Animal Models for *Salmonella* Pathogenesis: Studies on the Virulence Properties Using *Caenorhabditis elegans* as a Model Host

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1. Introduction

Studies associated with host-pathogen interaction and the principal mechanisms of pathogenesis including the systems adopted by the host for its defense has always been a topic of interest to the scientific community that primarily deals with pathogenic microbes causing human diseases. More importantly the post-genomic era has set a milestone in basic and applied science research by proper identification and validation of potential human “disease-causing” or “disease-associated” genes. Although the host-pathogen interaction is a complex biological system (Huffman et. al., 2004) it is equally important to understand the characteristic features of microbes and their respective hosts that always may not culminate into a disease process. So present day researchers are also working with microbes that may exist within hosts without causing any obvious disease, and at the same time trying to explore why some microbes only cause disease in certain hosts. Besides, a wide range of microbes those are pathogenic to the mammals, like bacteria and fungi, have also been known to manifest diseases in simple non-vertebrate hosts as well. In order to understand a pathogen, researchers would preferably screen the microbe's genome at length to identify all its virulence genes. On the other hand, screening in mammalian experimental hosts, such as mice, rats, or other mammals *per se*, sometimes seems unfeasible since they would be required in large numbers and thus quite expensive. In recent days use of simple non-vertebrate hosts, such as the round worm *Caenorhabditis elegans*, the fruit fly *Drosophila melanogaster*, and the plant *Arabidopsis thaliana*, are becoming common for convenience in investigating the virulence strategies adopted by several mammalian pathogenic microbes (bacteria and fungi) (Sifri et al., 2005). Also uses of these model organisms are in great practice to comprehend the conserved molecular pathways that are related to human diseases caused by microbial pathogens.

There is a wide variety of pathogens (both bacteria and fungi) that are known to affect the human health including animals. Amongst them those that have been extensively studied with different laboratory model hosts are, Gram-negative bacteria *Burkholderia, Pseudomonas,*
Salmonella, Serratia and Yersinia; Gram-positive bacteria Enterococcus, Staphylococcus and Streptococcus; and the fungus Cryptococcus neoformans. However there are some that are not pathogenic to mammals, but pose as insect pathogens, like Bacillus thuringiensis and the nematode-specific Microbacterium nematophilum.

Caenorhabditis elegans (commonly known as C. elegans) is a free living soil nematode that feeds on bacteria. Under laboratory conditions C. elegans feeds on E. coli (strain OP50). In order to study the effects of pathogenic strains on animals, researchers have started to use C. elegans as an excellent and a convenient model to explore the bacterial pathogenesis on animals by making the worms feed on the pathogens. Amongst the different categories of pathogens that are known to infect the worms in the same manner as in humans / animals, Salmonella is one that has been broadly studied in the worm system. The present chapter shall focus the attribute of the nematode C. elegans as a convenient model to study host pathogen interaction with special emphasis to Salmonella.

2. Caenorhabditis elegans as a simple model system to study human diseases

For the past few decades, the free-living bacteriovorous nematode Caenorhabditis elegans (Caeno, recent; rhabditis, rod; elegans, elegant) has emerged lately as a powerful model for study of developmental genetics, neurobiology and aging. Ever since it was introduced by Sydney Brenner, this simple multicellular eukaryote has been studied intensively with comprehensive genotypic and phenotypic information now available. This free-living nematode has the following features: small body length (1.5 mm adults), quick generation time (three days), large brood size (approximately 300 progeny per gravid adult), short lifespan (~3 weeks), ease of maintenance, reduced cost, a small genome (one half that of Drosophila melanogaster), ability to be stored for long periods by freezing, and the fact that it is a simple and genetically tractable model have made this nematode species an ideal model to study longevity and process of ageing (Ewbank, 2002; Houthoofd et al., 2003; Kurz et al., 2007), and as well as a model organism for molecular and developmental biology. Moreover, under the microscope, the unique transparent body of the worm allows one to observe many biological processes, including organogenesis, behavior, and as well as, pathogenesis. The life cycle is short, temperature dependent and consists of embryogenesis (development from fertilization to hatching) and post-embryonic development that has four larval stages separated by molts followed by the adult stage (Figure 1). Also, at every larval stage a new cuticle of stage-specific composition is secreted and the older one is shed. In L1 larvae, the nervous system, the reproductive system, and the digestive tract begin to develop, and this is completed by the L4 stage. Sometimes these nematodes can adopt another non developmental stage known as the Dauer stage instead of the normal third larval stage (Cassada and Russell, 1975). Entry into dauer is induced by stress like high temperature, starvation or overcrowding at the second molt (Fig. 1).

