THE INTERACTION BETWEEN *CAENORHABDITIS ELEGANS* AND THE BACTERIAL PATHOGEN *STENOTROPHOMONAS MALTOPHILIA*

by

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B.S., Spelman College, 2010

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

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Abstract

Nematodes play an important role in various habitats where numerous factors serve to shape their communities. One such factor is the potentially pathogenic nematode-prey interaction. This project is focused on the elucidation of the genes that the bacterivorous nematode *Caenorhabditis elegans* employs to respond to the emerging nosocomial bacterial pathogen *Stenotrophomonas maltophilia.* A virulent *S. maltophilia* strain JCMS requires the action of several *C. elegans* conserved innate immune pathways that serve to protect the nematode from other pathogenic bacteria. However, insulin-like DAF-2/16 signaling pathway mutants that are typically pathogen resistant are susceptible to JCMS, and several DAF-2/16 regulated genes are not significantly differentially expressed between JCMS and avirulent *E. coli* OP50. We have determined the complete set of mRNA transcripts under different bacterial treatments to identify genes that might explain this JCMS specific DAF-2/16 pathway evasion. The identified set included 438 differentially expressed transcripts among pairwise comparisons of wild-type nematodes fed OP50, JCMS or avirulent *S. maltophilia* K279a. Candidate genes were nominated from this list of differentially expressed genes using a probabilistic functional connection model. Six of seven genes that were highly connected within a gene network generated from this model showed a significant effect on nematode survival by mutation. Of these genes, C48B4.1, *mpk-2*, *cpr-4*, *clec-67* and *lys-6* are needed for combating JCMS, while *dod-22* was solely involved in K279a response. Only *dod-22* had a documented role in innate immunity, which merits our approach in the identification of gene candidates. To a lesser extent, we have also focused on the identification of virulence factors and the mode of action employed by *S. maltophilia*. JCMS virulence requires *rpfF*, *xps* and involves living bacteria that accumulate in the intestinal lumen. Additionally, the bacterial secretion encoding genes *cs*, *p773*,

p1176, *pi1y1* and *xdi* are involved in JCMS evasion of *daf-2*. In summary, we have discovered a novel host-pathogen interaction between *C. elegans* and *S. maltophilia* JCMS, revealed genes that are involved in each partner of the interaction, and established a new animal model for the study of *S. maltophilia* mode of action.

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Dedication

I would like to dedicate this work to God, my family, especially, my loving and extremely supportive mother (Rita Miller) and my friends. I would not have completed this degree program and project without their love and support.

Chapter 1 - Introductory Literature Review

Nematode Ecology

Nematodes thrive in a variety of habitats and are the most abundant multicellular organisms on Earth (Platt, 1994). This phylum consists of 25,043 species that are free living or parasitic (Zhang, 2013). Generally, Nematoda activity and niche are thought to be limited by excessive or insufficient moisture (Curry, 1994). In grasslands, nematodes are a significant part of the fauna with bacterial feeding nematodes forming 30 - 50% of the nematode community (Curry, 1994). The functional role of soil nematodes can be broadly categorized as ecosystem effect-neutral, disservice and service (Ferris, 2010). Characteristics such as destructive plant herbivory and overgrazing of a limited microbial resource qualify as ecosystem disservices (Ferris, 2010). In terms of ecosystem service, nematodes are involved in the recycling of microbial biomass and soil organic matter (Ingham *et al.*, 1985, Yeates, 2003). For example, nematodes take in excess amounts of nitrogen (N) that is mineralized as ammonia which is then, excreted and available for bacteria and plant uptake (Ferris *et al.*, 1998). In fact, soil mineral N levels are increased by at least 20% by bacterial or fungal feeding nematodes (Chen *et al.*, 1999, Ferris *et al.*, 1998). In agreement, there is a positive relationship between nematode abundance and primary production (Yeates, 1979). Nematodes are also involved in the relocation of organisms to new resources. For instance, bacterial feeding nematodes may distribute bacteria such as the gram positive soil bacterium *Bacillus thuringiensis* (Wei *et al.*, 2003) via digestion (Ingham *et al.*, 1985).

Due to the ecological influence and importance of nematodes, there is also interest in their development as biological indicators. For example, one study found that microbial-feeding nematode abundance changes in response to the addition of N, annual burning and/or season (Jones *et al.*, 2006). Another study found that an increase in nematode community complexity

was linked to a decrease in heavy metal content (Šalamún *et al.*, 2012). Nematode community structure has also been linked to a number of soil properties including pH buffering, electrical conductivity, bulk density and surface organic matter (Pattison *et al.*, 2004). Thus, nematodes respond to a number of ecological disturbances and may be useful in the development of biotic alternatives to chemical tests of environmental quality.

The model organism *Caenorhabditis elegans*

Caenorhabditis elegans is a genetically tractable organism that has been a model system for more than fifty years. Adult *C. elegans* have 959 somatic cells, and the cell lineage is known from egg to adult (Kimble *et al.*, 1979, Sulston *et al.*, 1977, Sulston *et al.*, 1983). *C. elegans* has proven to be an excellent model for developmental biology, neurobiology and aging. More recently, *C. elegans* has been used for the study of microbial pathogenesis, drug development/discovery and innate immunity (reviewed in Ewbank *et al.*, 2011, Irazoqui *et al.*, 2010c, Kim, 2013 and Marsh *et al.*, 2012a). The tractability of *C. elegans* is due to its short generation time and ease of rearing on agar plates with the standard lab food *E. coli* OP50 (Brenner, 1974). Additionally, the natural selfing of hermaphrodites make the propagation of strains and the establishment of homogeneous populations quick and simple (Altun *et al.*, 2009). The use of *C. elegans* is also advantageous due to presence of numerous molecular tools for functional assays. For example, the well accepted method for gene target knock-down, RNA interference (RNAi) was discovered in this model (Fire *et al.*, 1998). *C. elegans* was the first multicellular organism whose genome was sequenced and there are double-stranded RNA libraries for RNAi covering almost 90% of the transcriptome (reviewed in Lamitina, 2006). Other approaches such as genome wide microarray analysis are also highly advanced in this system and offer the ability to gain genome-wide insights on genes associated with a phenotype of interest (Lamitina, 2006). Additionally, transgenic strains can be generated via DNA

microinjection and fluorescent reporter constructs or microbes can easily be visualized within the nematode. The transparency of the nematode also allows observation of the histology of aging and, in relation to nematode-pathogenic interactions, nematode pathology.

C. elegans as an emerging innate immune model

C. elegans is naturally found in microbial infested habitats such as compost heaps and rotting fruits (Barrière *et al.*, 2005, Barrière *et al.*, 2007, Felix *et al.*, 2012). In such habits, there is potential for a number of ecological interactions including host-pathogen. As pathogen avoidance is a favorable trait, it has been postulated to be the origin of a diverse set of microbial virulence factors (reviewed in Irazoqui *et al.*, 2010a). Thus, there is an evolutionary "arms race" present in which nematodes feeding on microbes must evolve defense mechanisms to combat the potential disadvantageous effects of feeding on pathogens. Such mechanisms include the evolution of host innate immune genetic pathways that are reminiscent of those employed by more complex organisms. Recently, it has become realized that the study of invertebrate innate immunity in model systems as *C. elegans* can aid in the understanding of mammalian immune response (reviewed in Hoffmann *et al.*, 1999 and Kurz *et al.*, 2003b). In *C. elegans*, the intestine is the largest somatic organ and it is typically full of microbes (Felix *et al.*, 2012, McGee *et al.*, 2011). In terms of bacteria, the diverse set of gut bacterial flora is reminiscent of microbial communities in higher organisms (Bumbarger *et al.*, 2013, Felix *et al.*, 2012). As in humans, the intestine is lined with microvilli (Troemel *et al.*, 2008), likely involved in nutrient extraction and the first line of defense against potential pathogens (Ewbank *et al.*, 2011, Liévin-Le Moal *et al.*, 2006). In fact, the *C. elegans* intestine responds to microbial cues, signaling to other tissues which influence life history traits such as lifespan (Rera *et al.*, 2013). Thus, the rising use of *C. elegans* for the study of innate immunity has primarily focused on epithelial defense (due to the lack of phagocytes and adaptive immune response) (Sifri *et al.*, 2005). In sum, the use of *C.*

elegans as a model system has its advantages with the study of host-microbe interactions being particularly attractive due to physiological and genetic similarities to higher organisms.

The nematode-bacterial interaction

The dynamic interaction of nematodes with bacteria involves the potential transition from prey-predator to host-pathogen. There are three principal modes of interaction: 1) bacteria as a nutritional source, 2) pathogenic infection and 3) neuronal responses to bacteria (reviewed in Kim, 2013). As a food source, bacteria provide nutrition indirectly through their metabolism (commensalism) or directly through digestion (prey-predator). Furthermore, pathogenic infection involves two different interaction types: bacterial pathology and host innate immunity. Kim suggests the three modes of interaction are distinct and can influence the aging and longevity of *C. elegans* (Kim, 2013). However, these modes of interaction are not mutually exclusive. Neuronal response involves the detection of bacteria and their categorization as food or pathogen, which may or may not elicit immune response. There is also the possibility of bacteria initially being considered prey and later becoming pathogenic. For example, the standard laboratory food *E. coli* OP50 was originally viewed solely as a food source for nematodes but now is considered to be mildly pathogenic (Garigan *et al.*, 2002). The transition of *E. coli* from prey to predator is reviewed by Cabreiro and Gems and involves three stages: predation, symbiosis and dysbiosis (Cabreiro *et al.*, 2013b). The transition between these stages is partially dependent on elements of nematode digestion such as the effectiveness of the nematode anterior grinder at crushing bacterial cells (Portal-Celhay *et al.*, 2012b). Other factors include elements of the aforementioned nematode-bacterial interaction modes such as the host innate immune response and bacterial proliferation within the gut. During predation, bacteria primarily serve as food and are efficiently crushed by the grinder. In the middle stage, the living bacteria that escape digestion are commensals and provide the nematode with nutrients. Dysbiosis involves

the breakdown of this commensalism with a proliferation of living bacteria that becomes detrimental. This break down can occur naturally with nematode immunosenescence (Youngman *et al.*, 2011) during aging and/or due to a metabolic change in one of the partners that is detrimental to the other (reviewed in Cabreiro et al., 2013b). A summary and interpretation of these two concepts is shown in Figure 1.1 that highlights the nematode-bacterial interaction. However, this model provides a framework that summarizes an array of nematode-microbial interaction scenarios.

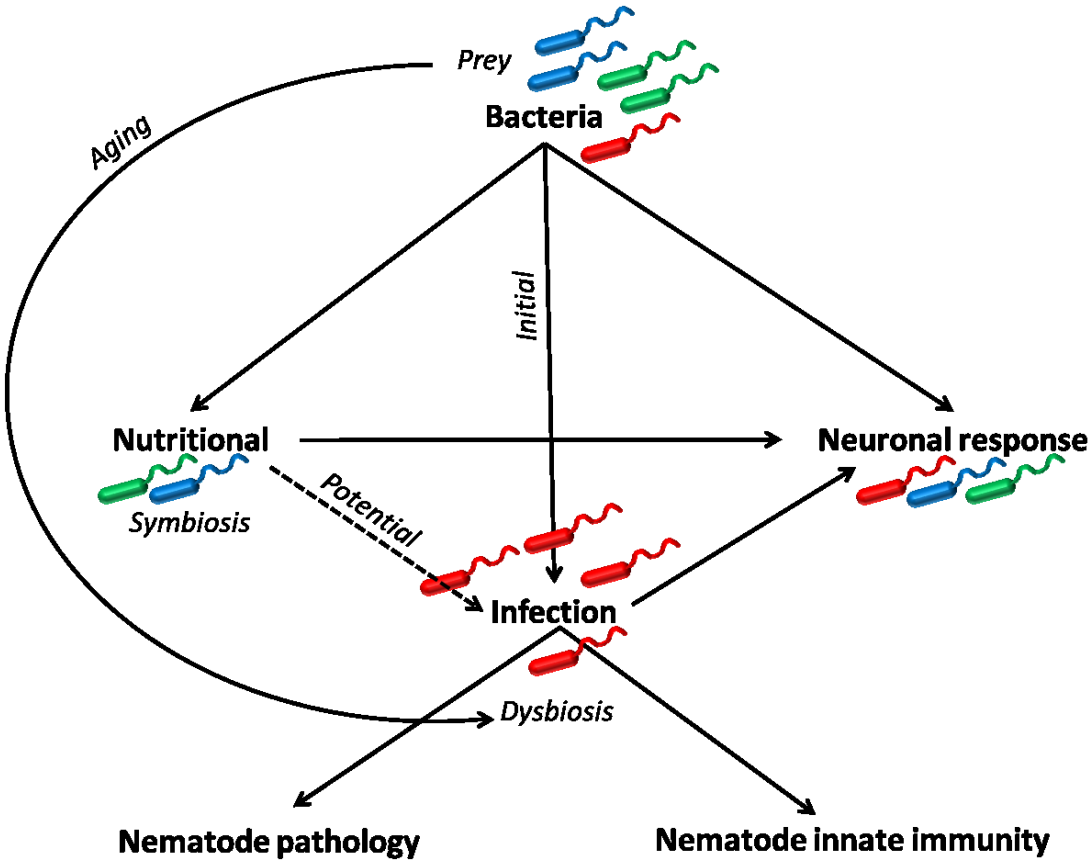


Figure 1.1 Modes of nematode-bacterial interaction.
Adapted from Kim, 2013 to include the potentially sharp or aging related transition from bacterial prey to pathogen in the nematode-bacterial interaction (reviewed in Cabreiro et al., 2013b). Blue bacteria represent non-pathogenic prey, green bacteria are commensals and red bacteria are pathogenic.

Applying the hologenome theory to the nematode-bacteria interaction

Another view of the bacterial-nematode interaction considers the partners as a single evolutionary unit called the holobiont. The hologenome theory states that the organism and its associated microbes are a unit of selection (Rosenberg *et al.*, 2011). Thus, nematodes and their associated bacteria may adapt to the environment through genetic interaction and cooperation. Although, this cooperation has not been thoroughly studied in the nematode-bacterial interaction, there is some evidence supporting the interaction of genes from both partners. For example, endogenous non-coding *E. coli* RNAs regulate *C. elegans* gene expression transcriptionally and/or post-transcriptionally that in turn affects nematode lifespan and behavior (Liu *et al.*, 2012). As postulated for mammals (reviewed in Fraune *et al.*, 2010), a recent study supports the selection of potential commensal bacteria within the *C. elegans* gut. Briefly, *E. coli* that was allowed to adapt to the *C. elegans* host environment was better at *in vivo* colonization and competition with the more pathogenic *Salmonella typhimurium* (Portal-Celhay *et al.*, 2012a). Although, the mechanism of selection is not understood, this study provides evidence that the *E.coli-C. elegans* interaction is favored and suggests that either partner benefits from cohabitation. Additionally, bacteria can be distributed by nematode digestion or external adherence (Ingham *et al.*, 1985), and, if the holobiont is selected upon by distribution, either partner may have genetic mechanisms that facilitate dispersal. In support of this hypothesis, *Microbacterium* (Gravato-Nobre *et al.*, 2005b, Hodgkin *et al.*, 2000) and *Yersinia* sp. (Darby *et al.*, 2002, Tan *et al.*, 2004) specifically colonize the nematode cuticle, and this colonization requires several *C. elegans srf* (SuRFace antigenicity abnormal) genes. Other bacteria such as *Stenotrophomonas maltophilia* produce pili or fimbriae that are implicated in the adhesion of

bacteria to biotic surfaces (De Oliveira-Garcia *et al.*, 2003). Thus, the nematode and bacteria contain genetic elements that could benefit both organisms and be selected on together.

The interaction between bacterial metabolism and nematode survival also supports selection on the holobiont. For instance, changes in bacterial metabolism are known to alter nematode lifespan: *E. coli* ubiquinone synthesis mutants that are defective in respiration increase *C. elegans* lifespan (Saiki *et al.*, 2008). A decrease in bacterial folate synthesis is also linked with extended lifespan in *C. elegans* fed *E. coli* HT115 mutants (Virk *et al.*, 2012). Lastly, the antidiabetic drug metformin causes different effects on *C. elegans* lifespan that is dependent on the metformin sensitivity of the *E. coli* strain consumed (Cabreiro *et al.*, 2013a). Therefore, *C. elegans* lifespan is linked to elements of bacteria metabolism, which, intuitively, must be related to the fact that *C. elegans* is a bacteriovore.

Bacteria as a nematode food source

As a bacteriovore, *C. elegans* naturally requires certain nutrients from the bacteria upon which it feds. Nematodes have increased lifespan on axenic (i.e. no microbial food source) medium (Houthoofd *et al.*, 2002) but, living *E. coli* is needed for optimal growth and reproduction (Lenaerts *et al.*, 2008). Specifically, the addition of metabolically active bacteria rescued the observed reduced brood size and developmental asynchrony exhibited by nematodes reared in axenic culture (Lenaerts *et al.*, 2008). Furthermore, *C. elegans* metabolism involves a number of "dietary response genes" that are differentially expressed in the nematode on non-pathogenic bacteria (MacNeil *et al.*, 2013a, Watson *et al.*, 2013). These dietary response genes also overlap with those that are expressed in response to pathogenic bacteria (MacNeil *et al.*, 2013a). Thus, even pathogenic bacteria provide some type of nutrition to the nematode. In fact, *C. elegans* reared on pathogenic strains of *Photorhabdus luminescens* (Sicard *et al.*, 2007) and *Pseudomonas aeruginosa* (Diaz *et al.*, 2015) have reduced developmental and reproductive rate.

A similar finding was observed for the nematode-*Micrococcus luteus* interaction as these bacteria reduce absolute fitness, a function of intrinsic growth rate and generation time (Coolon *et al.*, 2009). On the other hand, nematodes fed pathogenic *S. enterica* have faster development, reproduction and higher reproductive success than on *E. coli* (Diaz *et al.*, 2015). Lastly, *C. elegans* fed the avirulent soil bacteria *Comamonas* DA1877 have an accelerated growth rate, reduced progeny production and lifespan (MacNeil *et al.*, 2013a). Several of the described effects on nematode life history traits are characteristic of dietary restriction (Szewczyk *et al.*, 2006) and indicate that bacteria have varying nutritional quality (reviewed in MacNeil *et al.*, 2013b).

