2 Interactions Between Bacteria and Nematodes

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2.1 Introduction

Bacteria can interact with nematodes in several ways: (1) they can serve as a food source and thus are merely prey, (2) they can be pathogenic for the nematode and (3) they can live in symbiosis with the nematode host. In this review, we will restrict our discussion to the pathogenic and symbiotic interactions between bacteria and nematodes.

2.2 Pathogenic Interactions

Many nematodes are free-soil organisms that feed on bacteria and it therefore may not be too surprising that various bacteria have developed defence mechanisms against nematode grazing. In fact, recent work has shown that several bacteria are capable of killing nematodes either by the production of toxic compounds or by establishing a fatal infection. This finding has led to the development of a facile model system of host–pathogen interactions to identify conserved pathways associated with microbial pathogenesis (for recent reviews see Kurz and Ewbank 2000; Aballay and Ausubel 2002). The model involves the killing of the soil nematode *Caenorhabditis elegans* by a variety of human pathogens. *Pseudomonas aeruginosa*, an opportunistic pathogen that causes chronic infections in cystic fibrosis patients, was the first bacterium whose pathogenic interaction with *C. elegans* was studied in great detail (Mahajan-Miklos et al. 1999; Tan et al. 1999a, b). Depending on the strain and the culture conditions, *P. aeruginosa* kills *C. elegans* by at least three distinct mechanisms. When *P. aeruginosa* strain PA14 is grown on nematode growth (NG) medium cells colonize the nematode intestine and killing occurs over the course of 2 to 3 days ("slow killing"). In contrast,

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PA14 grown on high-osmolarity medium (PGS) kills worms much faster (within 4 to 24 h – "fast killing") due to the production of low-molecular-weight toxins that are secreted into the culture medium. As a consequence, heat-killed bacteria are capable of fast but not slow killing. Likewise fast but not slow killing can be effected by PA14 bacterial supernatants with death of *C. elegans* occurring similarly as with bacteria. The toxic compounds were identified as phenazines a class of tricyclic pigments that includes pyocyanin. In the case of slow killing, a screen of transposon insertion mutants of *P. aeruginosa* strain PA14 revealed that the *lasR-lasI* quorum-sensing system the *lemA* and *gacA* two-component regulators and *toxA* (encoding exotoxin A) were involved in PA14 lethality. In all 19 of 23 genes identified in a screen as being involved in *C. elegans* fast or slow killing were also shown to be required for full virulence in a mouse thermal injury and infection model (Mahajan-Miklos et al. 1999; Tan and Ausubel 2000). These results provide strong evidence for a tight correlation of genes required for virulence across evolutionarily disparate hosts. More recently, it has been shown that another *P. aeruginosa* strain PAO1 kills the nematode by cyanide poisoning ("paralytic killing") when it is grown on brain-heart infusion (BHI) broth (Darby et al. 1999; Gallagher and Manoil 2001).

Over the past few years is has become evident that *C. elegans* is a highly valuable model for the study of pathogenicity of a large number of pathogens (Ewbank 2002; Mylonakis et al. 2002), including *Salmonella typhimurium* (Aballay et al. 2000; Labrousse et al. 2000) and *Serratia marcescens* (Kurz and Ewbank 2000). Several members of the genus *Burkholderia* (O'Quinn et al. 2001; Gan et al. 2002), and the Gram-positive bacteria *Enterococcus faecalis, Staphylococcus aureus, Streptococcus pneumoniae* and *Streptococcus pyogenes* kill *C. elegans* (Garsin et al. 2001; Jansen et al. 2002). Furthermore, in a recent study, it has been demonstrated that a large number of bacteria are pathogenic to L4 *C. elegans* when grown on brain heart infusion (BHI) medium (Couillault and Ewbank 2002). Among these are the plant pathogens *Erwinia chrysanthemi*, *Agrobacterium tumefaciens, Erwinia carotovora* pv. *carotovora*; the enterobacteria *Shewanella frigidimarina* and *Shewanella massilia,* the fish pathogen *Aeromonas hydrophila* as well as the insect pathogens *Photorhabdus luminescens* and *Xenorhabdus nematophila*. Of these, *A. tumefaciens*, *E. carotovora* pv. *carotovora*, *A. hydrophila* and *P. luminescens* require live bacteria for lethality, whereas *X. nematophila* does not. Recently, the *C. elegans* model has been further expanded to include fungal pathogens, particularly the model human pathogen *Cryptococcus neoformans* (Mylonakis et al. 2002).