In 1998, C. elegans became the first metazoan to have a completely sequenced genome (The C. elegans Sequencing Consortium, 1998). More than 40% of the human disease genes have been predicted to have orthologs in the C. elegans genome. Overexpressing human genes in specific cell types in C. elegans using tissue- or cell- specific worm promoters, or studying the differential gene expression patterns of worms at the transcriptional level by
microarray analysis may well reveal the gene expression profiles (up- or down-regulated) for the worms. This helps the identification of certain members of the signaling cascades that are activated in diseases and may well act as candidates for drug therapies. Double-stranded RNA interference (dsRNAi) has also been another modern approach to study human diseases by treating worms with dsRNAs thus inactivating the function of specific C. elegans gene orthologs (Fire et al., 1998; Tabara et al., 1998; Timmons et al., 2001). Up to date several genomic data on human and animal microbial pathogens that are shown to harm and kill nematodes have been created. With these vast resources of genetic information, there is always a growing need for simple and innovative ways to study microbial virulence strategies and assay the role of individual genes to pathogenesis. Since both the host (i.e. C. elegans) and pathogens are amenable to genetic analysis and high throughput screening, in each of these pathosystems the worm has been successfully utilized both for the identification of microbial virulence factors and as well as the worm’s immune-defense mechanisms.

Fig. 1. Life cycle of C. elegans. Life cycle consists of four larval stages. At the first larval stage the life cycle can be interrupted by the “dauer” stage which is triggered by increased pheromone levels that result due to food scarcity, overcrowding and temperature (Courtesy: Introduction to C. elegans anatomy, Handbook-Hermaphrodite; http://www.wormatlas.org)
3. Bacteria as a food source for *C. elegans*: Effect of *Salmonella* and other pathogens as food

*C. elegans* has a life span of approximately two weeks at room temperature when fed on *Escherichia coli* OP50 bacteria (Brenner, 1974) grown on Nematode Growth Medium (NGM) agar (Garsin et al., 2001). However, when *C. elegans* is fed on other human pathogens, they exhibit a range of significant defects, like shorter life spans. Several human pathogens, including *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Serratia marcescens*, *Staphylococcus aureus*, *Vibrio cholerae*, and *Burkholderia pseudomallei*, kill *C. elegans* when supplied as a food source, and a diverse array of bacterial virulence factors have been shown to play a role in both nematode and mammalian pathogenesis (Aballay et al., 2000; Kurz & Ewbank, 2000; Labrousse et al., 2000; Tan et al., 1999). An important feature of *Pseudomonas aeruginosa*, a Gram-negative pathogen, is known to kill *C. elegans* and more particularly, under different media conditions. The *P. aeruginosa* strain PA14 kills *C. elegans* by “slow-killing” (in few days) or even by “fast killing” (few hours) (Tan et al., 1999). *Vibrio cholerae*, another Gram-negative bacterium kills *C. elegans* within few days (~5 days) by a “slow-killing” process (Vaitkevicius et al., 2006). A marked decrease in the life span was observed in worms feeding *V. vulnificus* as opposed to the regular food of *E. coli* OP50. In many cases, the intestines of the worms were found to get distended with clumps of pathogenic microorganisms that accumulate within it and probably happen to be primary cause of early deaths (Dhakal et al., 2006). Normally, the pharyngeal grinder of the worm efficiently disrupts the *E. coli* and essentially no intact bacterial cells are found within the intestinal lumen. However, virulent bacterial strains like *V. vulnificus* or *V. cholerae* have been shown to accumulate in both pharynx and the lumen of the worm intestines (Vaitkevicius et al., 2006; Dhakal et al., 2006) as evidenced under fluorescent microscope for the GFP-labeled bacterial strains.