Neuronal interactions with bacteria

There is substantial evidence for a role of the nervous system in *C. elegans* foraging/bacterial lawn behavior (Apfeld *et al.*, 1999). For example, *wildtype* egg laying is modulated by the presence of bacterial food, which requires the hermaphrodite-specific neurons (HSNs) (Trent *et al.*, 1983). The guanylate cyclases *gcy-35* and *gcy-36* are expressed in several body cavity neurons and function to promote nematode aggregative behavior on bacterial lawns (Cheung *et al.*, 2004). *C. elegans* also slows down upon encountering a bacterial lawn, and this behavior is mediated by a dopaminergic or serotonergic pathway depending on prior experience in environments with or without food (Sawin *et al.*, 2000).

The *C. elegans* nervous system is also required for recognition of bacterial peptides and bacterial pathogens. For instance, avoidance to some *Serratia* strains is regulated through the nematode AWB chemosensory neurons (Pradel *et al.*, 2007). Several genes have been directly implicated in pathogen avoidance, and include *npr-1* (de Bono *et al.*, 2002, Gloria-Soria *et al.*, 2008) which encodes a neuropeptide receptor, the serotonin-gated ion channel gene *mod-1* (Zhang *et al.*, 2005), and *tyra-3* (Bendesky *et al.*, 2011), which encodes a catecholamine

receptor. Mechanistically, the E3 ligase HECW-1 negatively regulates *P. aeruginosa* avoidance through the inhibition of NPR-1 in the OLL neurons (Chang *et al.*, 2011). In agreement with a neuronal role in pathogen avoidance, the nervous system also plays a role in nematode longevity. For instance, the neuromedin U receptor 2 ortholog *nmur-1* is expressed in the sensory and interneurons and is required for *wildtype* lifespan on *E. coli* (Maier *et al.*, 2010). Furthermore, DAF-2/16 insulin-like signaling in the neurons mediates longevity (Wolkow *et al.*, 2000), and dietary restriction induced long life requires the action of the transcription factor *skn-1* in the ASI head neurons(Bishop *et al.*, 2007). In sum, the nervous system plays a role in longevity, pathogen response and the detection of bacterial food. In some cases, the pathogen response has been shown to involve the recognition of specific bacterial molecules (Maier *et al.*, 2010, Pradel *et al.*, 2007); a process that is likely more prominent that currently documented.

Gene functions involved in nematode innate immunity

The *C. elegans* genome encodes a number of genes that are putatively involved in innate immune response. Most of these genes are annotated on the basis of homology and identified due to regulation upon infection (reviewed in Ewbank *et al.*, 2011). Such transcriptomic and proteomic studies typically identify hundreds of candidates with an array of associated gene ontology (GO) and protein domain and/or family terms. Some of the overlapping terms include: C-type lectin, lipid binding, fatty acid metabolism, F-box or CUB domain, ShK toxin, ion channel, cytochrome, protease, ribosome, lysozyme and lipase (Coolon *et al.*, 2009, Engelmann *et al.*, 2011, Irazoqui *et al.*, 2010b, Troemel *et al.*, 2006, Visvikis *et al.*, 2014, Wong *et al.*, 2007). Some commonly encountered broader GO terms include: collagen, development, membrane, metabolism, transcription and/or translation (Coolon *et al.*, 2009, Wong *et al.*, 2007). Several studies have taken this omic-level analysis a step further by determining which terms occur frequently (over-represented) in a list of differentially expressed genes in an effort to

describe what functions are important to the process of interest. Some of the terms identified for the nematode-bacterial response include: proteolysis, stress response, cell death, fatty acid metabolic process, lectin, lipid binding, lysozyme activity, translation and transcription (Engelmann *et al.*, 2011, Visvikis *et al.*, 2014, Wong *et al.*, 2007). In terms of genetic pathway regulation, a study comparing the expression of nematodes on *Serratia marcescens*, *E. faecalis* and *P. luminescens* found an over-representation of DKf-2, p38 MAPK and TGF-beta pathway gene effectors that were up-regulated upon infection (Engelmann *et al.*, 2011). This study also found that DAF-2/16 regulated genes were over-represented among the down-regulated genes (Engelmann *et al.*, 2011).

Several studies have used functional annotation to provide evidence for a nematode innate immune response that is specific and shared between microbial environments. For example, genes involved in proteolysis, stress response, insulin signaling and cell death are common to *E. faecalis*, *E. carotovora* and *P. luminescens* expression profiles while, only infection with *E. faecalis* is associated with a down-regulation of hormone receptors (reviewed in Wong *et al.*, 2007). Another study revealed that only some (11-26%) genes overlapped when comparing the *C. elegans* expression profiles on *S. aureus*, *P. aeruginosa* and/or the fungus *Candida albicans* (Pukkila-Worley *et al.*, 2011). Of the overlapping genes, many genes involved in carbohydrate binding i.e. lectins were up-regulated on both bacteria and down-regulated on the fungus (Pukkila-Worley *et al.*, 2011). This study also revealed a set of putative antifungal genes such as *abf-2*, *thn-1*and *cht-1* that are *C. albicans*-specific (Pukkila-Worley *et al.*, 2011). A similar situation is described in another expression study in which genes encoding putative and/or curated antimicrobial peptides such as *cnc*, *fip*, *fipr* and *nlp* genes are regulated on the

fungi *Drechmeria coniospora* and *Harposporium sp.* and not by bacterial infection (Engelmann *et al.*, 2011).

Nematode antimicrobial genes

Only a few of the putative antimicrobial genes in *C. elegans* have been functionally analyzed. The *C. elegans* antibacterial factor (ABF) peptides are similar to vertebrate defensins and expressed in the nematode intestine and pharyngeal tissue (Alper *et al.*, 2007, Kato *et al.*, 2002). ABF-2 exhibits microbicidal activity against various genera of bacteria and yeasts (Kato *et al.*, 2002). Additionally, ABF-2 may kill via cytoplasmic membrane disruption (Zhang *et al.*, 2000a) and, as expected, the knockdown of *abf-2* increases bacterial load in nematodes fed *S. typhimurium* (Alegado *et al.*, 2008). Higher bacterial load was also observed for mutants of the caenopore *spp-1* (Alegado *et al.*, 2008). Caenopores are a large family of 33 saposin-like proteins that share structural similarity with amoebapores and cytotoxic vertebrate proteins (reviewed in Ewbank *et al.*, 2011). These proteins are predominantly expressed in the intestine and can kill bacteria by permeabilizing their cytoplasmic membrane (Alper *et al.*, 2007, Hoeckendorf *et al.*, 2012, Roeder *et al.*, 2010). SPP-12 activity kills *B. megaterium*, *S. cerevisiae* and *D. discoideum* and is unique in that it is localized to the pharyngeal neurons (Hoeckendorf *et al.*, 2012). Mutants of *spp-12* are also short-lived on pathogenic *B. thuringiensis* but tolerant to non-pathogenic bacteria (Hoeckendorf *et al.*, 2012). Knockdown of another saposin gene *spp-5* caused significant distension, reduced fitness and a decreased lifespan in nematodes fed *E. coli* (Roeder *et al.*, 2010). The 15 member class of lysozymes (Schulenburg *et al.*, 2008) such as *lys-1*, *lys-7* and *lys-8* are needed for resistance to bacterial pathogens and are expressed in the intestine (Alper *et al.*, 2007, Mallo *et al.*, 2002, Murphy *et al.*, 2003, O'Rourke *et al.*, 2006, Portal-Celhay *et al.*, 2012b). C-type lectins are thought to be involved in pathogen recognition, and several genes enhance susceptibility when knocked down on *Staphylococcus aureus* and

Microbacterium nematophilum (Irazoqui *et al.*, 2010b). Intriguingly, another study found that C-type lectin over-expression enhanced resistance to *S. aureus* but caused hyper-susceptibility to *P. aeruginosa* (Irazoqui *et al.*, 2010b). Thus, C-type lectins have a putative antimicrobial role but, their expression is not always beneficial to the host. Lastly, the over-expression of neuropeptide-like peptides (NLPs) and caenacins (CNCs) confer enhanced resistance to the fungus *D. coniospora* (Pujol *et al.*, 2008, Zugasti *et al.*, 2009). NLP-31 has microbicidal activity against the fungi *D. coniospora, Aspergillus fumigatus* and *Neurospora crassa* and the bacteria *M. luteus* and *E. coli* (Couillault *et al.*, 2004). Thus, as suggested by the terms (protein domain or family and GO) that occur frequently in lists of *C. elegans* genes that are differentially expressed between microbial environments, proteins such as lysozymes and C-type lectins are involved in innate immune response. However, antibacterial factor peptides and/or other gene products reviewed above that are not commonly associated with over-represented terms should also be taken into consideration.

Nematode innate immune pathways

C. elegans antimicrobial genes are regulated by a number of different innate immune pathways and putative pathway components that act in various tissues (reviewed in Ewbank *et al.*, 2011, Irazoqui *et al.*, 2010a and Partridge *et al.*, 2010). As a major immune organ, the intestine is the site of action for numerous genetic pathways. These include the p38 MAPK, DAF-2/16, unfolded protein response (UPR), TGFβ, Wnt/Hox and ERK MAPK pathway. The DAF-2/16 and TGFβ pathway also act in the nervous system, and the p38 MAPK and TGFβ pathway are also required in the epidermis. Lastly, the UPR and Toll-like pathways are localized to the pharynx. Many of these pathways contain components with mammalian homologues that also may be important for innate immunity and/or stress response (Irazoqui *et al.*, 2010a, Kurz *et al.*, 2003b). These pathways can act in parallel, upstream or downstream of each other and other

putative immune response genes. The p38 MAPK pathway functions upstream of the UPR to modulate the response to bacterial pore-forming toxins (PFTs)(Bischof *et al.*, 2008), acts in parallel of *bar-1* and *egl-5* (Wnt/Hox) during host infection (Irazoqui *et al.*, 2008) and is thought to function in parallel of the putative immune receptor FSHR-1 [the sole leucine-rich repeat (LRR)-containing G-protein-coupled receptor (Powell *et al.*, 2009)]. The DAF-2/16 pathway is also thought to function in parallel to p38 MAPK signaling (Troemel *et al.*, 2006) and the DAF-2/16 pathway transcription factor *daf-16* is regulated by TGFβ signaling (Lee *et al.*, 2001). The following paragraphs review the known roles of the major *C. elegans* innate immune pathways. These pathways are involved in multiple types of stress response but this review is focused on the findings that are relevant to pathogen response. The pathways that were assessed for involvement on different bacteria in Chapter 2 are visualized in Figure 1.2 below:

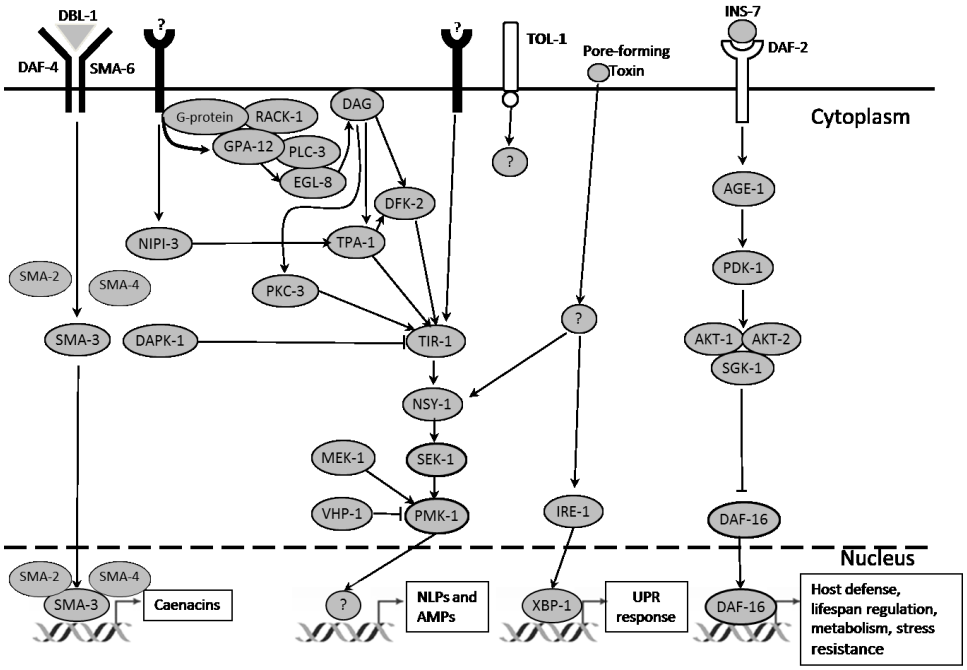


Figure 1.2 *C. elegans* innate immune pathways.
This figure (adapted from Irazoqui *et al.*, 2010a, Irazoqui *et al.*, 2010c and Partridge *et al.*, 2010) includes the p38 MAPK, DAF-2/16, unfolded protein response (UPR), TGFβ pathway and the Toll-like receptor (TLR) gene *tol-1* which were evaluated for involvement on *E. coli* OP50 and *S. maltophilia* JCMS in Ch. 2 of this dissertation.

DAF-2/16 insulin-like signaling pathway

In the conserved DAF-2/DAF-16 pathway, DAF-2 negatively regulates the forkhead box O (FOXO) transcription factor DAF-16 ((Lee *et al.*, 2001) and Figure 1.2). DAF-16 regulates hundreds of genes implicated in development, metabolism, stress response and aging (Henderson *et al.*, 2001, Lee *et al.*, 2003, Murphy *et al.*, 2003). The regulation of these genes is achieved by the tissue specific and overlapping endogenous expression patterns of different *daf-16* isoforms that regulate distinct and overlapping downstream genes (Kwon *et al.*, 2010). Some of the functional classes of genes strongly regulated by *daf-16* include protease, mitochondrial, heat shock and cytochrome P450 (McElwee *et al.*, 2003). As a result, *daf-2* loss of function mutants have extended lifespan and this longevity is dependent on the downstream transcription factor *daf-16* (Kenyon *et al.*, 1993, Lin *et al.*, 1997) and p38 MAPK pathway *pmk-1* (Troemel *et al.*, 2006). Mutants of *daf-2* are dependent upon several stress response genes such as those involved in endocytotic trafficking (Samuelson *et al.*, 2007), *sod-3* (superoxide dismutase) and catalase genes *ctl-1* and *ctl-2* for lifespan extension (Murphy *et al.*, 2003). Additionally, *sod-3* and *ctl-2* are needed for *daf-2* mutant resistance to *E. faecalis* (Chávez *et al.*, 2007) ,and several antimicrobial genes (*spp-12*, *spp-1*, *lys-8* and *lys-7*) were also found to regulate *daf-2* longevity (Murphy *et al.*, 2003). As one would expect given the necessity of antimicrobial genes in *daf-2* mutant longevity, *daf-2* mutants are also resistant to *P. aeruginosa* (Singh *et al.*, 2006). In fact, *daf-2* mutants are long lived on most bacteria including *Bacillus subtilis*, *E. coli* and the pathogen *Enterococcus faecalis* (Garsin *et al.*, 2003, Portal-Celhay *et al.*, 2012b). Mutants of *daf-2* also carry significantly less bacterial load than wild-type nematodes (Portal-Celhay *et al.*, 2012b), suggesting that DAF-2 is involved in preventing bacterial infection. The pathogen resistance phenotype exhibited by *daf-2* mutants also requires the activity of *daf-16* (Garsin *et*

al., 2003, Troemel *et al.*, 2006) and suggests that *daf-2* signaling to *daf-16* plays a role in *C. elegans* innate immunity.

Given the negative regulation of *daf-16* by *daf-2*, one would expect loss of function mutants of these genes to have an opposite phenotype when comparing survival on the same bacterial environment. As expected given the lifespan extension exhibited in *daf-2* mutants, *daf-16* mutants are susceptible to *E. coli* and S. *typhimurium* (Portal-Celhay *et al.*, 2012b). This study also found that *daf-16* is required to prevent pathogenic bacterial colonization (Portal-Celhay *et al.*, 2012b) and, another study found that nuclear localization of *daf-16* is induced by pathogenic bacteria (Wang *et al.*, 2012). Additionally, *daf-16* is required for the pathogen resistance exhibited by sterile nematodes (Miyata *et al.*, 2008). Together these data suggest that *daf-16* plays a role in *C. elegans* innate immune response that interacts with nematode reproduction. However, other studies have reported that *daf-16* loss of function mutants have lifespans that are not significantly different from *wildtype* on various bacteria (Kerry *et al.*, 2006, Troemel *et al.*, 2006). Thus, the role of *daf-16* is specific to bacterial environment.

The DAF-2/16 pathway is unique in that mutants of multiple pathway components are resistant to bacterial pathogens and involved in longevity. Mutants of the phosphoinositide 3-kinase encoding *age-1* are long-lived on bacterial pathogens (Evans *et al.*, 2008a, Garsin *et al.*, 2003) and resistant to bacterial colonization (Portal-Celhay *et al.*, 2012b). The downstream potentiated serine threonine kinases AKT-1 and AKT-2 are also resistant to pathogen killing and function with partial redundancy regulating unique and overlapping antimicrobial genes (Evans *et al.*, 2008a). The other DAF-2/16 pathway serine threonine kinases PDK-1 and SGK-1 have *wildtype* pathogen resistance but are involved in longevity and other stress responses (Evans *et al.*, 2008a, Hertweck *et al.*, 2004, Oh *et al.*, 2005).