It has also been reported that *C. elegans* can be chronically infected by *Microbacterium nematophilum* (Hodgkin et al. 2000). In this case, the bacteria adhere to the rectal and post-anal cuticle of susceptible nematodes and induce substantial local swelling of the underlying hypodermal tissue

known as the Dar (deformed anal region) phenotype. The swelling leads to constipation and slows growth in the infected worms but the infection is otherwise non-lethal.

2.3 Symbiotic Interactions

Bacteria can also live in symbiotic association with their nematode hosts. The majority of filarial nematodes including the major pathogenic species in humans *Wuchereria bancrofti, Brugia malayi* and *Onchocerca volvulus* (Taylor and Hoerauf 1999; Bandi et al. 2001) harbor bacterial endosymbionts that belong to the genus *Wolbachia*. Filarial nematodes are parasitic worms that cause some of the most devastating of all tropical diseases such as elephantiasis and river blindness. Studies on the inflammatory pathogenesis of filarial disease have shown that endotoxin-like activity derived from endosymbiotic *Wolbachia* bacteria is the major inflammatory stimulus of filarial nematodes. *Wolbachia* have so far been found in more than 20 species of filarial nematodes with only two species appearing to be uninfected (McGarry et al. 2003). Of those with *Wolbachia* the infection appears to be ubiquitous in all individuals developmental stages and populations throughout their global distribution (Taylor 2002). The bacteria reside in vacuoles and are restricted to the lateral cord cells and developmental stages within the female reproductive organs and intrauterine developmental stages as a consequence of their vertical transmission via the egg. Antibiotic depletion of bacteria shows that they are required for normal fertility and development of the worm and may even protect the parasites from host immunity. Given that filarial nematodes are major pathogens of humans throughout the tropics most research so far has focused on the contribution of *Wolbachia* to disease pathogenesis and as a novel target for antibiotic therapy (Taylor et al. 2000; Taylor and Hoerauf 2001).

Despite the obvious clinical importance of the symbiosis between *Wolbachia* and filarial nematodes, almost nothing is known about the underlying molecular mechanisms that are required for the interactions between the bacteria and the host nematode.

2.3.1 *Photorhabdus* and *Xenorhabdus*

Photorhabdus and *Xenorhabdus* are genera of Gram-negative bacteria that in addition to being pathogenic to insect larvae also have mutualistic interactions with nematodes from the families *Heterorhabditis* and *Steinernema*

respectively. The bacteria are found colonising the gut of a specialised free-living form of the nematode called the infective juvenile (IJ). The IJ migrates through the soil and infects susceptible insect larvae either by penetrating directly through the cuticle or through natural openings such as the mouth the anus and spiracles. Once inside the insect the IJ migrates to the insect circulatory system and releases the bacteria into the hemolymph. The bacteria proliferate and produce a wide range of toxins and hydrolytic enzymes that are responsible for the death and bioconversion of the insect larva into a nutrient soup that is ideal for nematode growth and development. The nematodes reproduce until the nutrient supply becomes limiting at which time they develop into IJs and are recolonised by the symbiotic bacterium.

This remarkable co-dependent reproductive cycle is the result of a highly evolved interaction between the bacterium and the nematode. The bacteria benefit from this interaction by being protected from the competitive environment of the soil and by being transported to the nutrient-rich hemolymph of an insect larva. In turn, the nematode takes advantage of the pathogenic potential of the bacteria to kill the insect host. The bacteria also supply the nutrient base for the growth and development of the nematode and suppress contamination of the insect cadaver by soil microorganisms by producing antibiotics. Recent studies in both *Xenorhabdus* and *Photorhabdus* have allowed us to identify genetic systems that play important roles in the tripartite association between the bacterium and its two eukaryotic hosts and these have been reviewed in recent publications (Forst and Nealson 1996; Forst et al. 1997; Forst and Clarke 2002; Ffrench-Constant et al. 2003). In this chapter, we will review our current understanding of the molecular mechanisms involved in the symbiosis between the bacteria and the nematode.