*Salmonella* is a gram-negative enteric bacterium that represents a major public health problem. *S. enterica* colonizes the *C. elegans* intestine as reported (Aballay et al., 2000; Labrousse et al., 2000). *S. enterica* serovar Typhi causes typhoid fever, a severe systemic infection. *S. enterica* serovar Typhimurium is known to be lethal to mice, causing a typhoid-like disease, and in humans it causes nonfatal infection restricted to the gastrointestinal tract and thus had been studied in the mouse model for systemic infections. When worms were exposed to *S. enterica* for only 3 h, then removed to plates seeded with OP50, there was significant early death. Invasion of host cells is an essential aspect of *Salmonella* sp. pathogenesis in mammalian systems, but *S. enterica* does not appear to invade *C. elegans* cells. Many novel strategies have been devised for understanding its mode of action and its interactions with host cells (Lee & Camilli, 2000; Chiang et al., 1999). The finding that *Salmonella* is capable of infecting *C. elegans*, and that genes important for its full pathogenicity in vertebrates also play a role during infection of *C. elegans*, opens the possibility of taking a new genetic approach to study *Salmonella*.

*Salmonella*, a gastrointestinal tract pathogen of humans, is responsible for approximately 2 million - 4 million cases of enterocolitis every year in the United States (Tampakakis et al., 2009). During infection, *S. enterica* serovar Typhimurium has the propensity to compete with normal intestinal flora. *Candida albicans* is another opportunistic human fungal pathogen that usually resides in the gastrointestinal tract and on the skin as a commensal and can also cause life-threatening invasive disease. Besides, both these organisms are pathogenic to the nematode *C. elegans*, causing a persistent gut infection and eventually leading to death of the worms (Fig. 2). Mylonakis and his group developed *C. elegans* as a polymicrobial infection
model to assess the interactions between S. Typhimurium and C. albicans (Tampakakis et al., 2009). They reported that when C. elegans is infected with C. albicans and S. enterica serovar Typhimurium, C. albicans filamentation is inhibited. They further utilized the host, C. elegans, to identify the antagonistic interaction between two human pathogens that reside within the gastrointestinal tract.

Fig. 2. S. typhimurium kills C. elegans. (a) L4 stage (open circles) or 1-day-old adult hermaphrodite (solid triangles and solid circles) worms fed either on S. typhimurium SL1344 (solid triangles and open circles) or on E. coli OP50 (solid circles). (b) C. elegans fed on S. typhimurium SL1344 (open circles) or E. coli OP50 (solid circles) for 5 h, then shifted to E. coli OP50. The inset shows the percentages of dead worms after transfer to OP50-containing plates after feeding for 1, 3 or 5 h on SL1344 (Courtesy Aballay et al., 2000).

4. Intestine as the store house of bacterial infection in C. elegans

Numerous bacteria infect the intestine of C. elegans. In many cases, the intestine becomes inflated; but it is not clear whether this is due to physical pressure exerted by the growing pathogen or as a physiological response of the nematode. The standard laboratory food, i.e. E. coli OP50 and Cryptococcus laurentii (Tan et al., 1999; Garsin et al., 2001; Mylonakis et al., 2002) does not colonize wild-type C. elegans, but various pathogens do. For example, Enterococcus faecalis, a gram-positive bacteria, colonizes in C. elegans and kills very rapidly (Garsin et al., 2001). It was eventually shown that genes involved directly or indirectly with the quorum-sensing system are involved in killing (Sifri et al., 2002). On the other hand, Pseudomonas aeruginosa can kill C. elegans rapidly by toxin-mediated mechanisms or slowly in an infectious process. In the “slow killing” model, bacteria colonize the intestine, but within a day exposure to the bacteria, no strong disease symptoms were observed (Tan et al., 1999). With continued exposure, the worms gradually cease pharyngeal pumping, become immobile, and eventually die. Moreover, large quantities of live bacteria like Salmonella enterica, Burkholderia cepacia, Serratia marcescens, Staphylococcus aureus, Vibrio vulnificus, V. cholerae, and C. neoformans are known to kill worms by colonization (Aballay et al., 2000; Garsin et al., 2001; Mylonakis et al., 2002; Kothe et al., 2003; Kurz et al., 2003; Rhee et al., 2006; Vaitkevicius et al., 2006). A screen of 960 transposon insertions in S. enterica produced 15 mutations with reduced killing of C. elegans, of which only some were virulent.
Salmonella – A Diversified Superbug