Toll-like receptor (TLR) and Toll and interleukin 1 receptor (TIR)

Although, the Toll-like receptor (TLR) gene(s) play a major role in vertebrate (reviewed in Kopp *et al.*, 1999 and Medzhitov *et al.*, 2000) and invertebrate, such as *Drosophila* immunity (reviewed in Valanne *et al.*, 2011), their role is less conserved in *C. elegans*. The single Toll-like receptor (TLR) gene *tol-1* (Figure 1.2) does not play a role in the *C. elegans* innate immune response to *M. nematophilum*, *P. aeruginosa* and *D. coniospora* (Pujol *et al.*, 2001) and does not regulate antimicrobial gene expression on *D. coniospora* (Couillault *et al.*, 2004). However, *tol-1* mutants and mutants of three putative TLR-associated signaling components *pik-1, ikb-1* and *trf-1* all are susceptible to *S. marcescens* and *E. coli* (Pujol *et al.*, 2001). This study also found that *tol-1* is required for *S. marcescens* avoidance (Pujol *et al.*, 2001) which is mediated through the two AWB chemosensory neurons (Pradel *et al.*, 2007). TLR pathway components *tol-1*, *ikb-1* and *trf-1* are also involved in resistance to *S. enterica* pharyngeal evasion and *tol-1* regulates the pharyngeal expression of defensing-like *abf-2* (Tenor *et al.*, 2008). Therefore, the function of *tol-1* in immune response is conserved but, in *C. elegans*, this gene is only involved in the response to specific bacteria. The function of TIR-1, a Toll and interleukin 1 receptor (TIR) domain containing protein is less specific in that this gene product is required for *D. coniospora* and *S. marcescens* resistance (Couillault *et al.*, 2004). Intriguingly, TIR-1 is actually a component of the p38 MAPK pathway and acts upstream of the MAP3K NSY-1 to regulate olfactory neuron asymmetry (Chuang *et al.*, 2005).

p38 MAPK pathway

The mitogen activated protein kinase (MAPK) PMK-1 is a central innate immune response regulator that controls the expression of many putative and several established antimicrobial genes including lectins and lysozymes (Troemel *et al.*, 2006). Thus, there have been a number of studies that implicate *pmk-1* and p38 MAPK signaling (Figure 1.2) in the

innate immune response. For example, loss of *pmk-1* causes hyper-susceptibilty on a number of bacterial pathogens (Kim *et al.*, 2002, Troemel *et al.*, 2006) and is associated with an increase in *S. typhimurium* intestinal colonization (Portal-Celhay *et al.*, 2012b). This hyper-susceptibility and increase in bacterial load dissipates when nematodes are exposed to non-pathogenic bacteria (Portal-Celhay *et al.*, 2012b). Additionally, the gonadal programmed cell death phenotype caused by *S. enterica* requires *pmk-1 (Aballay et al., 2003).* Therefore, like in mammals (Ichijo *et al.*, 1997), *C. elegans* has a p38 MAPK-dependent cell death pathway which might be connected to the interaction of this gene and cellular stress response pathways (Bischof *et al.*, 2008). Downstream, the basic-region leucine zipper transcription factor ATF-7 switches from repressor to activator when phosphorylated by PMK-1 (Shivers *et al.*, 2010). Intriguingly, this study found that both *pmk-1* and *atf-7* are needed for response to *P. aeruginosa* and *S. marcescens* but response to *E. faecalis* requires a *pmk-1* dependent and *atf-7* independent mechanism (Shivers *et al.*, 2010). ATF-7 is also downstream of the MAP2K SEK-1 and MAP3K NSY-1 which are also required for response to pathogens (Kim *et al.*, 2002, Shivers *et al.*, 2010). Thus, the evolutionary conserved p38 MAPK pathway is clearly involved in *C. elegans* innate immunity. The consistent hyper-susceptibility of p38 MAPK pathway components on varied bacterial pathogens is likely due to the many roles of MAPK signaling in organismal development, survival and stress response (reviewed in Keshet *et al.*, 2010).

Wnt/Hox pathway

Although, the p38 MAPK pathway has a multitude of evidence supporting a role in innate immunity, a recent study found that *pmk-1* did not regulate immune effector genes on *S. aureus* (Irazoqui *et al.*, 2008). In this case, the nematode bacterial-interaction requires the action of *bar-1* and *egl-5*. The transcriptional cofactor *bar-1*/β-catenin and the transcription factor *egl-5* are implicated in development and function as components and/or downstream of Wnt signaling

(Chisholm, 1991, Eisenmann *et al.*, 2000, Jiang *et al.*, 1998). Both genes are needed for the regulation of immune effectors genes such as the C-type lectin encoding *clec-52*, *clec-60* and *clec-71*; *bar-1* regulates the expression of cysteine-protease related *cpr-2* and invertebrate lysozyme *ilys-3* while, *egl-5* does not (Irazoqui *et al.*, 2008). Additionally, *bar-1* and *egl-5* mutants are both more susceptible to *S. aureus* than *wildtype*, and *bar-1* mutants also exhibit increased intestinal degradation (Irazoqui *et al.*, 2008). EGL-5 has also been shown to be involved in the response to *M. nematophilum*. Specifically, *egl-5* acts in the rectal cells and is required for *M. nematophilum* tail swelling pathology (Nicholas *et al.*, 2009). Thus, Wnt/Hox signaling is implicated in *C. elegans* innate immunity and the specific role is hypothesized to involve the control of immune effectors and/or the development of pathogen sensory tissue (Irazoqui *et al.*, 2008).

Transforming growth factor beta (TGFβ) signaling

The TGFβ Sma/Mab pathway (Figure 1.2) includes the ligand *dbl-1*, the type one and type two receptor genes *sma-6* and *daf-4*, and the Smad genes *sma-2*, *sma-3* and *sma-4* (Savage-Dunn *et al.*, 2003, Savage *et al.*, 1996, Suzuki *et al.*, 1999). This pathway is known to be involved in body size development (Savage-Dunn *et al.*, 2003, Savage *et al.*, 1996, Suzuki *et al.*, 1999) and the regulation of reproductive aging (Luo *et al.*, 2009). TGFβ signaling is also required for *S. marcescens* infection induced antimicrobial gene expression (Mallo *et al.*, 2002). This pathway regulates genes that are unique and overlap with those regulated by p38 MAPK components *tir-1* and *nsy-1* (Alper *et al.*, 2007). The TGFβ ligand *dbl-1* is needed to prevent pathogenic bacterial colonization and mutants of this gene were susceptible to *E. coli* and *S. typhimurium* (Portal-Celhay *et al.*, 2012b). DBL-1 is also involved in the induction of caenacin genes independent of the p38 MAPK in response to fungal infection (Zugasti *et al.*, 2009). In response to pathogenic bacteria, *dbl-1* regulates the expression of putative immunity effectors:

clec-85, *dod-22*, K08D8.5 and F55G11.7 (Alper *et al.*, 2007). The TGFβ pathway receptor DAF-4/SMA-6 and the downstream signaling component SMA-3/SMAD are also all needed for the induction of caenacins (Zugasti *et al.*, 2009).

C. elegans pathogen-induced damage response

The host response to pathogens involves two mechanisms: the tolerance of infection and the more frequently studied removal of infection (reviewed in Ayres *et al.*, 2012). Recently, researchers have provided evidence supporting a role for a "tolerance of infection" or damage-induced response to pathogens in *C. elegans* (Bakowski *et al.*, 2014, Visvikis *et al.*, 2014). In fact, intestinal infection with *N. parisii* is unique in that it does not require the p38 MAPK and DAF-2/16 pathway for resistance (Troemel *et al.*, 2008). In this case, resistance to infection involves ubiquitin ligase complex components, the ubiquitin-proteasome system and autophagy (Bakowski *et al.*, 2014). Other genes and the unfolded protein response (UPR) pathway are also involved in cellular stress response and have been implicated in innate immunity. Nematodes that lack the *atf-6* or *ire-1* arm of the UPR have increased sensitivity to PFT Cry5B (Bischof *et al.*, 2008). The UPR *ire-1* arm transcription factor *xbp-1* is essential for the maintenance of endoplasmic reticulum (ER) integrity during *P. aeruginosa* infection (Richardson *et al.*, 2010). Additionally, the apoptotic receptor CEP-1 regulates the expression of PQN/ABU proteins involved in a non-canonical UPR to pharyngeal invasion of *S. enterica* (Haskins *et al.*, 2008). Lastly, the endo and exocytosis regulators *rab-5* and *rab-11* are required for pore forming toxin resistance and involved in the restoration of intestinal cell plasma membrane integrity (Los *et al.*, 2011).

Mechanisms involved in more broad homeostatic processes are also important in the response to bacteria. A recent study found that JNK kinase signaling is involved in the surveillance of core processes that, if disrupted, stimulate bacterial avoidance (Melo *et al.*, 2012).

The DAF-2/16 pathway is postulated to regulate (positively for *daf-16* and negatively for *daf-2*) heat shock proteins and oxidative stress enzymes to maintain protein homeostasis during infection (Mohri-Shiomi *et al.*, 2008). This opposing regulation by *daf-2* and *daf-16* continues even as protein homeostasis decreases during nematode aging (Hsu *et al.*, 2003, Morley *et al.*, 2002). Specifically, HSF-1 is required for *C. elegans* defense to a number of bacterial pathogens and is needed for *daf-2* mutants to be resistant to *P. aeruginosa* (Singh *et al.*, 2006). In fact, pathogen resistant *daf-2* mutants have higher levels of HSP90 than *wildtype* (Singh *et al.*, 2006). Lastly, *C. elegans* produces reactive oxygen species (ROS) that are localized to the intestine in response to pathogens (Chávez *et al.*, 2007). As expected, the oxidative stress effectors *ctl-2* (peroxisomal catalase) and *trx-1* (thioredoxin) are needed for resistance to *S. typhimurium* killing and colonization (Portal-Celhay *et al.*, 2012b). These data support a less specific response to bacteria in which the damage caused by pathogenic interaction is recognized and triggers genes that play a role in damage clearance and homeostasis.

Nematode-bacterial pathology

In the natural environment, bacteria encounter a number of organisms including nematodes that serve as predators. This circumstance makes the production of bacterial virulence factors and/or characteristics that are advantageous in the nematode-bacterial interaction essential for bacterial survival. Over the last 15 years, many studies have focused on identifying and distinguishing the mode of bacterial pathogenic action in a variety of nematode-bacterial interactions. Table 1.1 (adapted and updated from Darby, 2005) summarizes these nematode-bacterial interactions and their known pathology in *C. elegans*. The study of several fungal-nematode interactions (Couillault *et al.*, 2004, Engelmann *et al.*, 2011, Pukkila-Worley *et al.*, 2011, Troemel *et al.*, 2008) has provided useful information on the specificity of the nematode-pathogen interaction and were also included. The listed pathologies are not mutually exclusive

and some pathogens were placed in multiple categories if evidence was found to support the given pathology. Many of the listed pathogens are also human opportunistic pathogens which means "one that utilizes the opportunity offered by weakened defense mechanisms to inflict damage to the host" (Von Graevenitz, 1977). This concept relates back to the idea of dysbiosis (Cabreiro *et al.*, 2013b) in which the nematode-microbial relationship shifts from prey to predator and the microbe becomes detrimental (Figure 1.1).

Table 1.1 Nematode-pathogen interaction *C. elegans* pathologies.

C. elegans pathology	Organisms
Diminished lifespan	*Aeromonas hydrophila* (Bogaerts *et al.*, 2010, Couillault *et al.*, 2002), *Agrobacterium tumefaciens* (Couillault *et al.*, 2002), *Burkholderia cenocepacia* (Huber *et al.*, 2004), *Burkholderia cepacia-like* (Wopperer *et al.*, 2006), *Burkholderia pseudomallei* (Gan *et al.*, 2002, Ooi *et al.*, 2012), *Candida albicans* (Pukkila-Worley *et al.*, 2011), *E. faecalis* (Garsin *et al.*, 2001, Sifri *et al.*, 2002), *Erwinia carotovora* (Couillault *et al.*, 2002), *Erwinia chrysanthemi* (Couillault *et al.*, 2002), *Nematocida parisii* (Troemel, 2011), *P. luminescens* (Sicard *et al.*, 2007), *P. aeruginosa* (Garsin *et al.*, 2003, Tan *et al.*, 1999a), *Salmonella enterica* (Garsin *et al.*, 2003, Tenor *et al.*, 2004), *S. typhimurium* (Labrousse *et al.*, 2000), *S. marcescens* (Kurz *et al.*, 2003a, Mallo *et al.*, 2002), *Shewanella frigidimarina* (Couillault *et al.*, 2002), *Shewanella massilia* (Couillault *et al.*, 2002), *S. aureus* (Garsin *et al.*, 2001), *Streptococcus pneumoniae* (Garsin *et al.*, 2001), *Streptococcus pyogenes* (Jansen *et al.*, 2002) and *Xenorhabdus nematophila* (Couillault *et al.*, 2002)
Toxin	*B. thuringiensis* (Marroquin *et al.*, 2000), *B. cenocepacia* (Köthe *et al.*, 2003), *B. pseudomallei* (Gan *et al.*, 2002, Ooi *et al.*, 2012), *P. luminescens* (Hu *et al.*, 1999, Sato *et al.*, 2014), *P. aeruginosa* (Darby *et al.*, 1999, Gallagher *et al.*, 2001), *S. pneumoniae* (Jansen *et al.*, 2002), *Streptococcus agalactiae* (Bolm *et al.*, 2004), *Streptococcus dysgalactiae* (Bolm *et al.*, 2004), *Streptococcus mitis* (Bolm *et al.*, 2004), *Streptococcus oralis* (Bolm *et al.*, 2004), *S. pyogenes* (Jansen *et al.*, 2002) and *X. nematophila* (Brillard *et al.*, 2001).
Gut infection	*B. cenocepacia* (Köthe *et al.*, 2003), *C. albicans* (Pukkila-Worley *et al.*, 2011), *E. faecalis* (Garsin *et al.*, 2001, Sifri *et al.*, 2002), *N. parisii* (Bakowski *et al.*, 2014), *P. aeruginosa* (Tan *et al.*, 1999a, Tan *et al.*, 1999b), *S. enterica* (Aballay *et al.*, 2003, Tenor *et al.*, 2004), *S. marcescens* (Kurz *et al.*, 2003a) and *S. aureus* (Begun *et al.*, 2007).
Germline cell death	*S. enterica* (Aballay *et al.*, 2003).
Cuticle infection	*M. nematophilum* (Hodgkin *et al.*, 2000), *X. nematophila* (Drace *et al.*, 2008), *Yersinia pestis (Darby et al., 2002)*and *Yersinia pseudotuberculosis* (Joshua *et*

	al., 2003).
Full body parasitism	*D. coniospora* (Jansson, 1994).

The updated *C. elegans* pathology of bacterial and significant fungal (*C. albicans, N. parisii* and *D. coniospora)* interactions (Darby, 2005). Most of the organisms listed above are human pathogens and have clinical importance (Beck-Sagué *et al.*, 1993, Kuroki *et al.*, 2009, Mahajan *et al.*, 1995, Sifri *et al.*, 2005) and (reviewed in Darby, 2005).

As seen in Table 1.1, many of the microbial processes at work in the nematode are similar to those seen in human-pathogen interactions. For example, *Salmonella* has been found to infect the gut of both humans and *C. elegans* (McCormick *et al.*, 1993, Tenor *et al.*, 2004). In agreement, there is a notable overlap between *Salmonella* type II secretion system-associated virulence factors required for nematode and human pathogenesis (Tenor *et al.*, 2004). In *E. faecalis*, a putative quorum-sensing system, cytolysin (Singh *et al.*, 1998) and a sucrose-6-phosphate hydrolase are implicated in mammalian and nematode killing (Garsin *et al.*, 2001).

The use of the nematode to study bacterial pathogens has led to the discovery of a number of additional bacterial virulence requirements. Generally, virulence seems to require living bacteria for pathogens such as *S. marcescens* that cause gut infection but have not been found to secrete a toxin (Table 1.1and (Kurz *et al.*, 2003a).Wild-type *Salmonella* lipopolysaccharide (LPS) is needed for *C. elegans* gut infection and gonadal programmed cell death (Aballay *et al.*, 2003). *S. marcescens* virulence also seems to require LPS for virulence along with hymolysin and iron uptake (Kurz *et al.*, 2003a). These virulence requirements differ among bacterial genera and change according to the environment. For example, *P. aeruginosa* colonizes the intestine (Tan *et al.*, 1999a), but unlike other pathogens (Table 1.1), this colonization is not thought to be required for nematode killing in liquid (Kirienko *et al.*, 2013). In this case, *P. aeruginosa* virulence requires the bacterial siderophore pyoverdin which sequesters host iron and causes a hypoxic response in *C. elegans* (Kirienko *et al.*, 2013). Killing

in liquid also does not require quorum-sensing (Kirienko *et al.*, 2013), however, quorum-sensing regulators LasR and RhIR control hydrogen cyanide (Gallagher *et al.*, 2001) that lethally paralyzes *C. elegans* on *P.aeruginosa* lawns grown on brain heart infusion (BHI) agar (Darby *et al.*, 1999). Quorum-sensing gene products GacA and LemA are also *P. aeruginosa* virulence factors (Tan *et al.*, 1999b) and, *P. aeruginosa* evades the *C. elegans* immune system via stimulation of the DAF-2/16 pathway, which suppresses the expression of immune effector genes (Evans *et al.*, 2008b).Thus, *C. elegans* pathology involves mechanisms that facilitate bacteria-bacteria contact, nematode degradation and exploitation of the host environment.

Stenotrophomonas maltophilia

Stenotrophomonas maltophilia are heterogeneous, gram negative, non-fermentative obligate aerobes, which can live in a variety of environments *(Rocco et al., 2009, Ryan et al., 2009)*. These bacteria are found in plant rhizospheres, oil brines, a variety of soil types and a number of water sources including rivers, sewage, wells and bottled water (reviewed in Denton *et al.*, 1998). Although ever-present in nature, *S. maltophilia* are most commonly found in association with plants. Not surprisingly, *S. maltophilia* were once considered to be a part of the plant pathogenic genus *Xanthomonas* (Palleroni *et al.*, 1993). However, the plant - *S. maltophilia* interaction can be beneficial to plants in that the bacteria produce protective antimicrobial compounds (Kai *et al.*, 2007, Suma *et al.*, 2013) and generate factors that promote plant growth (reviewed in Ryan *et al.*, 2009). Such factors include the provision of sulfate through the oxidation of sulfur (Banerjee *et al.*, 2009) and nitrogen fixation (Park *et al.*, 2005). *S. maltophilia* are also distinct from *Xanthomonas spp.* in their resistance to antibiotics, growth at human body temperature and pathogenic association with humans (reviewed in Palleroni *et al.*, 1993).

