are further supported by the fact that *tdk* mutants, defective in salvaging exogenous thymine, have a competitive colonization defect. Because *tdk* mutants cannot salvage exogenous thymine they must synthesize it using a pathway that requires folate, a cofactor that is also necessary for the synthesis of methionine. Thus the competitive colonization defect of the *tdk* mutant could be explained by higher cellular demand for limited folate pools causing a reduced efficiency of methionine biosynthesis (Orchard and Goodrich-Blair, 2005).

Overall these data support the model that the nematode receptacle has numerous amino acids and vitamins available for bacterial use, but is limiting for methionine and threonine. The precise nature of the nutrient source in the receptacle remains obscure. One study found that an *X. nematophila opp* mutant, defective in uptake of oligopeptides, displayed wild type colonization levels, leading to the conclusion that if exogenous oligopeptides are present in the receptacle they are not the only source of amino acids (Orchard and Goodrich-Blair, 2004). Whether the nutrient source derives from dead sibling bacteria or directly from the nematode remains to be elucidated. Also, to date, the metabolic requirements in the receptacle have been analyzed only for *X. nematophila*, and it is Unknown if methionine and threonine limitation is a general aspect of nematode receptacles. Continued metabolic studies on a diversity of nematode-bacterium symbionts should shed light on these important questions.

8. Release of Symbiont Bacteria from Nematodes during Insect Infection

The IJ is encased in a second outer cuticle, retained from the second-stage juvenile. The outer cuticle maintains the IJs in a non-feeding state and protects them from harmful environmental conditions when the nematodes are in the soil awaiting for a suitable insect hosts. IJs encountering an insect larva penetrate the insect either through the host's natural openings (mouth, spiracles, anus) or through the cuticle by using a cuticular tooth (only present in *Heterorhabditis* spp.) and enter the hemocoel.

Exposure to insect hemolymph stimulates "recovery" of *Steinernema* spp. and triggers release of *Xenorhabdus* through the intestine and out the anus (Poinar, 1966; Sicard et al., 2004a; Martens et al., 2004; Snyder et al., 2007). Recent findings have shown that bacterial motility is not required for movement out of the receptacle and that hemolymph-induced pharyngeal pumping provides a force for the release of *X. nematophila* out of the receptacle and into the intestinal lumen, since anesthetized nematodes did not regurgitate the bacteria (Snyder et al., 2007). Therefore, the release of pathogenic *Xenorhabdus* spp. is likely a nematode-controlled event.

9. Future Directions

Although major advances have been made over the past years in understanding several aspects of entomopathogenic nematode interactions, there is still a great deal to be learned about their ecology, behavior, and molecular biology. Such knowledge has the potential to inform innovative approaches to control insects, such as through creation of nematode-bacterium pairs with increased soil persistence and bacterial virulence. Furthermore, such studies could have implications across a broader front by revealing fundamental aspects animal-bacterium relationships that can be applied to understanding such important pathogens as filarial nematodes and vector-borne diseases (e.g. *Yersina pestis*). To ensure that such knowledge is frequently and clearly transmitted it will be important to initiate and continue dialogue among scientists from diverse disciplines.

The genetic tractability of the *Xenorhabdus* and *Photorhabdus* bacteria has allowed tremendous progress on understanding the genetic elements involved in nematode-bacterium symbiosis. Progress studying the molecular biology of the nematode partner in this relationship has been comparatively slow. However, such work has been and will continue to be aided by technologies developed for *Caenorhabditis elegans*. This nematode is a close relative of Heterorhabditidae, and has been exhaustively studied as a model system for multicellular organisms (Hobert, 2003; Kamath and Ahringer, 2003; Stringham and Candido, 1993). The genome of this nematode is fully sequenced (Chervitz, 1998) and cutting-edge technology developed for its study is available to apply to entomopathogenic nematodes (Burnell, 2002). For example, transposon mutagenesis (for identification and cloning genes), EST (expressed sequence tags) screening (for identification of EPN gene transcripts), and RNAi technology (to suppress nematode gene expression) are being developed for use by several labs (Burnell, 2002; Ciche and Steinberg, 2007).

Technological developments and increasing interest in the fascinating biology of the tripartite interaction between entomopathogenic nematodes, their bacterial symbionts, and their insect hosts will undoubtedly bring many new insights in the coming years, including in such areas as (1) the pathogenic interactions between the nematodes and their insect hosts; (2) the development and maintenance of relationships between the nematodes and their symbiotic bacteria; (3) the environmental signals and genetic processes involved in sex determination and (4) chemoreception and host detection.

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