(Tenor et al., 2004). Although bacterial colonization is greatly correlated with worm killing, it is not adequate for killing. For instance, aerobically grown *Enterococcus faecium* although accumulates to high levels, it does not kill (Garsin et al., 2001). *S. enterica*, *S. marcescens*, and *E. faecalis* are pathogens also known to cause persistent infections (Aballay et al., 2000; Labrousse et al., 2000; Garsin et al., 2001; Kurz et al., 2003) in *C. elegans* in contrast to *Pseudomonas aeruginosa* and *S. aureas*.

Different strains of *Salmonella*, such as *S. typhimurium* as well as other *Salmonella enteric* serovars including *S. enteritidis* and *S. dublin* are all effective in killing *C. elegans* (Aballay et al., 2000). When worms are placed on a lawn of *S. typhimurium*, the bacteria have been shown to accumulate in the intestinal lumen and the nematodes die over the course of several days (Fig. 3). This killing in particular requires direct contact with live bacterial cells. Interestingly, the worms die in the same manner even when placed on a lawn of *S. typhimurium* for a relatively short period of time (3–5 hours) before transfer to a lawn of *E. coli*, their natural food. A high titer of *S. typhimurium* still persists in the *C. elegans* intestinal lumen for the rest of the worms’ life even after their transfer to an *E. coli* lawn. Killing is directly correlated with an increase in the titer of *S. typhimurium* in the *C. elegans* lumen. Even a small inoculum of *S. typhimurium* has been shown to be enough to establish a persistent infection *C. elegans* which is probably due to the presence of *C. elegans* intestinal receptors to which bacteria might adhere (Fig. 4).

![Fig. 3. Bacterial colonization of the *C. elegans* intestine. Confocal images showing young adult hermaphrodite worms fed on (a,b) *E. coli* DH5α–GFP for 72 h, (c,d) *S. typhimurium* SL1344–GFP for 72 h, or (e,f) *P. aeruginosa* PA14–GFP for 24 h. (a,c,e) Transmission images showing the intestinal margins (indicated with arrows). (b,d,f) Merged images showing bacterial fluorescence (green channel) and the gut autofluorescence (red channel). Scale bar, 50 μm (Courtesy Aballay et al., 2000).](www.intechopen.com)
Bacterial proliferation and persistence can be easily determined by monitoring the worms in due course under microscope for the presence of GFP-labeled bacteria. In particular, pathogenic strains expressing green fluorescent protein (GFP) are therefore extremely useful in examining the fate of such microbes upon ingestion by the worms (Fig. 5). A virulent strain of

Fig. 4. *S. typhimurium* colonizes the worm intestine. Young adult worms were fed on (a) *E. coli* DH5α–GFP or (b, c) *S. typhimurium* SL1344–GFP for 5 h and then transferred to *E. coli* OP50 for (a, b) 24 h or (c) 96 h. Scale bar, 50 μm (Courtesy Aballay et al., 2000).