S. maltophilia are considered emerging opportunistic nosocomial pathogens, are inherently resistant to antibiotics and have been associated with a number of diseases and

infections (reviewed in Brooke, 2012, Denton *et al.*, 1998 and Quinn, 1998). These bacteria are not highly pathogenic but mortality rates range from 14 to 69% in patients with bacteremia (Jang *et al.*, 1992, Victor *et al.*, 1994). This bacterium has been isolated from necropsy specimens, blood culture, skin lesions, oropharyngeal swabs of healthy adults and immunocompromised patients (Denton *et al.*, 1998). *S. maltophilia* also accounts for a small percentage of nosocomial pneumonias (A'Court *et al.*, 1992), the pathogenesis of Crohn's disease and ulcerative colitis (Parent *et al.*, 1978, Parent *et al.*, 1976) and has been found to colonize 30% of patients with cystic fibrosis (Steinkamp *et al.*, 2005). Other common infections/diseases associated with *S. maltophilia* include biliary sepsis, endocarditis, urinary tract, soft tissue, eye, chronic obstructive pulmonary disease, meningitis and patients with obstructive lung cancer (reviewed in Brooke, 2012). From 1993 to 2004, *S. maltophilia* was among the 11 most frequently isolated gram negative bacteria in insensitive care unit (ICU) patients (Lockhart *et al.*, 2007).There are incidents of community acquired *S. maltophilia* infection (Falagas *et al.*, 2009), but a recent study from 2001 to 2007 revealed that most cases were hospital acquired with some being health-care associated (Garazi *et al.*, 2012). In this study, intensive care unit stay and intubation were associated with mortality. This finding is likely correlated with the propensity of *S. maltophilia* to adhere to plastics and form biofilms (Brooke, 2012).

S. maltophilia **virulence factors**

Despite the biomedical importance of *S. maltophilia*, only a handful of virulence factors have been identified. *S. maltophilia* have nematotoxic activity against the nematodes *Panagrellus redivivus* and *Bursaphelenchus xylophilus* (Huang *et al.*, 2009). This nematotoxic activity is mediated by a serine protease which also degrades several human proteins isolated from blood serum and connective tissue (Hagemann *et al.*, 2006). On the other hand, the killing of *C. elegans* requires the action of a diffusible signaling factor system (Fouhy *et al.*, 2007). This

rpf/DSF (diffusible signaling factor)-quorum sensing system regulates the expression of extracellular enzymes (endoglucanase and protease) (Fouhy *et al.*, 2007) that might play a role in *C. elegans* pathogenicity. Additionally, the bi-functional mutase SpgM is required for virulence in a rat lung infection model (McKay *et al.*, 2003), the Xps type II secretion system is required to induce death of human lung epithelial cells (Karaba *et al.*, 2013) and *S. maltophilia* are putatively lethal to protozoa as these bacteria encode refractile inclusion bodies (R bodies) (Ryan *et al.*, 2009) that are toxic to freshwater *Paramecium* (Heruth *et al.*, 1994).

In terms of additional putative virulence factors, *S. maltophilia* strains produce a variety of hydrolytic enzymes including DNases, RNases, lipases and chitinases (reviewed in Ryan *et al.*, 2009). *S. maltophilia* can also efficiently capture siderophores (iron sequesteration) produced by other microorganisms (Jurkevitch *et al.*, 1992) and produce pili that are implicated in bacterial cell ahseion to epithelial and abiotic surfaces (De Oliveira-Garcia *et al.*, 2003). Other presumed virulence factors include peptidoglycan synthetase, haemagglutinin, LPS O antigen and genes encoding type I, II (Sec), IV, V and arginine transporter (TAT) secretion systems (reviewed in Ryan et al., 2009).

Towards the development of a *S. maltophilia* model system

Despite its medical importance, there are few model systems to investigate the mode of *S. maltophilia* action (Fouhy *et al.*, 2007, Looney *et al.*, 2009, Ryan *et al.*, 2009, Steinert, 2011) and we are just beginning to understand the requirements of *S. maltophilia* virulence. As determined via homology, the *S. maltophilia* genome contains a number of putative virulence factors. However, as summarized above, only a few of these putative virulence factors have a demonstrated role in pathogenicity. Furthermore, little is known about the degree in which these virulence factors and/or bacterial mode of action is conserved between strains. Here, we develop *C. elegans* as a model for the study of *S. maltophilia* strain specific responses. *S. maltophilia* has

been found in association with the nematode *Pristionchus pacificus* (Rae *et al.*, 2008) and is detected in the natural environment of *C. elegans* (B. Samuel, personal communication), suggesting that the investigation of this nematode-bacterial interaction is ecologically and evolutionarily significant. Furthermore, given that an association with *S. maltophilia* is detrimental to both humans and nematodes, the elucidation of the genetic basis of this interaction has biomedical implications. The use of *C. elegans* as a model system also has a number of advantages including the availability of genetic and whole genome tools, and its emergence as a model system for innate immunity. This emergence is attributed to the many similarities between human and nematode microbial pathology (Table 1.1) and the discovery of conserved genes and genetic pathways that play an imperative role in *C. elegans* immune response. Chapter 2 and Chapter 3 of this dissertation are primarily focused on the nematode innate immune response and Chapter 4 discussed our findings on the identification of *S. maltophilia* virulence factors. Together, these studies shed some light on the host-pathogen evolutionary arms race by investigating a novel interaction between *C. elegans* and *S. maltophilia* strain JCMS.

Chapter 2 - A *S. maltophilia* strain evades a major *C. elegans* defense pathway

Introduction

Stenotrophomonas maltophilia, a gram negative bacillus previously classified as both *Pseudomonas* and *Xanthomonas* (Denton *et al.*, 1998), is an emerging opportunistic human pathogen. From 1993 to 2004, *S. maltophilia* was found to be among the 11 most frequently recovered organisms from ICU patients in U.S. hospitals (Lockhart *et al.*, 2007). A more recent study of patients with *S. maltophilia* bacteremia revealed that most cases were hospital acquired with some being health-care associated (Garazi *et al.*, 2012). In this study, intensive care unit stay and intubation were associated with mortality. This is likely correlated with the propensity of *S. maltophilia* to adhere to plastics and form biofilms (De Oliveira-Garcia *et al.*, 2003) and the infection of patients that are already ill. In fact, *S. maltophilia* can cause nosocomial pneumonia (A'Court *et al.*, 1992), enhances the pathogenesis of Crohn's disease and ulcerative colitis (Parent *et al.*, 1978, Parent *et al.*, 1976) and colonizes 30% of patients with cystic fibrosis (Steinkamp *et al.*, 2005). Recent studies found that *S. maltophilia* infections result in mortality in up to 69% of patients with bacteremia and a number of infections such as meningitis and endocarditis are *S. maltophilia* associated (reviewed in Brooke, 2012). Thus, *S. maltophilia* is a medically important pathogen that has significant effects on human health. Furthermore, the characterization of various *S. maltophilia* strains is imperative as the genome sequences of environmental (including R551-3) and clinical (including K279a) *S. maltophilia* isolates contain heterogeneity that might help mediate adaptations to different environments (Ryan *et al.*, 2009).

Despite its medical importance, there are few model systems to investigate the mode of *S. maltophilia* action (Fouhy *et al.*, 2007, Looney *et al.*, 2009, Ryan *et al.*, 2009, Steinert, 2011). Furthermore, little is known about what is needed for *S. maltophilia* virulence and if these

features are bacteria and/or strain specific. Here, we develop *C. elegans* as a model for the study of *S. maltophilia* strain specific responses. Previous work has demonstrated that *S. maltophilia* K279a can kill *C. elegans* (Fouhy *et al.*, 2007) and another strain, *S. maltophilia* G2, was shown to have nematocidal activity (Huang *et al.*, 2009). *S. maltophilia* has also been detected in association with the nematode *Pristionchus pacificus* (Rae *et al.*, 2008) as well as in the natural environment of *C. elegans* (B. Samuel, personal communication). Accordingly, it is likely that *C. elegans* encounters *S. maltophilia* strains in natural settings and suggests their interaction is evolutionarily significant.

Caenorhabditis elegans has proven to be an excellent model for understanding development, neurobiology, behavior, and more recently, innate immunity (Irazoqui *et al.*, 2010a). Like other metazoans, *C. elegans* has evolved in the presence of microbes. The interaction between *C. elegans* and its associated microbes is multifaceted, as these nematodes feed on potentially pathogenic bacteria. Consequently, *C. elegans* has evolved both conserved and unique innate immune pathways to deal with the microbial world. Whereas, the conserved Toll-like receptor (TLR) pathway plays a central role in innate immunity in other animals, the single *C. elegans* TLR gene *tol-1*, is not involved in the response to many pathogens, including *D. coniospora*, *P. aeruginosa* and *M. nematophilum* (Pujol et al., 2001) nor, in the control of antimicrobial gene expression (Couillault et al., 2004). Uniquely, *tol-1* plays a role in the protection of pharyngeal tissue upon challenge with *Samonella enterica* (Tenor et al., 2008), suggesting a specific response that differs from usual TLR signaling. Conversely, the functions of other innate immune pathways are conserved and the study of nematode immune response can be informative in understanding how higher organisms mount pathogen defenses (Irazoqui *et al.*, 2010c, Tan *et al.*, 2011). For example, the highly conserved p38 mitogen-activated kinase

(MAPK) pathway plays a major role in response to human bacterial pathogens (Marsh *et al.*, 2012a) such as *P. aeruginosa* (Kim et al., 2002) and *Staphylococcus aureus* (Sifri et al., 2003). The unfolded protein response (UPR) *ire-1-xbp-1* arm is a downstream target of the p38 MAPK pathway in response to pore-forming toxin (PFT) (Bischof et al., 2008), a virulence factor for a number of bacteria pathogens including *B. thuringiensis* (Bravo et al., 2007). Other conserved pathways such as the DBL-1/TGFβ pathway, play a role in nematode response to a number of pathogens including *D. coniospora* (Zugasti et al., 2009) and *S. marcescens* (Mallo et al., 2002). In the conserved DAF-2/16 pathway, the insulin-like receptor DAF-2 negatively regulates the transcription factor DAF-16/FOXO and, activation of DAF-16 induces expression of downstream effector genes. As a result, *daf-2* mutants are long-lived on most bacteria tested to date including *E. coli*, *E. faecalis* and other human pathogens (Garsin et al., 2003, Pujol et al., 2001). In addition to a role in innate immunity, the DAF-2/16 pathway also has overlapping functions in regulating longevity, aging and diapause (Dillin *et al.*, 2002, Gems *et al.*, 1998, Huang *et al.*, 2011). The regulation of longevity and innate immunity is distinct, involving pathway components that play a role in both processes or have an exclusive role in longevity (Evans *et al.*, 2008a). Each of the conserved innate immune pathways specifically regulate downstream effectors such as lysozymes, lectins, neuropeptide-like peptides (NLPs) and antimicrobial factors (reviewed in Marsh et al., 2012a). For example, *dbl-1* regulates caenacins but not the structurally related NLPs (Zugasti et al., 2009). Innate immunity effector genes such as members of the lysozyme and C-type lectin family are also pathogen specific (Alper et al., 2007) and innate immune pathway components can exhibit effector regulation independent of other pathway constituents (Evans *et al.*, 2008b). Lastly, the p38 MAPK and DAF-2/16 pathway appear to function in parallel but, lack substantial overlap in positively regulated downstream

genes (Troemel et al., 2006). Thus, in addition to sharing conserved pathways with human innate immunity, *C. elegans* seemingly shares a conserved genetic architecture employing multiple pathways and corresponding effectors that function in parallel to combat varied microbial assault. Therefore, *C. elegans* is a valuable model to study conserved innate immune pathway function and specificity.

In this study, we report our discovery of an *S. maltophilia* strain, JCMS, that kills *C. elegans*. Our results indicate that *S. maltophilia* JCMS accumulates in the *C. elegans* intestine, does not appear to require an extracellular toxin to confer virulence and needs to be alive for maximum virulence. Our data also showed that the UPR, p38 MAPK and DBL-1/TGF-beta pathways are involved in a general bacterial innate immune response. Furthermore, *S. maltophilia* JCMS was virulent to normally pathogen-resistant *C. elegans* mutants such as *daf-2*, *akt-1* and *ins-7*, suggesting that JCMS evades the downstream effects of these DAF-2/16 pathway components. These findings correlate with our observation that *daf-2* regulated effector genes were not differentially expressed when nematodes were fed *S. maltophilia* JCMS, as compared to *E. coli* OP50. These findings demonstrate the value of evaluating several conserved genetic pathways as a whole and provide evidence for bacterial strain specificity in the *C. elegans* innate immune response.

Material and Methods

Nematode strains

C. elegans strains containing the following alleles were obtained from the *Caenorhabditis* Genetics Center: LG I: *daf-16(mu86)*, *tol-1(nr2033)*, LG II: *age-1(hx546)*, *ire-1(v33)*, *nsy-1(ag3)*, *sma-6(wk7)*, LG III: *atf-7(qd137)*, *daf-2(e1368)*, *sma-4(e729)*, *sma-3(e491)*, *tir-1(qd4)*, *xbp-1(zc12)* LG IV: *daf-18(ok480)*, ZK1251.1& *ins-7(ok1573)*, *pmk-1(km25)*, *sma-2 (e502)*, LG V: *akt-1(ok525)*, *dbl-1(nk3)*, LG X: *akt-2(ok393)*, *pdk-1(sa680)*, *sek-1(km4)*, *sgk-1(ok538)*.

Strain ZD350 [agIs219 *atf-7(qd137)* III] was provided by D. Pagano (MIT). N2 was used as the wild-type strain and was thawed yearly from frozen stock for experimentation.

Bacterial strains and growth

S. maltophilia JCMS was isolated by our laboratory from a culture of *Mesorhabditis sp.* nematodes found in soils from Konza Prairie, near Manhattan, KS. Briefly, nematodes were isolated from soil cores, washed in sterile M9 buffer and allowed to crawl on nematode growth medium (NGM) plates without any bacteria for 1 hour. Nematodes were then moved to a plate seeded with *E. coli* OP50 for rearing. Bacteria that grew on the initial NGM plate were considered to be "nematode associated bacteria". Despite our efforts to ensure that JCMS was indeed associated with native soil nematodes, it is possible that this strain could have been present in the soil from which the nematodes were isolated or a laboratory contaminant. *E. coli* OP50 and OP50-GFP were obtained from the *Caenorhabditis* Genetics Center, *S. maltophilia* K279a from R. Ryan (University College Cork), *S. maltophilia* R551-3 from D. van der Lelie (Brookhaven National Laboratory), *E. faecalis* V583 from L. Hancock (Kansas State University) and *P. aeruginosa* PA14 from F. M. Ausubel (Harvard Medical School). Transformation of *S. maltophilia* strains was completed via the insertion of a mini-Tn7 expression cassette that expresses GFP (Ciche *et al.*, 2007), miniTn7KSGFP (pURR25), obtained from T. Ciche (Michigan State University) into the genomes of each *S. maltophilia* strain. All bacterial strains were frozen at - 80˚C upon retrieval and were thawed regularly for use in experimentation. *S. maltophilia* strains are naturally Ampicillin resistant and were streaked for colony isolation from frozen stock on Luria Broth (LB) agar containing 100µg/mL Ampicillin to selectively prevent growth of other bacterial contaminants. *E. coli* OP50 was streaked on LB agar for colony isolation. For each bacterial strain, liquid LB was inoculated and shaken overnight at 32˚C.

Bacterial lawns used for survival were seeded on nematode growth medium (NGM) with bacterial culture at log/lag phase and grown overnight at room temperature.

Nematode survival assays

Nematodes were reared and synchronized as L4s at 20˚C on *E. coli* OP50 lawns. For survival analysis, 10 to 15 L4s are picked onto three to six replicate lawns of bacteria and maintained at 25˚C. The number of surviving nematodes was recorded daily and death was determined by lack of motion in response to prodding with a platinum wire pick. Nematodes were picked to new bacterial lawns for the first five to six days after the start of the experiment to separate them from their progeny. Dead nematodes were removed upon discovery. Sample sizes (N = number of nematodes) vary due to contamination and the removal of specimens that died via means other than the specified bacterial treatment, such as desiccation that occurs when nematodes leave the bacterial lawn and die at the plate edge. The infrequent presence of contamination was determined by observing bacterial lawn morphology and contaminated replicates were discarded. Kaplan-Meier estimates of survival over time and survival curve statistics using Cox proportional hazards models were performed in R (Vienne, Austria: R Foundation for Statistical Computing). Survival curves can be statistically compared using the log-rank and Cox proportional hazard tests. Cox proportional hazards models were used to test the effect of independent variables such as genotype and bacteria on the hazard, a dependent variable defined as the probability of dying at a given time (Goel *et al.*, 2010). The model used for analysis is indicated in the legends of the relevant tables and the effect of the designated independent variable was considered significant if the *p* value was less than 0.05. Some models included a categorical variable that specified the date in which the experiment was completed. This categorical variable and the interaction between this variable and genotype or bacteria were included in the model if found to be significant. Models were evaluated by testing for a non-zero

slope and visualizing the Schoenfeld residuals (UCLA: Statistical Consulting Group). A non-zero slope is an indication of proportional hazard assumption violation and models were fit to the data aiming to meet that assumption.

Effect of bacterial viability and presence on nematode killing

Overnight cultures of *E. coli* OP50 and *S. maltophilia* strains were heat-killed for one hour at 92˚C using a Thermolyne DryBath or killed with a one and a half hour 120μg/mL doxycycline treatment at 32˚C. The treated cultures were concentrated 20-fold as described previously (Gruber *et al.*, 2007) and used to seed NGM plates containing 100 μg/mL Ampicillin to prevent growth of *E. coli* OP50 transferred from nematode rearing plates. Prior to use, bacterial lawns were examined for colonies to determine whether any bacteria survived the killing treatment. For these experiments, OP50-GFP was used instead of OP50 since it is resistant to Ampicillin. To test whether *S. maltophilia* presence and/or secretions might impact nematode viability we performed a filter assay as previously described (Twumasi-Boateng *et al.*, 2012) using bacterial cultures grown on a 0.2 μM mixed cellulose esters filter (Millipore) placed on NGM plates at room temperature overnight. Prior to survival analysis the filter containing the treatment strain was removed and the plate was seeded with *E. coli* OP50.