Although the *Photorhabdus-Heterorhabditis* and *Xenorhabdus-Steinernema* systems are remarkably similar in many ways, there is little doubt that these systems have emerged by convergent evolution. *P. luminescens* and *X. nematophilus* are the most extensively studied species of *Photorhabdus* and *Xenorhabdus* respectively and comparisons of major phenotypic traits of these bacteria reveal important differences (Forst and Clarke 2002). Several salient differences also exist between the nematode families *Heterorhabditidae* and *Steinernematidae* (Forst and Clarke 2002). The first generation adults of *Heterorhabitis* are hermaphroditic and the infective juveniles carry the symbiotic bacteria in the intestine below the basal bulb. In contrast the first generation adults of *Steinernema* exist as amphimictic males and females and the bacteria are carried in a specialised intestinal vesicle. While both nematodes belong to the Rhabditida family *Heterorhaditis* branch in the clade containing *Caenorhabditis elegans* and are most closely related to the *Strongylida* group, whilst *Steinernema* branch in a separate

clade and are more closely related to the *Strongyloides* group (Blaxter et al. 1998).

Significant differences also exist in the growth characteristics of the respective nematodes. *Steinernema* nematodes that do not contain the symbiotic bacteria (i.e. axenic nematodes) can grow on artificial medium while an artificial medium does not exist that supports axenic growth of *Heterorhabditis*. *Steinernema carpocapsae* can grow axenically and develop into infective juveniles when injected into *G. mellonella* whilst axenic *Heterorhabditis* develop into J1 progeny but these juveniles die, thus infective juveniles are not formed (Han and Ehlers 2001). Taken together, these observations support the idea that the congruence observed in the life cycles of these bacteria-nematode associations is the result of convergent evolution.

A remarkable feature of both *Xenorhabdus* and *Photorhabdus* is the occurrence of variant cell types that arise during prolonged culturing of the bacteria (Akhurst1980). The primary variant is characterised by the presence of numerous phenotypic traits that are generally greatly reduced or absent from the secondary variant (Boemare and Akhurst 1988; see Table 2.1). These traits include the formation of non-mucoidy colonies, the loss of dye-binding ability, a reduction in the amount of pigments antibiotics and siderophores produced by the bacteria. In addition, the production of proteases and lipases and in the case of *Photorhabdus* bioluminescence is also affected by phenotypic variation. Interestingly, the primary variant is always found associated with the infective juvenile and, in addition to being virulent, the primary variant supports nematode growth and development in vivo and in vitro. On the other hand, the secondary variant whilst remaining virulent to insect larvae shows a strikingly reduced ability to support nematode growth and development. The ability

Table 2.1. Phenotypes used to distinguish phenotypic variants of *Photorhabdus* and *Xenorhabdus*

Phenotypic trait	*Photorhabdus*	*Xenorhabdus*
Colony morphology	+	–
Dye uptake	+	+
Bioluminescence	+	–
Motility	+	+[a]
Pigment production	+	–
Antibiotic production	+	+
Crystal proteins	+	+
Protease production	+	–
Lipase production	+	–
Catalase	+	–

[a] Depending on strain

of *Heterorhabditis* and *Steinernema* nematodes to grow on the respective secondary variant cells is also strikingly different. *Steinernema* can grow to varying degrees on secondary cells of *Xenorhabdus* in vivo and in vitro, while *Heterorhabditis* is unable to grow on secondary cells of *Photorhabdus* (Volgyi et al. 1998; Han and Ehlers 2001; Joyce and Clarke 2003). Taken together, these findings indicate that primary-specific products and properties are involved in the symbiotic interaction between the nematode and the bacterium.

In *Photorhabdus* the primary-specific phenotypes are required for symbiosis with the nematode but do not appear to be necessary for pathogenicity, suggesting that different sets of genes (i.e. regulons) are required for each interaction (Forst and Clarke 2002; Joyce and Clarke 2003). Moreover, an understanding of how phenotypic variation is regulated could be extrapolated into an understanding of the regulatory pathways that control symbiosis. To this end, it has recently been reported that a single insertion in the genome of the secondary variant of *P. temperata* K122 that restores the production of many of the primary-specific phenotypes (Joyce and Clarke 2003). This insertion is within a gene with homology to *hexA*, a gene encoding a repressor of exoenzyme production in *Erwinia* (Mukherjee et al. 2000) and it has been shown that the *hexA*$^{-}$ secondary variant supports nematode growth and development, suggesting that *hexA* encodes a repressor of symbiosis. (Joyce and Clarke 2003).