Fig. 5. Accumulation of *S. typhimurium* within the intestine and pharynx of *C. elegans*. (a,c) Nomarski and (b,d) fluorescence photomicrographs of the (a,b) posterior and (c,d) anterior of a worm after contact for 5 days with GFP-expressing *S. typhimurium*. The intestine and terminal bulb of the pharynx are seen to be full of intact bacteria (Courtesy Labrousse et al., 2000).
Salmonella – A Diversified Superbug

*S. typhimurium* expressing GFP (12023 ssaV-GFP) is known to kill *C. elegans* as the wild-type strain. The grinder which is located in the terminal bulb of the pharynx of the worms normally breaks bacteria (Albertson & Thomson, 1976). However, with increasing infection, the number of *S. typhimurium* significantly increases beyond the terminal bulb and gradually starts to mount up within the intestinal lumen (Fig. 5). Increase in the intestinal lumen of the worms is accompanied by the decrease in the volume of the intestinal cells. Nonetheless, the cells of the terminal bulb of the pharynx get progressively destroyed and their place is taken up by bacteria. Also worms with defective grinders have been found to be more susceptible to *Salmonella* infection and therefore less resistant to the pathogenic effects. For example, *phm-2* worm mutants possess abnormal terminal bulb and therefore are more susceptible to bacterial attack than the N2 worms (Fig. 6) (Labrousse et al., 2000).

![Graph showing survival of C. elegans fed on E. coli and S. typhimurium](image)

Fig. 6. Survival of *C. elegans* fed on *E. coli* and *S. typhimurium*. Wild-type worms (circles) or *phm-2* mutants (triangles) fed on *E. coli* strain OP50 until the larval L4 stage and then kept on OP50 (open circles), or transferred to *S. typhimurium* strain 12023 (black symbols), or after 8 h Thimerosal sterilization and returned to OP50 (grey circles). Dead worms were scored accordingly. (Courtesy Labrousse et al., 2000).

5. Assessment of pathogenicity of microbes to *C. elegans*

Both genetic and environmental factors play an important role in determining the virulence of a pathogen. Host mortality assays are generally performed to assess the pathogenicity of the microbes. This is generally done by measuring the time (TD50: time to death for 50% of the host) required by the microbe to kill a fixed percentage of host (Mahajan-Miklos et al., 1999; Garsin et al., 2001). As already mentioned earlier, *S. enterica* serovar Typhimurium colonizes the nematode intestine (Aballay et al., 2000; Labrousse et al., 2000). Adult worms transferred plates seeded with *S. enterica* and incubated at 25° C, the TD50 was shown to be 5.1 days, compared to 9.9 days for control animals fed on *E. coli* OP50 (Aballay et al., 2000). When worms were exposed to *S. enterica* for merely 3 h, then removed to OP50, there was a significant early death in the worm population suggesting the pathogenic effect of *S. enterica* on *C. elegans*. Although invasion of host cells is an essential aspect of *Salmonella* sp. pathogenesis in higher animal systems, yet it has been demonstrated that *S. enterica* does not appear to invade *C. elegans* cells.
Salmonella pathogenicity islands -1 and -2 (SPI-1 and SPI-2), PhoP and a virulence plasmid are required for the establishment of a persistent infection (Alegado & Tan, 2008). It was observed that the PhoP regulon, SPI-1, SPI-2 and spvR are induced in C. elegans and isogenic strains lacking these virulence factors exhibited significant defects in the ability to persist in the worm intestine. Salmonella infection also led to induction of two C. elegans antimicrobial genes, abf-2 and spp-1, which operate to limit bacterial proliferation. Thus resistance to host antimicrobials in the intestinal lumen has been found to be a key mechanism for Salmonella persistence. Apart from genetic factors there are environmental factors, such as, the composition of the media on which the pathogen is grown that has been shown to have influence on the host’s mortality rate. For example, Escherichia coli OP50, which is non pathogenic otherwise can be rendered pathogenic almost as pathogenic as Enterococcus faecalis when it is grown on brain heart infusion (BHI) agar (Garsin et al., 2001). Salmonella enterica strains grown on NGM are rendered infectious depending on their serotypes (Table 1).