Bacterial accumulation, distension and pharyngeal pumping

Synchronized L4s were fed GFP bacteria for 11 days and maintained at 25˚C. Prior to observation, nematodes were fed non-GFP bacteria for one hour to clear the intestinal lumen of non-adhering bacteria that we reasoned would be swept away during this feeding period. Nematodes were anesthetized (10mM sodium azide) for observation daily at 1000X magnification using a Zeiss Axioplan II equipped with epifluorescence and differential interference contrast (DIC) optics. GFP accumulation pattern (punctate or diffuse) was scored and the degree of intestinal distension was quantified using a micrometer. Only living nematodes

were scored. The sample size depended upon the length of time nematodes survived on the different bacteria. GFP accumulation pattern was observed and distension was recorded for the anterior section of the intestine (most proximal to the pharynx) of 30 worms for each bacterial treatment. To measure pharyngeal pumping rates, synchronized L4s were picked onto each treatment bacteria. Each treatment included three replicates of 10 to 15 nematodes. During the survival analysis, six nematodes were randomly selected and observed from each treatment daily. Pumping of the posterior bulb of the pharynx was counted for 30 seconds and extrapolated to 60-second intervals. Mean pumping rate is an average of pharynx bulb pumps per minute observed on each day of the experiment.

CFU counts

Bacterial load was determined using methods modified from previous studies (Garsin *et al.*, 2001, Portal-Celhay *et al.*, 2012b). Synchronized L4s were fed non-GFP (except for *E. coli* due to the presence of Ampicillin resistance) strains from 0.5 to 144 hours on NGM plates at 25˚C. Triplicates of 10 nematodes were picked after 0.5, 1, 6, 12, 24, 48, 96 and 144 hours of exposure to *E.coli* OP50 and *S. maltophilia* JCMS, R551-3 and K279a and fed non-GFP *E. coli* OP50 for one hour of clearing. Nematodes were then placed on un-seeded NGM doxycycline (120µg/mL) plates for washing: once with 25mM levamisole/M9 (LM) buffer, twice with LM buffer with doxycycline (120µg/mL) and twice with M9 buffer. Washed nematodes were then placed in a 1.7mL microcentrifuge tube containing 50µL of M9 buffer + 1% Triton^{TM} X-100, Sigma-Aldrich and homogenized using a pestle motor. Crushed nematodes were diluted and plated on LB agar containing 100µg/mL Ampicillin to select for growth of adherent strains.

Germline removal

To assess the *E.coli* OP50 specific dependence of *age-1(hx546)* mutant survival extension on the absence of the germline, we used RNAi to knockdown *cdc-25.1* as previously

reported (Shapira *et al.*, 2006). Briefly, adult nematodes were picked onto RNAi plates (1mM isopropyl β-D-1-thiogalactopyranoside (IPTG) and 50μg/mL Ampicillin) seeded with *E. coli* HT115 (DE3) either expressing double-strand (ds) RNA or the empty vector (L4440) and allowed to lay eggs. Prior to seeding, each bacterial strain was shaken overnight at 32˚C in LB Ampicillin (50μg/mL) and dsRNA expression was induced via shaking in 2ml of fresh LB AMP in the presence of IPTG (1mM) for three hours. Treated adult nematodes were removed and the eggs were allowed to develop into normal adult nematodes, without a proliferating germline (Glp) that were picked onto NGM plates seeded with *E. coli* OP50 or *S. maltophilia* JCMS for survival analysis.

Reverse transcription quantitative polymerase chain reaction (RT qPCR)

Synchronized *wildtype* and *daf-2(e1368)* L4s were grown on *E. coli* OP50 or *S. maltophilia* JCMS at 25˚C for 24 hours, collected in M9 buffer and lysed in TRIzol® reagent (Life Technologies). RNA extraction and DNAse treatment were completed using the PureLink RNA Mini Kit (Invitrogen) and on-column PureLink® DNase Treatment (Invitrogen). RNA quality was checked by visualizing 28S and 18S rRNA bands using gel electrophoresis and checking 260/280 and 260/230 absorbance ratios using a NanoDrop™ 8000 Spectrophotometer. Intact RNA was used for cDNA synthesis using a SuperScript® VILO cDNA Synthesis Kit (Invitrogen). RT qPCR was completed using 96 well plates and the CFX96 Touch™ Real-Time PCR Detection System (BIO RAD). Each amplification reaction was performed in triplicate and three biological replicates were done for each bacterial nematode combination. Primer sequences for *clec-85* (5'- CCTGTGCTACTCAATTTCCGC - 3' and 5'-CTGGAAGAAGCTCGGCTCAA - 3') and *spp-1* (5'- GCCAATCCAGCTAACCCACT- 3' and 5'-AACGGCAACAGCATAGTC CA - 3') were designed using NCBI Primer3 and checked for specificity using NCBI BLAST. Primer sequences for *csq-1* (5'- AACTGAGGTTCTGACCGAGAAG - 3' and 5'-TACTGG

TCAAGCTCTGAGTCGTC - 3') were designed in Geneious and also checked for specificity using NCBI BLAST. Published primer sequences for *dod-22*, K08D8.5, *lys-7* and *lys-1* were used (Alper *et al.*, 2007). The efficiency of each primer pair was determined using a standard curve on a pooled sample of cDNA. The efficiencies of the target and reference gene *csq-1* were determined to be approximately equal (Applied Biosystems) and were assumed to be 100% during normalization and ΔC_T quantification. The reference gene *csq-1* was chosen due to its low variance between bacterial treatments used. Differential expression was determined by comparing the $2^{-\Delta CT}$ values for biological replicates of the target gene on JCMS or OP50 in a *daf-2* mutant background versus *wildtype* and in wild-type nematodes on JCMS versus OP50 (Schmittgen *et al.*, 2008). Statistical significance (p <0.05) was determined with a Student's t test assuming equal variance.

Accessions

The full-length *S. maltophilia* JCMS 16S rRNA gene sequence was deposited in GenBank with accession number KF724885.

Results

S. maltophilia JCMS kills *C. elegans* and is more virulent than *S. maltophilia* R551-3 and K279a

S. maltophilia strain JCMS was isolated in our laboratory (see Material and Methods). We amplified the complete 16S rRNA gene and sequence comparisons indicated that JCMS is more similar to the clinical strain K279a than to the environmental isolate R551-3 (Figure A.1). S. *maltophilia* strains R551-3, K279a and JCMS display different levels of virulence to *C. elegans* with JCMS being the most severe (Figure 2.1 and Table A.1), as seen by comparing the hazard ratios, as determined by the corresponding Cox proportional hazards model (see Material and Methods) between bacterial treatments. Briefly, a hazard is the probability that an individual

nematode dies at a given time. Therefore, in this case, the hazard ratio compared the relative hazards of two bacteria. Nematodes fed JCMS were 9.8 and 4.1 times more likely to die than those fed K279a and R551-3, respectively (Table A.1). Survival of nematodes on JCMS was not significantly different from *E. faecalis* V583 and was significantly (as determined by the corresponding Cox proportional hazards model, see Material and Methods) higher than on *P. aeruginosa* PA14 (Figure 2.1, Table A.1), both well-studied *C. elegans* and human pathogens. *S. maltophilia* K279a has been reported to kill *C. elegans* within 24 hours (Fouhy *et al.*, 2007). However, K279a was avirulent in our hands, as nematode survival on K279a was not significantly different from the *C. elegans* laboratory food *E. coli* OP50 (Figure 2.1, Table A.1). Similarly, and in contrast to a previous report (Fouhy *et al.*, 2007), we observed that *P. aeruginosa* was significantly more virulent than K279a (Figure 2.1, Table A.2). Although, the source of this experimental discrepancy was unclear, this difference prompted us to test the effect of bacterial lawn growth medium. As previously demonstrated (Tan *et al.*, 1999a), *P. aeruginosa* PA14 was significantly more virulent to nematodes when grown on the "fast" killing peptone-glucose-sorbitol (PGS) medium and *E. coli* OP50 was more virulent when grown on brain heart infusion (BHI) medium (Garsin *et al.*, 2001) (Table A.2). K279a was more virulent when grown on the enriched media BHI or PGS (Table A.2) but was not as virulent as previously reported (Fouhy *et al.*, 2007). PA14 was still more virulent than K279a when grown on PGS, but the difference in survival was reduced. Intriguingly, nematodes were more likely to die when fed JCMS grown on NGM than JCMS grown on PGS or BHI (Table A.2). This media dependent difference in JCMS tolerance was interesting; however, the mechanism of virulence was not explored as the richer media did not cause an increase in virulence as observed for the other *S.*

maltophilia strains (Table A.2). In summary, neither K279a nor OP50 are considered virulent and JCMS is the most virulent *S. maltophilia* strain in our hands.

S. maltophilia JCMS accumulates in the intestine

Given the diverse levels of virulence displayed by the different *S. maltophilia* strains, we sought to determine whether whole-nematode bacterial load was related to pathogenicity. In order to quantify bacterial load, we performed a titer assay on nematodes exposed to *E. coli* OP50-GFP (used instead of OP50 because of its Ampicillin resistance, see Material and Methods), *S. maltophilia* JCMS, K279a or R551-3. Nematodes exposed to JCMS carried a 10-fold greater bacterial load than any other strain tested at 30 minutes (Figure 2.2A). Bacterial load exponentially increased on JCMS after 12 hours and roughly correlated with the degree of pathogenic effect for all the bacterial strains tested. *C. elegans* feeds by the pharynx pumping bacteria from the mouth into the intestine. It is possible that the observed difference in bacterial load was related to feeding behavior, as rapid uptake of bacterial cells could cause an increased load. To address this question, we measured the pharyngeal pumping rates of nematodes fed each bacterial strain daily until death. Since pumping rates are known to decline with age (Huang *et al.*, 2004), we only included data from the first four days. Mean pumping rates were similar for all bacterial treatments on days one and two and were slightly diverged on days three and four, with nematodes fed K279a having the highest mean pumping rate on day four (Figure 2.2B). Nematodes fed JCMS did not have a substantially higher pumping rate on any of the days observed. Thus, nematode bacterial uptake does not correlate with pathogenicity or contribute to the observed strain specific differences in bacterial load.

The observed *S. maltophilia* strain specific and pathogenicity related differences in bacterial load prompted us to determine where bacteria were localized within the nematode and if localization was also coordinated with pathogenicity. To visualize and track bacteria within *C.*

elegans, we introduced a GFP plasmid into *S. maltophilia* JCMS, K279a and R551-3. Integration of GFP did not cause a significant difference in nematode survival (Table A.3). Bacterial pathogenicity has previously been correlated with bacterial accumulation within the *C. elegans* intestine and distension of the intestinal lumen (Garsin *et al.*, 2001, Sifri *et al.*, 2005). Thus, the extent of intestinal lumen distension (Figure 2.2C) was measured while observing the pattern of GFP accumulation (Figure 2.3). We focused on the anterior portion of the intestine since it has been shown to be sensitive to the effects of pathogenic bacteria (Irazoqui *et al.*, 2010b, Spanier *et al.*, 2010). A large degree of intestinal distention was observed for all *S. maltophilia* strains with distention occurring earlier and to a greater extent in nematodes fed more pathogenic strains (Figure 2.2C). Nematodes fed *E. coli* OP50-GFP accumulated bacteria within the anterior intestinal lumen primarily in a "punctate" pattern that appeared to involve intact bacterial cells (Figure 2.3B,C and A.2A). Worms fed JCMS-GFP accumulated bacteria primarily in a "diffuse" pattern that appeared to involve higher numbers of tightly packed bacteria, some of which might have been lysed, releasing GFP into the intestinal lumen (Figure 2.3D-F and A.2B). Efforts to characterize the exact cellular mechanism responsible for the "diffuse" GFP accumulation pattern have been inconclusive. However, the "diffuse" pattern of GFP bacterial accumulation is characteristic to all *S. maltophilia* strains and roughly correlates with degree of virulence (Figure A.2B). Few GFP-labeled *S. maltophilia* cells were observed in tissues outside the intestinal lumen and not until nematodes were older and deteriorating, suggesting that *S. maltophilia* pathogenesis is primarily an intestinal disease which becomes systemic after prolonged exposure. The extent of this effect, and the degree of intestinal distension, appeared to be generally consistent for all sections of the nematode (data not shown), signifying that the accumulation and distention patterns are representative.

Intriguingly, the bacterial load data (titer assay) showed a quick increase in bacterial load (within 30 min), but the accumulation of GFP- labeled bacteria and intestinal lumen distension occurred more slowly. When accumulation in all parts of the intestine was measured, 7 to 17 percent of nematodes accumulated GFP expressing bacteria on day one, depending upon the strain (Figure A.2C). These data (Figure A.2C) correlate better with bacterial load (Figure 2.2A) data, but differences remain. One possible explanation for these differences is that nematodes in the accumulation experiments were cleared from non-adherent bacteria on the same non-GFP bearing strain while, in the titer assay, nematodes were cleared on *E. coli* OP50. To investigate this experimental difference, we compared JCMS-GFP accumulation after clearing on non-GFP bearing OP50 or JCMS. JCMS-GFP initially accumulated to a greater extent when cleared on *E. coli* OP50 (Figure A.2D). Thus, JCMS is better at dislodging itself than OP50. This bacterial specific difference in ability to clear non-adherent bacteria may have contributed to the discrepancy between the GFP accumulation and bacterial load experiments. However, taken together these data indicate that the bacterial load and GFP accumulation experiments are distinct. Moreover, it has been previously shown that the presence of a GFP signal and/or intestinal distension does not necessarily indicate an established infection (Hsiao *et al.*, 2013). Thus, although, GFP accumulation, distension and bacterial load should intuitively correlate, these phenomena can be uncoupled and represent different aspects of *S. maltophilia* pathogenicity.

S. maltophilia **JCMS virulence is not mediated by a toxin and requires live bacteria**

The ability of *S. maltophilia* JCMS to colonize and accumulate within *C. elegans* (Figure 2.2A) suggested that living bacteria were involved in *S. maltophilia* virulence. To test whether this was the case, we used heat to kill *E. coli* OP50 and *S. maltophilia* JCMS, R551-3 and K279a. Nematodes fed heat-killed JCMS survived longer than those fed living bacteria while,

survival on heat-killed R551-3 and K279a was not statistically different from non-killed bacteria (Figure 2.4A, Table A.4). Surprisingly, heat-killed OP50 was more hazardous to nematodes than living bacteria. This effect is not likely due to a lack of nutrition as these bacteria were concentrated in the same manner as the *S. maltophilia* strains. While the curves shown in Figure 2.4A appear to indicate that heat-killed R551-3 and K279a were also more hazardous to nematodes, these differences were not statistically significant. These data suggest that a heat stable compound might contribute to the virulence of the mildly pathogenic *S. maltophilia* R551-3. Additionally, JCMS mediated killing appeared to be distinct from other *S. maltophilia* isolates in that it does not involve a toxin but required un-treated living bacterial cells. To confirm these data and determine if a heat-labile toxin could be involved in *S. maltophilia* JCMS virulence, we used the antibiotic doxycycline to treat *E. coli* and each *S. maltophilia* strain. Doxycycline inhibits protein synthesis and the treatment was optimized to remove all proliferating cells. As expected (Garigan *et al.*, 2002), nematodes fed antibiotic-treated OP50 survived significantly longer than those fed living bacteria (Figure 2.4B, Table A.4). In fact, nematode survival was extended for nematodes on all the antibiotic-treated bacteria tested, except R551-3. These data provide additional evidence that a toxin might be involved in the virulence of R551-3. Furthermore, it appears that proliferating bacteria and/or a factor associated with bacterial growth is required for wild-type nematode survival on non-treated JCMS, K279a and OP50. Since antibiotic treatment should not denature peptides or other putative virulence factors, we conclude that a toxin does not play a significant role in *S. maltophilia* JCMS pathogenicity. We also attempted to use UV treatment as another means to kill bacteria without denaturing proteins. However, we were unable to find a UV dose that completely killed all cells (data not shown).

We next used a filter assay to test for the contribution of *S. maltophilia* secretions on pathogenicity and did not observe a decrease in survival of nematodes fed secretion-treated OP50 for any *S. maltophilia* strain (Figure 2.4C, Table A.5). In fact, we observed a significant extension in survival for K279a secretions. Nevertheless, it is possible that the filter bound the toxic secretion and/or the molecule is too large to pass through the filter. While, this issue could be addressed through the use of non-filtered, bacteria free supernatant obtained via centrifugation, we were unable to remove all viable *S. maltophilia* cells from the supernatant (data not shown). Therefore, we cannot rule out the role of a large and/or filter bound toxin. However, taken together, these data strongly suggest that living bacteria, rather than an enterotoxin, play a larger role in nematode pathogenesis by *S. maltophilia* JCMS.

DAF-2/16 insulin-like signaling pathway component mutants are not resistant to *S. maltophilia* JCMS

C. elegans daf-2 mutants display increased survival and increased pathogen resistance to practically all bacteria tested to date (Evans *et al.*, 2008a, Garsin *et al.*, 2003, Irazoqui *et al.*, 2010a). To determine whether this was the case for *S. maltophilia* JCMS, we analyzed the survival of various *daf-2* pathway mutants on JCMS and *E. coli* OP50 as a control. As expected, both *daf-2(e1368)* and *daf-2(e1370)* mutants were resistant to OP50 and neither was resistant to *S. maltophilia* JCMS (Figure 2.5A and Table 2.1). Specifically, *daf-2(e1370)* mutants were more susceptible to *S. maltophilia* JCMS, while *daf-2(e1368)* mutants were similar to *wildtype*. While, we observed increased susceptibility for *daf-2(e1370)* mutants, they displayed pleiotropic effects such as reduced brood size and abnormal development (Gems *et al.*, 1998), which might have contributed to the observed JCMS specific increased susceptibility. Additionally, previous work has shown that the bacterial load of *daf-2* mutants versus *wildtype* is reduced in *C. elegans* fed bacterial pathogens (Portal-Celhay *et al.*, 2012b). However, the bacterial load of *daf-2* mutants

on JCMS was not different from *wildtype* (Figure A.3). Thus, on JCMS, *daf-2* is not involved in combating bacterial infection. Mutants of other DAF-2/16 pathway components including the insulin-like ligand *ins-7* and an ortholog of the serine/threonine kinase Akt/PKB *akt-1* had increased survival on OP50 but, were also not resistant to JCMS, with *ins-7* mutants being more susceptible (Figure 2.5B and Table 2.1). Intriguingly, mutants of another serine/threonine kinase Akt/PKB ortholog *akt-2* were resistant to JCMS, suggesting a role that is similar to that on OP50. The differential roles of *akt-1* and *akt-2* on JCMS may be linked to their differential regulation of antimicrobial gene expression (Evans *et al.*, 2008a). Contrary to previous reports (Evans *et al.*, 2008a, Garsin *et al.*, 2003), we observed that *age-1* mutants were not resistant to OP50. This anomaly led us to ask whether previously reported effects of sterility on lifespan and pathogen resistance (Hsin *et al.*, 1999, Miyata *et al.*, 2008) might be responsible. We used *cdc-25.1(RNAi)* to remove the germline (Shapira *et al.*, 2006) in *age-1* mutants and found that *age-1; cdc-25.1(RNAi)* nematodes had significantly longer survival than *cdc-25.1(RNAi)* nematodes on OP50 (Figure 2.5C and Table 2.1). There was not a significant difference between *wildtype* and *age-1* mutants fed the RNAi vector only, confirming that the knockdown of *cdc-25.1* was the cause of lifespan extension. Furthermore, there was not a significant difference in survival between *age-1; cdc-25.1(RNAi)* and *cdc-25.1(RNAi)* nematodes on JCMS. When the germline-dependent effects of *age-1* mutants are taken into account, like most other DAF-2/16 pathway components, *age-1* mutants were not resistant to JCMS. Lastly, forkhead box O (FOXO) homolog *daf-16* mutants were not significantly different from *wildtype* on JCMS or OP50, which is consistent with previous results (Garsin *et al.*, 2003, Kerry *et al.*, 2006, Troemel *et al.*, 2006). In summary, all tested components of the DAF-2/16 pathway except *daf-16* were similarly involved in response to OP50. We observed that most of the pathway mutants were not

significantly different from *wildtype* on *S. maltophilia* JCMS suggesting that DAF-2/16 signaling plays little or no role in the *C. elegans* defense response on these bacteria. That the more severe *daf-2(e1370)* mutant and the *ins-7* ligand mutant are slightly susceptible to JCMS clouds the picture somewhat. However, as these mutants are usually resistant to most *C. elegans* pathogens, our data indicate their role in response to JCMS is different than that for other bacterial pathogens.