Under standard laboratory conditions, it has been shown that the primary variant of *Photorhabdus* is motile whilst the secondary variant is non-motile (Akhurst 1980). We have shown that motility is important for the colonisation of the nematode gut (H.P.J. Bennettand D.J. Clarke DJ, unpubl. data), supporting the correlation between the production of primary-specific phenotypes and symbiosis. Interestingly, motility was one of the phenotypes unaffected in the *hexA*$^{-}$ mutant and, recently, it has been reported that motility in the secondary variant of *P. temperata* NC19 is derepressed when the bacteria are cultured under anoxic conditions (Hodgson et al. 2003). This suggests that there are least two independent signalling pathways HexA-dependent and oxygen-dependent controlling phenotypic variation and symbiosis in *Photorhabdus*. The molecular characterisation of these pathways will be important for a clear understanding of the bacterial commitment to the association with the nematode.

Steinernema can grow on secondary variants of *Xenorhabdus*, suggesting that phenotypic variation in this bacteria-nematode association is not as tightly correlated with symbiosis as it is in the *Photorhabdus-Heterorhabditis* system (Volgyi et al. 1998). Moreover, there is evidence suggesting that unlike *Photorhabdus* secondary variants of some *Xenorhabdus* strains are attenuated in their virulence to insect larvae (Volgyi et al. 1998). Nonetheless, genetic studies have identified loci in *Xenorhabdus* that

are involved in both phenotypic variation and symbiosis, suggesting that there is at least some degree of functional overlap (Volgyi et al. 2000). Indeed, as phenotypic variation (and thus symbiosis) in *Photorhabdus* is controlled by environmental signals, there is evidence that the expression of genes involved in the symbiotic interaction between *Xenorhabdus* and *Steinernema* is also controlled by the environment. The EnvZ-OmpR pathway has been extensively studied in *Escherichia coli* and *Salmonella*, where it has been shown to be involved in motility, biofilm formation, adaptation to acidic conditions and virulence (Pratt et al. 1996; Shin and Park 1995; Vidal et al. 1998; Lee et al. 2000). EnvZ is a membrane-spanning sensor kinase that responds to increasing osmolarity by phosphorylating the response regulator OmpR. OmpR-P then binds to DNA and alters the expression of certain genes. Functional copies of both *envZ* and *ompR* have been identified in *X. nematophila* and it has recently been shown that the *ompR* gene is required for the normal interaction between *Xenorhabdus* and *Steinernema* (Leisman et al. 1995; Tabatabai and Forst 1995; Forst and Tabatabai 1997; Boylan and Forst 2002; Kim et al. 2003). This suggests that *Xenorhabdus* is regulating the expression of genes required for symbiosis in response to uncharacterised environmental signals.

Recent studies into the association between *Xenorhabdus* and *Steinernema* have focused on the molecular mechanisms of the bacterial colonisation of the specialised vesicle in the nematode gut. It has been shown that the vesicle is initially colonised by a small number of bacteria and that these bacteria then grow inside the lumen of the vesicle (Martens et al. 2003). Moreover, growth of the bacteria in the gut appears to be limited by the nematode either through structural modification of the vesicle or nutrient availability in the gut. This highlights the active role played by the nematode in the association and suggests that there might be a cost to the nematode for supplying these nutrients, indicating that there is a strong selective pressure on the nematode to facilitate the colonisation of its gut by the bacteria. A recent genetic study identified 15 bacterial loci that were required for colonisation of the nematode by *Xenorhabdus* (Heungens et al. 2002). Several of these loci were identified as genes encoding proteins with homology to regulatory proteins in other bacteria, e.g. *rpoS rpoE* and *lrp.* These genes all encode proteins that are involved in controlling the bacterial response to environmental stresses such as starvation and membrane perturbations (Hengge-Aronis 1999; Raivio and Silhavy 2001; Helmann 2002). Other genes identified were predicted to encode proteins involved in amino acid and siderophore biosynthesis. This suggests that *Xenorhabdus* may be required to scavenge for the limited amounts of iron available in the nematode gut. Interestingly siderophore production is also required for *Heterorhabditis* growth and development when cultured with *P. temperata* NC19 (Ciche et al. 2001) and K122 (Watson and Clarke, unpublished

data) pointing to an important role for iron in controlling the mutualistic interactions in both of these systems.

2.4 Conclusions

In this review, we have described some examples of symbiotic and pathogenic interactions that occur between bacteria and soil-dwelling nematodes. Recent genetic analysis of these bacteria-nematode interactions has led to a significant increase in our understanding of the molecular mechanisms controlling how bacteria infect their hosts and, more importantly, the role of the host in determining the output of the infection, i.e. symbiotic or pathogenic. This research will continue to provide important information in our fight against pathogenic infections of humans and other mammals.

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