<table>
<thead>
<tr>
<th>Strain</th>
<th>Growth media</th>
<th>Pathogenicity status</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. enterica ser. Paratyphi</td>
<td>NGM</td>
<td>Non-pathogenic</td>
<td>Aballay et al., 2000</td>
</tr>
<tr>
<td>S. enterica ser. Typhi</td>
<td>NGM</td>
<td>Non-pathogenic</td>
<td>Aballay et al., 2000</td>
</tr>
<tr>
<td>S. enterica ser. Dublin</td>
<td>NGM</td>
<td>Infectious</td>
<td>Aballay et al., 2000</td>
</tr>
<tr>
<td>S. enterica ser. Enteritidis</td>
<td>NGM</td>
<td>Infectious</td>
<td>Aballay et al., 2000</td>
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Table 1. Effect of media on C. elegans exposed to Salmonella (Adapted from Alegado et al., 2003).

6. C. elegans inherent immune response to Salmonella infection

Innate immunity consists of a variety of defense machinery used by metazoans to avert microbial infections. These nonspecific defense responses used by the innate immune system in animals are governed by interacting and intersecting pathways that not only directs the immune responses but also governs the longevity and responses to different stresses. Even though ample research on C. elegans immune response is still ongoing, yet there has not been enough information on the worms’ innate immune response towards bacterial pathogens in contrast to the fruit fly, Drosophila, and mammals where a fundamental feature like Toll signaling pathway exists. For example, isolation of a strain carrying a mutation in nol-6, which encodes a nucleolar RNA-associated protein in C. elegans or RNAi-mediated depletion of nol-6 as well as other nucleolar genes led to an enhanced resistance to S. enterica mediated killing that was associated with a reduction of pathogen accumulation. These results also demonstrated that animals deficient in nol-6 are more resistant to infections by Gram-negative and Gram-positive pathogens signifying that
nucleolar disruption activates immunity against different bacterial pathogens (Fuhrman et al., 2009). Studies also indicated that nucleolar disruption through RNAi ablation of ribosomal genes resulted in an increased resistance to pathogen that requires P53/CEP-1. Thus from the reports it is quite evident that *C. elegans* activates innate immunity against bacterial infection in a *p53/cep-1*–dependent manner (Fig. 7). Furthermore, *C. elegans* mutants which exhibited reduced pathogen accumulation (Rpa), displayed enhanced resistance to *S. enterica*-mediated killing (Fig. 8).

![Fig. 7.](image1.png) rpa-9 mutants are resistant to both *S. enterica* accumulation and *S. enterica*-mediated killing (Courtesy Fuhrman et al., 2009).

![Fig. 8.](image2.png) rpa-9 mutation activates immunity against *S. enterica* in a *p53/cep-1*–dependent manner. (Courtesy Fuhrman et al., 2009).

To date different molecular approaches, including forward genetics screens and RNAi have facilitated the identification of certain signaling pathways involved in the response of *C. elegans* to infection. For example, *Salmonella enterica* serovars is also known to trigger programmed cell death (PCD), and *C. elegans* cell death (ced) mutants have been shown to be more susceptible to *Salmonella*-mediated killing (3) (Aballay et al., 2003). *Salmonella*-elicited PCD was shown to require p38 mitogen-activated protein kinase (MAPK)
encoded by the pmk-1 gene. On the other hand inactivation of pmk-1 by RNAi blocked Salmonella-induced cell death. C. elegans innate immune response triggered by S. enterica was thus shown to require intact lipopolysaccharide (LPS) and is mediated by a MAPK signaling pathway. Besides innate immunity in C. elegans is known to be regulated by neurons expressing NPR-1/GPCR, a G-protein-coupled receptor related to mammalian neuropeptide Y receptors that functions to suppress innate immune responses (Styser et al., 2008).