The degree to which mutations of DAF-2/16 pathway components affect survival was inferred from the value of the mutant to wild-type hazard ratio (from the corresponding Cox proportional hazard model, see Material and Methods). Thus, the hazard ratio is an indication of the level of involvement the mutated gene plays on a given bacteria. Values near one suggest a gene is not involved in response to a given bacteria while, values that deviate from one suggest involvement. Values greater than one indicates that the mutation shortens survival and values less than one indicate that the mutation increases survival. We did not consider mutant hazard ratios that were not significantly different from *wildtype* in these analyses of differential degrees of mutant effects. The hazard ratios for DAF-2/16 pathway mutants on OP50 were all less than and deviated the most from one (Table 2.1). These results suggest that this pathway plays a larger role than the other tested defense pathways on OP50. We only observed significant extended survival on JCMS for *akt-2* mutants, which are about half as likely to die as wild-type nematodes. In contrast, *akt-2* mutants are nearly one fifth as likely to die as wild-type nematodes on OP50, indicating a smaller role on JCMS. In fact, the hazard ratios of all DAF-2/16 pathway mutants deviated further from one on OP50 than on JCMS, suggesting that this pathway plays a greater role on OP50. Furthermore, the hazard ratios of DAF-2/16 pathway mutants on JCMS

deviated the least from one as compared to the other defense pathway mutants, suggesting that this pathway as a whole is more expendable.

A conserved role for other *C. elegans* defense pathways in combating *S. maltophilia* JCMS

Mutations affecting the UPR, p38 MAPK and DBL-1/TGFβ pathway components *ire*-1, *pmk-1* and *dbl-1* had increased susceptibility on JCMS (Figure 2.6 and Table 2.1). These results suggested involvement of these *C. elegans* defense pathways and were bolstered by our analysis of mutants affecting multiple genes within each pathway (Figs. 6, A.3 and A.4, Table 2.1). Mutants of the p38 MAPK pathway signaling components *sek-1*, *nsy-1*, *tir-1* and *atf-7* were each susceptible to OP50 and JCMS, but *pmk-1* mutants were only susceptible to JCMS (Figure 2.6A, A.4 and Table 2.1). These results agreed with previous data in that *pmk-1* mutants were susceptible to pathogenic *P. aeruginosa* and that the absence of *pmk-1* in nematodes fed *E. coli* is irrelevant (Troemel *et al.*, 2006). The hazard ratios of p38 MAPK mutants were higher than for other pathway mutants on both JCMS and OP50 (Table 2.1). In addition, the hazard ratio for a given mutant was higher on JCMS than on OP50 for these genes. For example, the hazard ratios for *sek-1* and *nsy-1* on JCMS were 228 and 31.3, while on OP50 they were 7.18 and 13.9 (Table 2.1). Thus, loss of the p38 MAPK pathway is the most detrimental on both bacteria with loss on JCMS being more severe.

Mutations in the DBL-1/TGFβ pathway components that form the SMAD complex (*sma-2*, *sma-3* and *sma-4*) also increased susceptibility on both OP50 and JCMS (Figure A.5 and Table 2.1). Thus, the SMAD complex components have a similar role on JCMS and OP50. However, mutants of upstream components *dbl-1* and *sma-6* were specifically susceptible to JCMS and long-lived on OP50 (Figure 2.6B, Table 2.1). The hazard ratios of these mutants were closer to one on OP50 than on JCMS. Thus, it appears that these components are necessary for

the response to JCMS and, perhaps, less important on OP50. In fact, with the exception of *sma-2*, the hazard ratios for DBL-1/TGFβ pathway mutants deviated more from one on JCMS. Comparatively, the deviations from one for this pathway are not as great as for the p38 MAPK pathway. Thus, loss of the DBL-1/TGFβ pathway as a whole is only mildly detrimental on JCMS.

UPR pathway components were also found to play similar roles in response to JCMS and OP50. Mutants of *ire-1* and *xbp-1* had significantly increased susceptibility on both OP50 and JCMS (Figure 2.6C, Table 2.1). Similar to the p38 MAPK and DBL-1/TGFβ pathways, the hazard ratios for UPR pathway mutants were higher on JCMS. In addition, the hazard ratio of *ire-1* mutants was greater than that of *xbp-1* mutants on JCMS and OP50, suggesting a greater role for this serine/threonine protein kinase on both bacteria. Lastly, Tol-like receptor (TLR) *tol-1* mutant survival was not significantly different from *wildtype* on OP50, but was slightly increased on JCMS (Table 2.1). The resistance of *tol-1* mutants to JCMS was only marginally significant with the hazard ratio indicating a little involvement.

Differential regulation of immune effector genes on *S. maltophilia* JCMS

We used RT qPCR to investigate the expression of several innate immune effector genes on *S. maltophilia* JCMS and *E. coli* OP50. We chose to focus on *clec-85*, *lys-1*, *lys-7*, *dod-22*, K08D8.5 and *spp-1* due to their differential regulation by p38 MAPK, DBL-1/TGFβ and DAF-2/16 signaling (Alper *et al.*, 2007, Murphy *et al.*, 2003). These genes were also of interest because they are expressed in the intestine and pharynx, sites of pathogen contact (Alper *et al.*, 2007). Our analysis of DAF-2/16 pathway mutant survival suggested these genes would not play a role in the *C. elegans* innate immune response to *S. maltophilia* JCMS. Accordingly, we sought to determine the dependence of each effector gene on DAF-2/16 signaling by comparing expression in *wildtype* and *daf-2* mutants. Of the putative DAF-2/16 regulated genes chosen,

clec-85, *lys-7* and *dod-22* were significantly differentially expressed on JCMS (Figure 2.7A). Consistent with previous work (Alper *et al.*, 2007, Murphy *et al.*, 2003), *clec-85* and *lys-7* were up-regulated and *dod-22* was down-regulated in *daf-2* mutants on OP50. However, the expression of *spp-1* and K08D8.5 in the *daf-2* background was not significantly different from *wildtype* on either bacteria and these genes were not evaluated further since their expression trends did not agree with previous studies (Alper *et al.*, 2007, Murphy *et al.*, 2003). Additionally, as expected for a *daf-2* independent gene, the expression of *lys-1* in the *daf-2* background was not significantly different from *wildtype* on either bacteria. The expression of *lys-1*, *lys-7*, *dod-22* and *clec-85* are known to be up-regulated when *C. elegans* comes into contact with pathogenic bacteria (Alper *et al.*, 2007, Mallo *et al.*, 2002). Thus, the expression of these immune effector genes is typically expected to be up-regulated on pathogenic bacteria such as JCMS versus a less or non-pathogenic condition like OP50. However, since *clec-85*, *dod-22*, and *lys-7* are regulated by DAF-2/16 signaling and DAF-2/16 signaling was not primarily involved in JCMS response; we hypothesized that these effectors would not be differentially expressed. As expected, only *lys-1* was significantly up-regulated on JCMS versus OP50 (Figure 2.7B). These results further indicate that the DAF-2/16 pathway plays little or no role in *C. elegans* innate immune response to JCMS, while other pathways that regulate effectors such as *lys-1* play a larger role.

Discussion

S. maltophilia JCMS is a *C. elegans* bacterial pathogen

We have isolated a strain of the emerging nosocomial pathogen *S. maltophilia* that kills *C. elegans* via the accumulation of live bacteria in the intestine. *S. maltophilia* JCMS was the most virulent *S. maltophilia* strain tested (Figure 2.1). In addition, virulence was not enhanced on "fast killing" media as seen previously for *P. aeruginosa* (Tan *et al.*, 1999a) and here for *S. maltophilia* K279a (Table A.2). Like *E. faecalis*, *P. aeruginosa* and *S. marcescens* (Garsin *et*

al., 2001, Marsh *et al.*, 2012a), *S. maltophilia* JCMS accumulates in the gut (Figure 2.2A, 2.3 and A.2) and this is accompanied by intestinal distention (Figure 2.2C). We also observed an association between bacterial load or GFP-labeled bacterial accumulation and survival for all *S. maltophilia* and *E. coli* strains examined (Figure 2.2A and A.2). Unlike *P. aeruginosa* (Tan *et al.*, 1999a), the mode of action of *S. maltophilia* JCMS and K279a involves the presence of living bacteria (Figure 2.4A,B). Furthermore, in contrast to *P. aeruginosa* (Mahajan-Miklos *et al.*, 1999) and *B. thuringiensis* (Bravo *et al.*, 2007), *S. maltophilia* JCMS virulence likely does not involve a toxin (Figure 2.4). Thus, establishment of intestinal infection is a common mode of action employed by a number of bacterial pathogens while, the presence of living bacteria and a putatively virulent secretion is less common.

Overall, these data suggest that the mode of *S. maltophilia* action is generally conserved across strains. However, there are several differences that suggest strain specific modes of action. Virulence of *S. maltophilia* JCMS involves the presence of living bacteria. To our surprise, this requirement does not hold true for *S. maltophilia* R551-3 as, heat-killing does not make a difference in survival while, antibiotic treatment strangely decreases survival (Figure 2.4A,B). Together, these data suggest that R551-3 produces a toxin that kills nematodes in the absence of proliferating bacterial cells. However, to our surprise, R551-3 secretions did not have an effect on *C. elegans* survival (Figure 2.4C). One possibility is that R551-3 produces a toxin upon antibiotic treatment that is absent in un-treated secretions from living bacteria. Another possibility is that the toxin is large and/or became bound to the nitrocellulose filter used in the filter assay. Comparatively, heat-killed *P. aeruginosa* are as virulent as living bacteria on fast killing media (Tan *et al.*, 1999a) and employ heat stable diffusible toxins called phenazines (Mahajan-Miklos *et al.*, 1999). However, unlike R551-3, antibiotic treatment of *P. aeruginosa*

attenuates nematode killing (Tan *et al.*, 1999a). Thus, upon heat-killing, the mode of action may be similar for *P. aeruginosa* and *S. maltophilia* R551-3. However, if R551-3 produces a toxin, it is heat resistant and induced upon antibiotic treatment, further distinguishing this strain from other *S. maltophilia* strains and *P. aeruginosa.*

Differences in the onset of bacteria accumulation also suggest differences in the mode of action for *S. maltophilia* strains. Specifically, JCMS initially caused the highest bacterial load and diffuse GFP accumulation pattern (Figure 2.2A and A.2). We could not discern individual cells within the area of diffuse GFP accumulation, suggesting that the GFP exists extracellularly and was derived from lysed cells. However, the GFP variant expressed in the bacteria is not known to be active extracellularly. One possibility is that the diffuse GFP is trapped within a biofilm that protects it from the extracellular environment. *S. maltophilia* has been shown to form biofilms on a variety of biotic surfaces (Brooke, 2012) and *S. aureus* forms biofilms within the *C. elegans* intestine (Begun *et al.*, 2007). Thus, it is possible that the observed *S. maltophilia* GFP diffuse pattern corresponds to an intestinal biofilm. Biofilm formation is thought to be a survival mechanism of clinically relevant organisms such as *S. aureus* and *E. faecalis* (reviewed in Donlan *et al.*, 2002). *E. faecalis* is a well-known human commensal and opportunistic pathogen that has been shown to establish a proliferating intestinal infection in nematodes (Garsin *et al.*, 2001). Survival on *S. maltophilia* JCMS was distinct from *S. maltophilia* R551-3, *S. maltophilia* K279a and *P. aeruginosa* PA14, but not significantly different from survival on *E. faecalis* V583 (Figure 2.1 and Table A.1). Also, like *S. maltophilia* JCMS, *E. faecalis* does not kill nematodes when treated with antibiotics (Garsin *et al.*, 2001). This suggests that the mode of *S. maltophilia* JCMS and *E. faecalis* action could be similar, possibly involving opportunistic

proliferation and biofilm formation. This mode of action might be shared by other *S. maltophilia* strains that cause significant decreases in survival (Huang *et al.*, 2009, Thomas *et al.*, 2013).

The DAF-2/16 pathway plays a relatively small role in defense against *S. maltophilia* JCMS

Binding of the insulin/IGF receptor ortholog DAF-2 negatively regulates the transcription factor DAF-16/FOXO. When the functions of DAF-2 or other members of the DAF-2/16 pathway are disrupted, DAF-16 is free to enter the nucleus to promote the expression of numerous genes. Survival of *daf-16* mutants was not significantly different from *wildtype* on *S. maltophilia* JCMS or OP50 (Table 2.1) as previously seen on *E. faecalis* and *P. aeruginosa* (Garsin *et al.*, 2003, Troemel *et al.*, 2006). Also in agreement with previous results, we observed that the survival of most DAF-2/16 pathway mutants was extended on *E. coli* OP50 (Figure 2.5, Table 2.1) (Garsin *et al.*, 2003, Hamilton *et al.*, 2005). However, *ins-7*, *daf-2*, *akt-1* and *age-1* mutants are not long lived on JCMS; suggesting that these bacteria evade the downstream effects induced by removal of these gene activities. These downstream effects include the activation of *daf-16* regulated general stress effectors such as *mtl-1* (Murphy *et al.*, 2003) that provide protection from oxidative and other bacterial by-products. Thus, these data suggest that the activation of such stress effectors is insufficient in the protection against JCMS and that JCMS causes cellular stress that is distinct from other bacteria that require the action of the DAF-2/16 pathway. Previous work has shown that *P. aeruginosa* eludes the *C. elegans* defense response via activation of the DAF-2/16 pathway (Evans *et al.*, 2008b). *S. maltophilia* might evade the DAF-2/16 pathway in several ways. At one extreme, the response to JCMS may be completely independent of insulin-like signaling rendering the entire pathway indispensable and loss of pathway components negligible. On the other hand, a JCMS-specific response might involve

signaling through select pathway components in which only a few components would be necessary.

As reported in previous studies (Evans *et al.*, 2008b), we also observed that *ins-7* mutants are resistant to *E. coli* OP50 (Figure 2.5B and Table 2.1). However, *ins-7* mutants are slightly susceptible to JCMS; suggesting that this gene functions to activate defense functions on these bacteria. Yet, the role for *ins-7* appears to be relatively minor, as the hazard ratio was the smallest among the other significantly susceptible innate immune pathway mutants on JCMS. On the other hand, *akt-2* mutants are resistant to OP50 and JCMS, supporting a role for this gene in both bacterial environments. On JCMS, this result may be due to the dual role of *akt-2* in pathogen resistance and longevity (Evans *et al.*, 2008a). As *age-1* also has a dual role (Evans *et al.*, 2008a), it is possible that this resistance is dependent on the germline, as we demonstrated for *age-1* mutants. In fact, *akt-2* and *age-1* mutants with an intact germline had similar survival phenotypes and hazard ratios (Table 2.1). Still, the resistance of *akt-1* mutants on JCMS is curious but suggests that *akt-1* and *akt-2* might have different functions in response to these bacteria. For example, *akt-1* and *akt-2* have been shown to differentially regulate innate immune effectors such as *thn-2* on *E. coli* and *spp-1* on *E. coli* and *P. aeruginosa* (Evans *et al.*, 2008a). Thus, the differential survival phenotypes of *akt-1* and *akt-2* on JCMS could be attributed to them playing different roles, as seen in the regulation of effector genes. However, like *ins-7* mutants, the mutant to *wildtype* hazard ratio is fairly small as compared to other mutant hazard ratios (Table 2.1). Therefore, if *C. elegans akt-2* has a role on *S. maltophilia* JCMS, this role is nearly negligible.

Expression of down-stream innate immune effectors also supports the notion that JCMS specifically eludes the DAF-2/16 pathway. Previous work has shown that the immune effector

genes *dod-22* and *lys-7* are induced by *P. aeruginosa* and *S. marcescens* and that *clec-85* is induced by *P. aeruginosa*, *S. marcescens* and *S. aureus* (Alper *et al.*, 2007). Here we show that while *dod-22*, *lys-7* and *clec-85* were regulated by *daf-2* on JCMS they were not significantly differentially expressed between JCMS and the avirulent OP50 control (Figure 2.7). This is consistent with our observation that most DAF-2/16 pathway component mutants are not resistant to *S. maltophilia* JCMS. In summary, these data indicate that *S. maltophilia* JCMS evades the effects of the DAF-2/16 pathway, supporting a negligible role of this pathway on these bacteria.

Conserved defense pathways are involved in the *C. elegans* response to *S. maltophilia* JCMS. We performed a survey of the known *C. elegans* bacterial defense pathways and found that several conserved pathways were involved in the response to *S. maltophilia* JCMS and *E. coli* OP50 while the Toll-like receptor *tol-1* was not. As seen previously for several nematode pathogens (Pujol *et al.*, 2001), *tol-1* was not required for response to OP50 (Table 2.1). Furthermore, the marginally significant resistance and correspondingly small hazard ratio for *tol-1* mutants on JCMS suggests a diminished role in *C. elegans* defense response to these bacteria. On the other hand, the role of the UPR, p38 MAPK and TGFβ-like pathway is largely conserved as these pathways are involved in the response to other pathogenic bacteria (Kim *et al.*, 2002) (Bischof *et al.*, 2008, Kim *et al.*, 2002, Mallo *et al.*, 2002, Sifri *et al.*, 2003, Zugasti *et al.*, 2009) and *E. coli* (Table 2.1). All three pathways function in the nematode intestine, while, the p38 MAPK and TGFβ-like pathway also act in the hypodermis (reviewed in Irazoqui *et al.*, 2010a). Furthermore, the p38 MAPK TIR-1-NSY-1-SEK-1 module is required for the neuroendocrine regulation of pathogen avoidance (Shivers *et al.*, 2009) and the UPR pathway acts in the pharynx (Haskins *et al.*, 2008).Thus, pathogen response involves action by multiple pathways acting in

multiple tissues, likely making the absence of a more widely expressed pathway even more detrimental. Given that observation, it is not surprising that the mutant versus *wildtype* hazard ratios for the more universally acting p38 MAPK pathway were higher on both JCMS and OP50 (Table 2.1). On average, the hazard ratios for UPR pathway mutants were higher than TGFβ pathway mutants. Thus, of the conserved pathways assessed, loss of the p38 MAPK pathway is the most detrimental, followed by the UPR and TGFβ pathway. The largest hazard ratios were observed for genes encoding signaling proteins, such as *nsy-1*. A plausible explanation for these large hazard ratios might be that signaling through these proteins is imperative for multiple pathways and/or a number of biological processes.

The expression of the putative lysozyme *lys-1* on JCMS and OP50 further highlights the role of p38 MAPK and TGFβ signaling in *C. elegans* defense response. *lys-1* is regulated by p38 MAPK and TGFβ signaling (Alper *et al.*, 2007) and knock-down causes increased susceptibility to *S. aureus* (Jensen *et al.*, 2010). The expression of *lys-1* is up-regulated on the bacterial pathogens *S. marcesens* (Mallo *et al.*, 2002) and *P. aeruginosa* (Alper *et al.*, 2007), and we also observed significant up-regulation on JCMS as compared to OP50 (Figure 2.7B). This suggests that *lys-1* is required for the response to JCMS similar to its upstream regulators. Furthermore, the up-regulation of *lys-1* on JCMS versus OP50 was consistent with our observation that the hazard ratio values exhibited by p38 MAPK and TGFβ pathway mutants exhibited great deviations from one, indicating that these pathways play a large defense role on JCMS.

Closer inspection of the hazard ratios between *S. maltophilia* JCMS and *E. coli* OP50, suggest that p38 MAPK and TGFβ pathway components might play different roles in response to each bacteria. PMK-1 is the terminal kinase in the p38 MAPK pathway and is important for the switch of the leucine zipper transcription factor ATF-7 from repressor to activator in response to

P. aeruginosa (Shivers *et al.*, 2010). Both *atf-7* and *pmk-1* mutants are hyper-susceptible to *S. maltophilia* JCMS (Figure 2.6A and A.3, Table 2.1), suggesting that PMK-1 mediates this switch on these bacteria. However, *pmk-1* mutants are not susceptible, while *atf-7* mutants are susceptible, to *E. coli*, suggesting that the immune response to *E. coli* is *atf-7* dependent and *pmk-1* independent. In terms of the TGFβ pathway, *dbl-1* and *sma-6* mutants were slightly resistant to OP50, but the hazard ratios were relatively low compared to other resistant mutants on OP50 (Figure 2.6B, Table 2.1). This indicates that *sma-6* and *dbl-1* activities on OP50 are somehow disadvantageous in wild-type nematodes. On the other hand, all TGFβ-like pathway component mutants were susceptible to JCMS. These data agree with a previously established role of TGFβ signaling for response to *S. marcescens* infection (Mallo *et al.*, 2002). Thus, in wild-type nematodes, all p38 MAPK and TGFβ pathway components likely play a defense role on JCMS while, only some components are required on OP50, suggesting bacteria-specific action of individual pathway components. Intriguingly, we did not observe bacteria-specific action for the tested UPR pathway components as both mutants are susceptible and loss of *ire-1* was more detrimental than *xbp-1* on both JCMS and OP50.

Figures

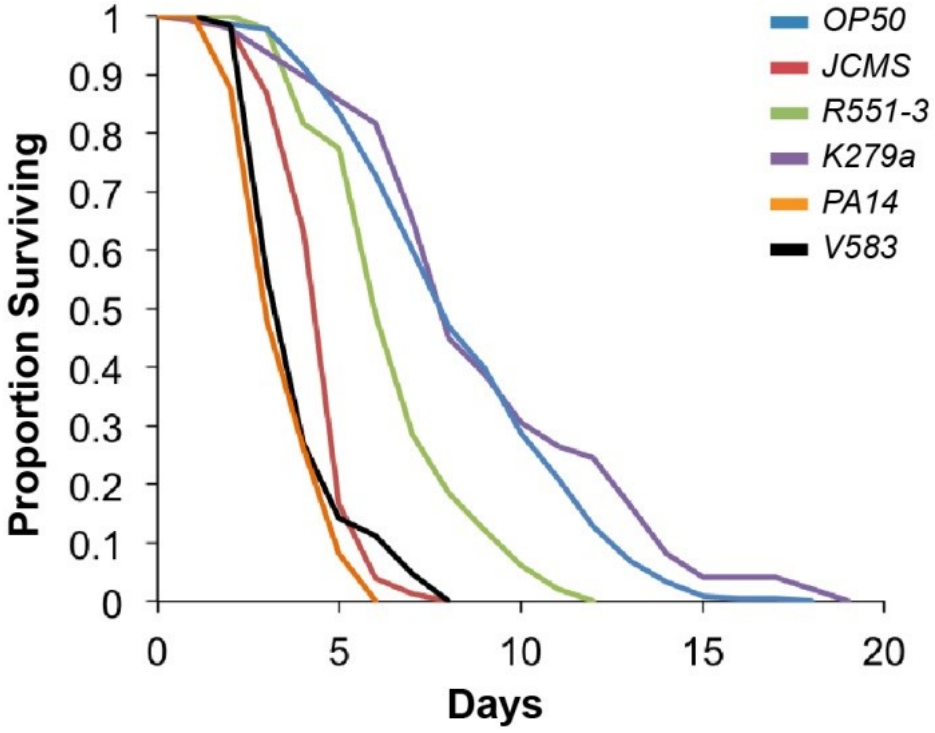


Figure 2.1 JCMS is the most virulent *S. maltophilia* strain.

Wildtype survival on *S. maltophilia* JCMS (red), *S. maltophilia* R551-3 (light green), *S. maltophilia* K279a (purple), *E. coli* OP50 (blue), *E. faecalis* V583 (black) or *P. aeruginosa* PA14 (orange). Results plotted are the proportion of surviving worms using Kaplan-Meier estimates for at least three replicate samples (10 to 15 nematodes per replicate) of the same nematode population. p values from the application of Cox proportional hazards models and sample sizes of each population are included in Table A.1. Survival on PA14, K279a and R551-3 was significantly different ($p < 0.05$) from survival on JCMS. JCMS was more virulent than OP50 and K279a and, PA14 was more virulent than JCMS. Survival on JCMS versus V583 was not significantly different ($p= 0.0663$).

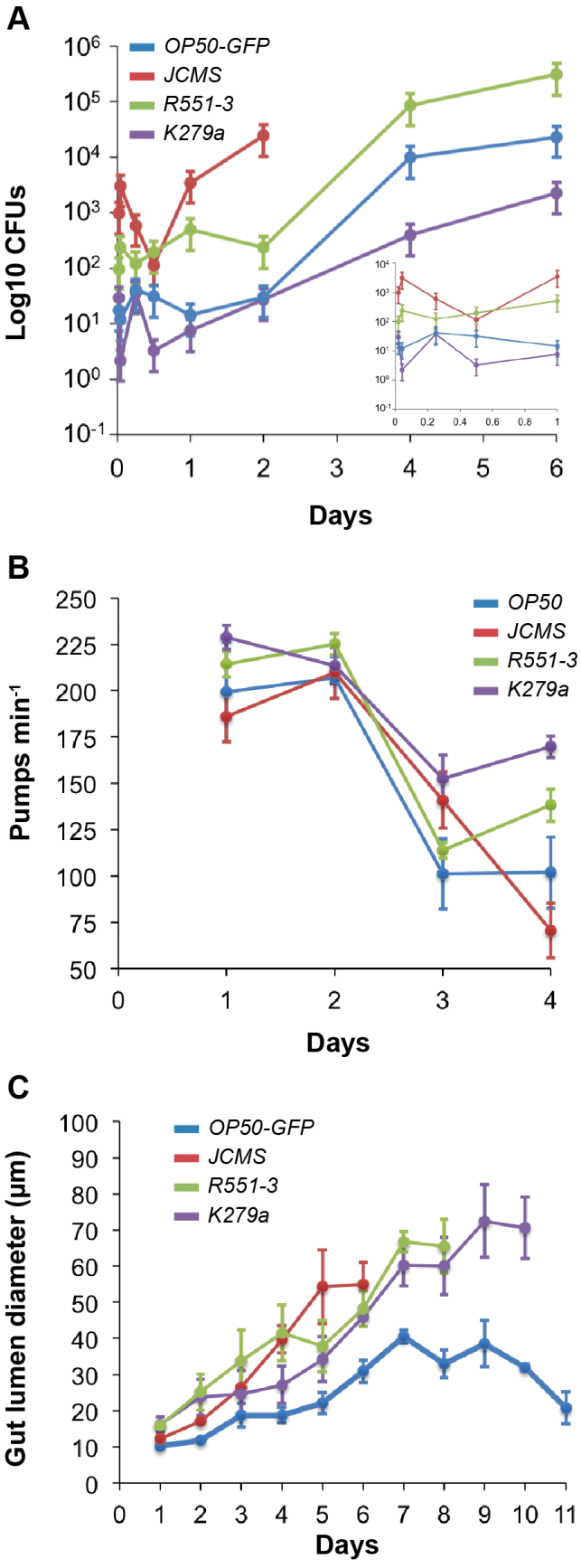

A
OP50-GFP
JCMS
R551-3
K279a
Log10 CFUs
Days
B
OP50
JCMS
R551-3
K279a
Pumps min-1
Days
C
OP50-GFP
JCMS
R551-3
K279a
Gut lumen diameter (µm)
Days

Figure 2.2 *S. maltophilia* JCMS persists in the nematode and causes intestinal distension.

Mean bacterial load (A), pharynx pumping rate (B) and intestinal lumen distention (C) for synchronized wild-type nematodes fed *E. coli* OP50 or OP50-GFP (blue), *S. maltophilia* JCMS (red), R551-3 (light green) or K279a (purple). A) Mean log CFUs (colony forming units) per worm for three replicates of 10 nematodes cleared of non-adherent bacteria for one hour on OP50 after feeding on the indicated bacterial strain. Inset shows an expanded view of the time points from the first day of feeding. B) Mean pumping rate (pharynx pumps per minute) for six nematodes picked at random per indicated bacterial treatment on days one - four. C) Adult worms were anesthetized for observation daily after exposure to each indicated bacterial strain. The width of the gut was scored in the anterior region of each worm for six to 11 days depending on nematode survival. n = 51 on JCMS, 63 on R551-3, 69 on K279a and 76 on OP50. All error bars indicate standard error of the mean.

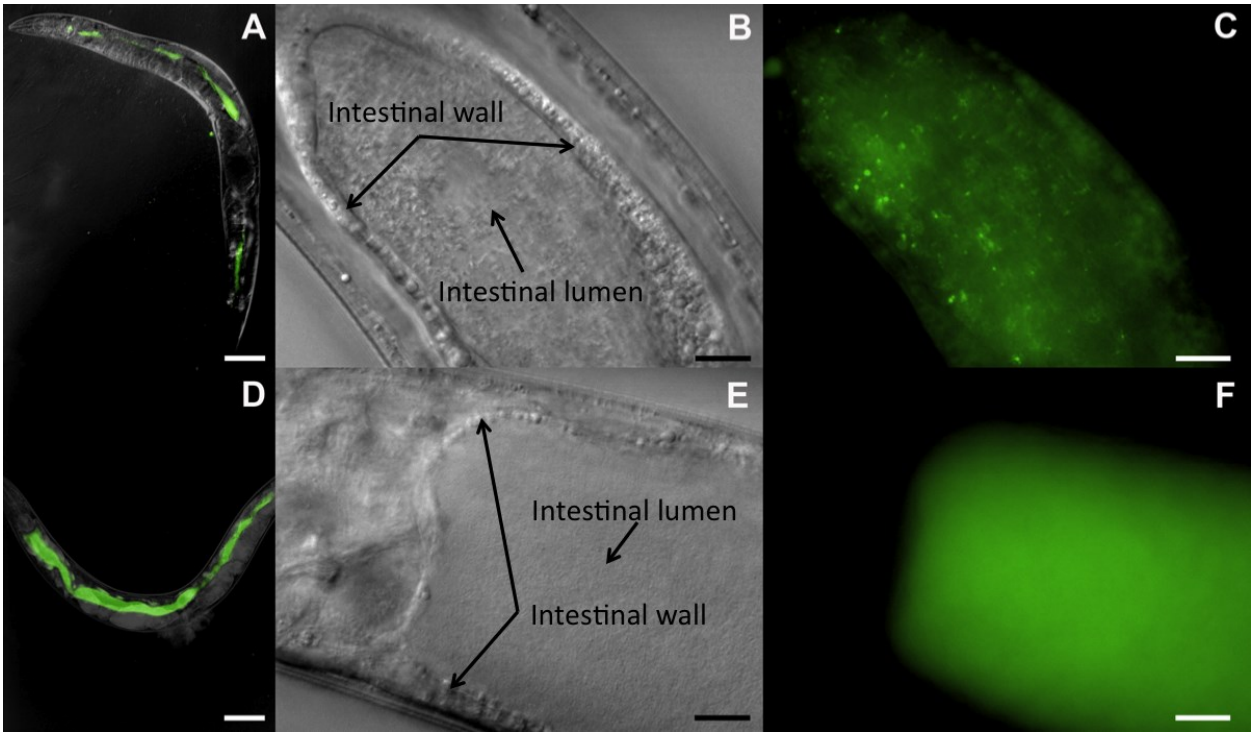


Figure 2.3 Accumulation of GFP expressing bacteria in the nematode intestine.

Micrographs of wild-type nematodes grown on either *E. coli* OP50-GFP (A-C) or *S. maltophilia* JCMS-GFP (D-F). Anterior is to the left in all panels. A and D) Overlay of DIC and fluorescent images on day six at 10× magnification, bars 100 µm. B and E) DIC images of the anterior intestine on day four at 1000× magnification of OP50-GFP (B) and JCMS-GFP (E) fed nematodes. Bars are 10 µm. Arrows on the DIC images indicate the intestinal wall. Both anterior intestines were distended, but JCMS-GFP fed animals appear to contain more bacteria. C and F) Fluorescence images of the same nematodes shown in B and E, respectively. Bars are 10 µm. The OP50-GFP fed nematode shown in C) displays the punctate pattern of GFP bacterial accumulation, while the JCMS-GFP fed nematode in F) shows the diffuse pattern.

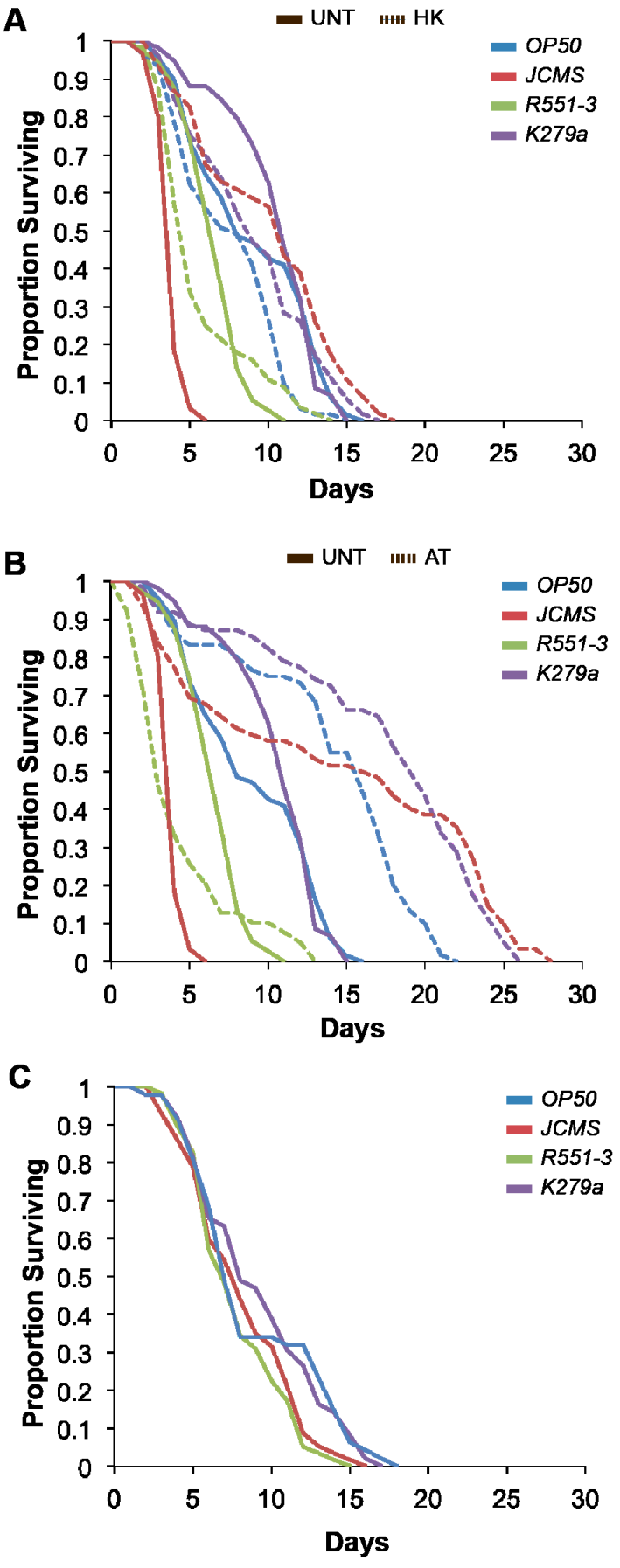

A
UNT
HK
OP50
JCMS
R551-3
K279a
Proportion Surviving
Days
B
UNT
AT
OP50
JCMS
R551-3
K279a
Proportion Surviving
Days
C
OP50
JCMS
R551-3
K279a
Proportion Surviving
Days

Figure 2.4 *S. maltophilia* JCMS virulence is not mediated by a toxin and requires living bacteria.