With regard to the conserved Toll signaling, C. elegans too possesses a toll-signaling pathway comparable to the innate immunity found in Drosophila or mammals. As opposed to the fly and mammalian tolls, C. elegans tol-1 (the C. elegans homolog of Toll) was previously stated to be required for the worm development and recognition of pathogens but not important for resistance to the pathogens (Pujol et al., 2001). However, later evidences subsequently support that TOL-1 is required to prevent Salmonella enterica invasion of the pharynx, which comprise one of the first barriers against pathogens in C. elegans. It was also illustrated that TOL-1 is required for the correct expression of ABF-2, which is a defensin-like molecule expressed in the pharynx, and heat-shock protein 16.41 (HSP-16.41), which is also expressed in the pharynx, and is part of a HSP superfamily of proteins required for C. elegans immunity. Thus, TOL-1 has been shown to have a direct role in C. elegans defence against pathogens (Tenor & Aballay, 2008).

7. Influence of probiotic bacteria on Salmonella-infected C. elegans

Probiotic bacteria have been defined as living microorganisms that exert useful effects on human health when ingested in sufficient numbers. Lactic acid bacteria (LAB) are the most frequently used probiotic microorganisms. LAB have been found to have a wide range of physiological influences on their hosts, including antimicrobial effects, microbial interference, supplementary effects on nutrition, antitumor effects, reduction of serum cholesterol and lipids, and immunomodulatory effects. Lactobacilli and bifidobacteria fed worms were shown to display increased life span and resistance to Salmonella clearly showing that LAB can enhance the host defense of C. elegans by prolonging the life span (Ikeda et al., 2007). Hence the nematode may once again emerge out as an appropriate model for screening useful probiotic strains or dietetic antiaging substances.

8. Role of NRAMPs and autophagy in bacterial infection

The C. elegans intestine also presents many advantages because this system can mimic the host-pathogen interactions that occur specially during phagocytosis. Macrophages play a pivotal role in the resolution of microbial infections via the process of phagocytosis. Nramp1 (Natural resistance-associated macrophage protein-1) is a functionally conserved iron-manganese transporter in macrophages and manganese, a superoxide scavenger, which is required in trace amounts and functions as a cofactor for most antioxidants. Nramp homologues, smfs, have been identified in the nematode C. elegans (Bandyopadhyay et al., 2009). We have demonstrated that hypersensitivity to the pathogen Staphylococcus aureus, an effect that was rescued by manganese feeding or knockdown of the Golgi calcium/manganese ATPase, pmr-1, indicating that manganese uptake is essential for the innate immune system. Reversal of pathogen sensitivity by
manganese feeding suggested a protective and therapeutic role of manganese in pathogen evasion systems thus proposing that the *C. elegans* intestinal lumen may mimic the mammalian macrophage phagosome and thus could be a simple model for studying manganese-mediated innate immunity. Similar experiments with *Salmonella enterica* in the near future may open more possibilities in favor of utilizing the nematode intestine as a model for manganese-mediated innate immunity.

Autophagy, a lysosomal degradation pathway, plays a crucial role in controlling intracellular bacterial pathogen infections. Jia et al., (2009) showed the outcome of autophagy gene inactivation by feeding RNAi techniques on *Salmonella enterica* serovar Typhimurium infection in *C. elegans*. Genetic inactivation of the autophagy pathway increased bacterial intracellular replication, decreased animal lifespan, and resulted in apoptotic-independent death. In *C. elegans*, genetic knockdown of autophagy genes abrogates pathogen resistance conferred by a loss-of-function mutation, *daf-2(e1370)*, in the insulin-like tyrosine kinase receptor or by overexpression of the DAF-16 FOXO transcription factor. Therefore, autophagy genes play an essential role in host defense in vivo against an intracellular bacterial pathogen and mediate pathogen resistance in long-lived mutant nematodes.

9. *C. elegans* as a target for drug discovery

By means of genomics technologies, *C. elegans* is growing into a prominent model organism for functional characterization of novel drugs in biomedical research. In fact many biomedical discoveries, for example diabetes type 2 diseases, depression (relating to serotonergic signaling) or the neurodegenerative Alzheimer’s disease have been made for the first time using the worms. The simple body plan of the worms has always made it an appropriate model for the fastest and most amenable to cost-effective medium/high-throughput drug screening technologies. Besides, *C. elegans* has always been a better choice over in vitro or cellular models to study drug-reporter interaction and in doing so monitoring the actual behavioral responses of the animals. Conventionally, antimicrobial drug discovery has brought about screening candidate compounds directly on target microorganisms (Johnson & Liu, 2000). In order to discover such novel antimicrobials, a series of antibiotics are therefore being screened to identify those that help in the survival of the worms or markedly reduce the number of bacteria colonizing the nematode intestine. For such high throughput screening of compound libraries, conventional agar-based infection experiments in *C. elegans* are later assessed in liquid media contained in standard 96-well microtiter plates for carrying out the curing assays. Interestingly, these simple infection systems may allow one to screen nearly 6,000 synthetic compounds and more than 1000 natural extracts. Moreover, the in vivo effective dose of many of these compounds was significantly lower than the minimum inhibitory concentration (MIC) needed to prevent the growth of the pathogens in vitro. More importantly, many of the compounds and extracts had not as much of affect on in bacterial growth in vitro. Screening synthetic compound libraries and as well as extracts of natural products for substances that cure worms from bacterial persistent infection allows one to identify compounds that not only blocks pathogen replication in vitro but in addition identifies virulence of the pathogen, may kill it, or may augment the host’s immune response. Nevertheless, activities of some these compounds or extracts are considerably high only in whole animal assay in vivo, and hence the rationale for using a whole-animal screen in a drug discovery program.
10. Closing remark

Attention must be given to the C. elegans natural bacterial food, pathogens and their virulence factors. A better understanding about the dietary behavior and the natural pathogenic organisms of the C. elegans shall open the gates for more information about this worm. Besides, the introduction of genomics and combinatorial chemistry has firmly enabled one to make use of defined targets to identify new antibiotics. The nematode C. elegans has undoubtedly proven to be a simple model for studying the interaction between microbial pathogens and host factors, and further examining the roles of specific gene products to virulence and immunity. It is apparent that there are conserved pathogenic genes involved in C. elegans killing and mammalian pathogenesis. An important experimental advantage of C. elegans as a model to study bacterial pathogenesis is that genetic analysis may as well be carried out in both the pathogens and in the host, simultaneously, a process termed as “interactive genetic analysis.” It would undoubtedly be more useful to further focus on the characterization of chemical suppressors of virulent factor expressions or secretions as candidate novel antibiotics, taking C. elegans as the model. Additionally the worm model would also be useful to address questions with regard to the pathophysiology of worm death in case of lethal infections and further extend to identify the groups of virulent factors that are important in C. elegans killing.

The various categories of experiments so far carried out has provided a proof-of-principle that screening experiments may be useful in identifying new bacterial virulence factors, not only in Salmonella, but perhaps other pathogens that are able to cause a persistent infection in C. elegans, such as S. aureus (Sifri et al., 2003). Until date several loci have been identified from screens not having direct implication in Salmonella virulence. Thus, a saturating genome-wide screen would be extremely fruitful in identifying the predominance of Salmonella genes that are required for persistent infection in C. elegans, some of which could also be important for pathogenesis in other hosts.

11. Acknowledgment

All publications and figures referred in this chapter have been cited to justify the theme of the present review article. We are deeply indebted to all the authors of the original papers. At the same time we sincerely regret for those references that have been left out unintentionally.

12. References


Animal Models for Salmonella Pathogenesis:
Studies on the Virulence Properties Using Caenorhabditis elegans as a Model Host


Salmonella – A Diversified Superbug


Salmonella is an extremely diversified genus, infecting a range of hosts, and comprised of two species: enterica and bongori. This group is made up of 2579 serovars, making it versatile and fascinating for researchers drawing their attention towards different properties of this microorganism. Salmonella related diseases are a major problem in developed and developing countries resulting in economic losses, as well as problems of zoonoses and food borne illness. Moreover, the emergence of an ever increasing problem of antimicrobial resistance in salmonella makes it prudent to unveil different mechanisms involved. This book is the outcome of a collaboration between various researchers from all over the world. The recent advancements in the field of salmonella research are compiled and presented.

How to reference
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