Survival of wild-type nematodes grown on untreated (UNT, solid lines), A) heat-killed (HK, dashed lines) or B) antibiotic-treated (AT, dashed lines) bacteria. C) Survival of wild-type nematodes grown on secretion (filtrate) treated *E. coli* OP50; bacterial secretions are indicated by color. *E. coli* OP50 (blue), *S. maltophilia* JCMS (red), *S. maltophilia* R551-3 (green) or *S. maltophilia* K279a (purple). Results plotted are the proportion of surviving worms using Kaplan-Meier estimates for at least three replicate samples (10 to 15 nematodes per replicate) of the same nematode population. p values from the application of Cox proportional hazards models and sample sizes of each population are included in Table A.4 and Table A.5. Living JCMS was significantly ($p < 0.05$) more virulent than heat-killed and antibiotic-treated JCMS while, OP50 treated with JCMS secretions (filtrate) was not significantly different from OP50 treated with OP50 secretions ($p= 0.401$).

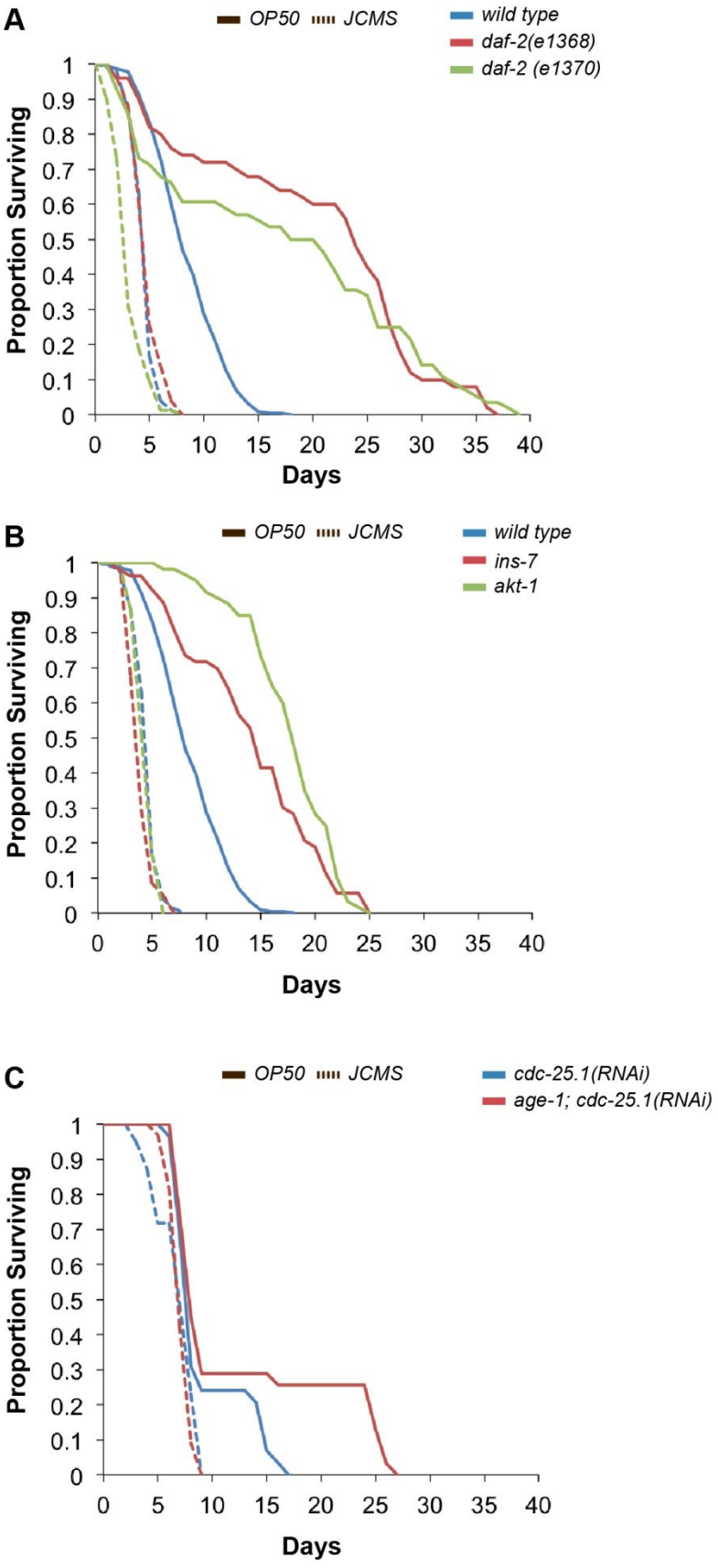
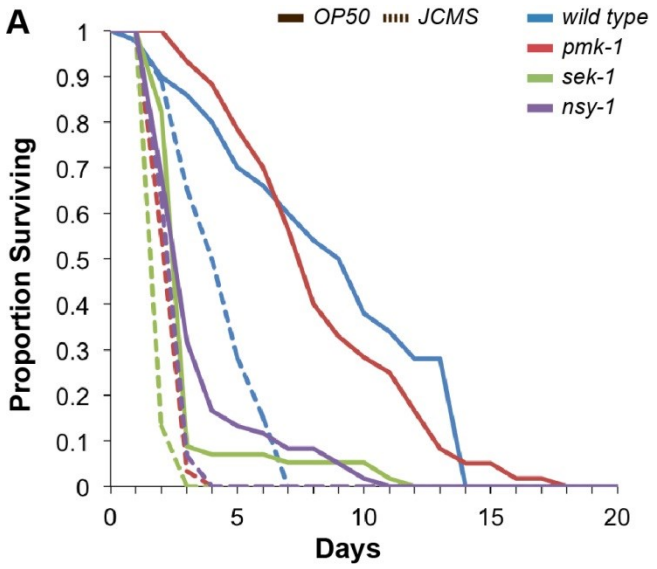
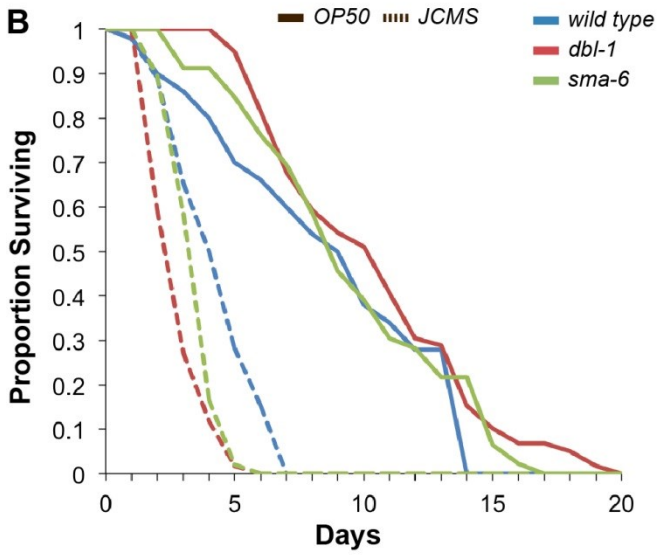

A
OP50
JCMS
wild type
daf-2(e1368)
daf-2 (e1370)
Proportion Surviving
Days
B
OP50
JCMS
wild type
ins-7
akt-1
Proportion Surviving
Days
C
OP50
JCMS
cdc-25.1(RNAi)
age-1; cdc-25.1(RNAi)
Proportion Surviving
Days

Figure 2.5 Survival of representative DAF-2/16 insulin-like signaling (IIS) pathway mutants.

A) Survival of wild-type nematodes (blue), *daf-2(e1368)* (red) and *daf-2(e1370)* (light green) mutants on *E. coli* OP50 (solid lines) and *S. maltophilia* JCMS (dashed lines). B) Survival of wild-type nematodes (blue), *ins-7(ok1573)* (red) and *akt-1(ok525)* (light green) mutants on *E. coli* OP50 (solid lines) and *S. maltophilia* JCMS (dashed lines). C) Survival of adult nematodes without a proliferating germline (*cdc-25.1(RNAi)*, blue) and *age-1; cdc-25.1(RNAi)* (red) mutants on *E. coli* OP50 (solid lines) and *S. maltophilia* JCMS (dashed lines). Results plotted are the proportion of surviving worms using Kaplan-Meier estimates for at least three replicate samples (10 to 15 nematodes per replicate) of the same nematode population. p values from the application of Cox proportional hazards models and sample sizes of each population are included in Table 2.1. On JCMS, survival of *daf-2(e1370)* and *ins-7* mutants was significantly different from *wildtype*, while the survival of *daf-2(e1368)* and *akt-1* mutants was not significantly different. The survival of *daf-2*, *ins-7* and *akt-1* mutants on OP50 was significantly extended. Survival of *age-1; cdc-25.1(RNAi)* mutants was significantly longer than *cdc-25.1(RNAi)* nematodes on OP50 but not on JCMS. p values less than 0.05 were considered significant.


A
OP50
JCMS
wild type
pmk-1
sek-1
nsy-1
Proportion Surviving
Days
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
0
5
10
15
20


B
OP50
JCMS
wild type
dbl-1
sma-6
Proportion Surviving
Days
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
0
5
10
15
20

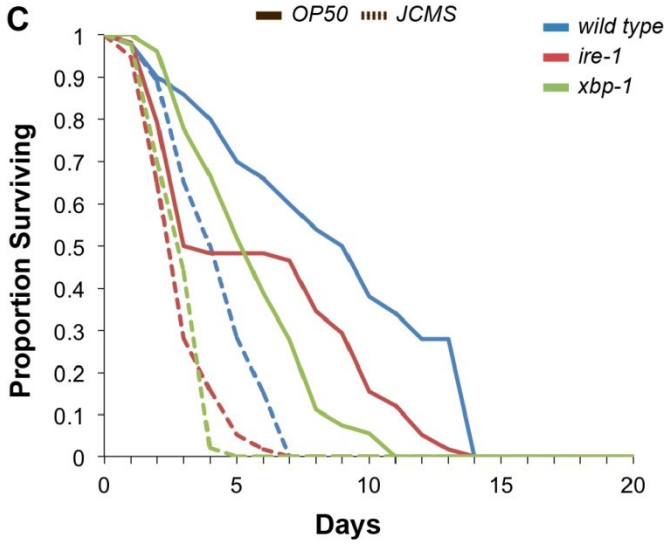

C
OP50
JCMS
wild type
ire-1
xbp-1
Proportion Surviving
Days
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
0
5
10
15
20

Figure 2.6 p38 MAPK, DBL-1/TGFβ and UPR defense pathway mutants.

A) Survivorship of wild-type nematodes (blue), *pmk-1(km25)* (red), *sek-1(km4)* (green) and *nsy-1(ag3)* (purple) mutants on *E. coli* OP50 (solid lines) and *S. maltophilia* JCMS (dashed lines). B) Survivorship of wild-type nematodes (blue), *dbl-1(nk3)* (red) and *sma-6(wk7)* (green) mutants on *E. coli* OP50 (solid lines) and *S. maltophilia* JCMS (dashed lines). C) Survivorship of wild-type nematodes (blue), *xbp-1(zc12)* (green), and *ire-1(v33)* (red) mutants on *E. coli* OP50 (solid lines) and *S. maltophilia* JCMS (dashed lines). Results plotted are the proportion of surviving worms using Kaplan-Meier estimates for at least three replicate samples (10 to 15 nematodes per replicate) of the same nematode population. p values from the application of Cox proportional hazards models and sample sizes of each population are included in Table 2.1. All pathway mutants had significantly decreased survival on JCMS. Survival for *pmk-1* mutants was not significantly different from *wildtype* and *dbl-1* and *sma-6* had significantly extended survival on OP50. All other immune pathway mutants were significantly susceptible to OP50. p values less than 0.05 were considered significant.

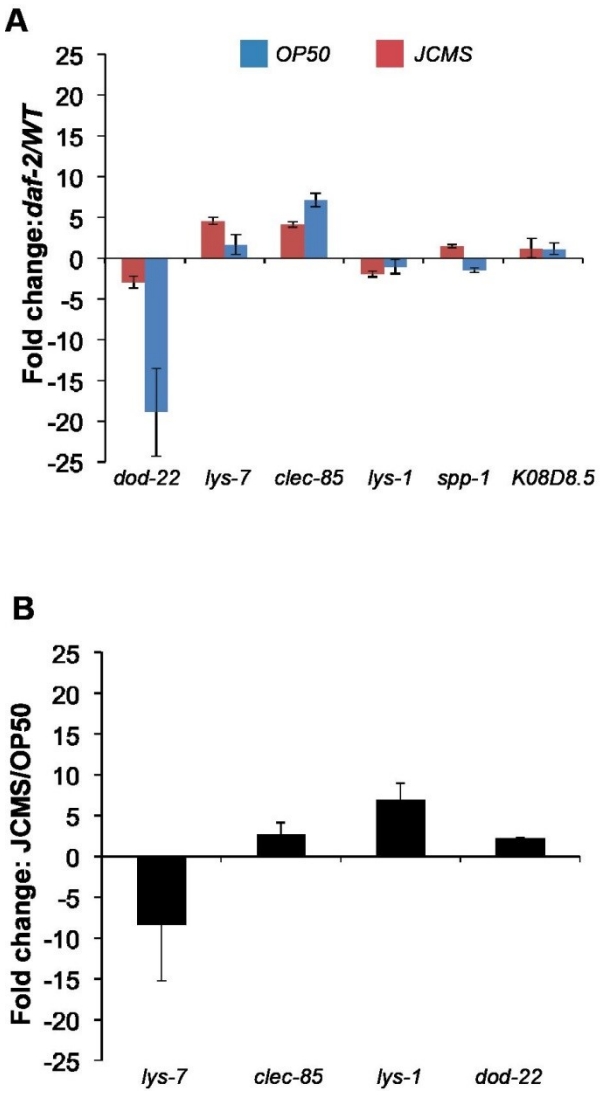


Figure 2.7 *daf-2* regulated genes are not regulated on *S. maltophilia* JCMS.

RNA was extracted from synchronized wild-type nematodes and *daf-2(e1368)* mutants that were grown on *E. coli* OP50 or *S. maltophilia* JCMS for 24 hours. Differential expression was determined by comparing biological replicates of the target gene in (A) *daf-2(e1368)* mutants versus *wildtype* nematodes (control) on OP50 (blue) or JCMS (red) and (B) *wildtype* nematodes on JCMS (black) versus OP50 (control). Fold change is shown in reference to expression in the control sample (*wildtype* on JCMS and/or OP50). Statistical significance ($p < 0.05$) was determined with a Student's t test assuming equal variance. The effector genes *dod-22*, *lys-7* and *clec-85* are significantly regulated by *daf-2* on JCMS. *lys-1* was marginally significantly ($p = 0.058$) up-regulated on JCMS versus OP50 and was not regulated by *daf-2* on either bacteria.

Table

Table 2.1 *C. elegans* defense pathway mutant responses.

	***S. maltophilia* JCMS**					***E. coli* OP50**				
Genotype	**Mean**	**SE**	**N**	**Hazard Ratio**	**p value**	**Mean**	**SE**	**N**	**Hazard Ratio**	**p value**
wildtype	4.69	0.088	150	N/A	N/A	8.53	0.187	244	N/A	N/A
cdc-25.1(RNAi)	6.97	0.163	33	N/A	N/A	9.48	0.624	29	N/A	N/A
DAF-2/16:										
ins-7	4.1	0.139	58	1.609	0.0023	11.04	0.426	53	0.210	3.66E-15
daf-2(e1368)	4.88	0.187	50	0.794	0.161	10.84	0.515	50	0.108	2.22E-16
daf-2(e1370)	3.26	0.167	74	2.163	8.6E-08	9.59	0.585	56	0.193	2.96E-13
akt-1	4.54	0.121	59	1.19	0.262	12.58	0.169	60	0.0741	<2E-16
akt-2	5.36	0.164	59	0.552	0.00016	12.13	0.278	47	0.195	<2E-16
age-1	5.28	0.2	210	0.594	0.00112	8.6	0.194	278	0.835	0.164
age-1;cdc-25.1(RNAi)	7.3	0.163	33	1.229	0.393*	11.77	1.119	31	0.304	0.0014*,**
daf-16	4.38	0.128	50	1.22	0.153	8.19	0.309	58	1.22	0.175
Unfolded protein response:										
xbp-1	3.11	0.17	57	2.747	1.9E-10	5.98	0.478	58	1.68	0.00042
ire-1	3.14	0.13	50	5.806	<2E-16	5.74	0.298	54	2.88	2.26E-11
p38 MAPK:										
nsy-1	2.7	0.076	60	31.296	<2E-16	2.43	0.208	60	13.9	<2E-16
tir-1	2.7	0.089	57	11.317	<2E-16	3.56	0.194	56	8.03	<2E-16
sek-1	1.13	0.044	60	227.79	<2E-16	3.28	0.228	57	7.18	<2E-16
pmk-1	2.58	0.072	60	16.492	<2E-16	8.3	0.404	60	0.948	0.711
atf-7	3.02	0.091	57	6.717	<2E-16	6.43	0.306	82	1.82	4.07E-06
DBL-1/TGFβ:										
dbl-1	3.0	0.138	59	3.841	<2E-16	9.8	0.374	59	0.543	6.98E-05
sma-6	3.54	0.125	48	3.297	1.2E-11	9.01	0.452	46	0.647	8.63E-03
sma-2	3.39	0.157	51	2.953	1.4E-10	5.5	0.216	59	3.67	<2E-16
sma-3	3.76	0.137	49	2.388	2.5E-07	6.52	0.377	50	1.51	8.74E-03

sma-4	3.65	0.128	46	3.073	4.1E-10	6.68	0.457	42	1.52	1.35E-02
Toll-like receptor:										
tol-1	5.07	0.144	60	0.729	0.041	8.38	0.341	60	0.878	0.377

Mean= mean survival of nematodes in days. Number of nematodes tested =N. p values less than 0.05 were considered significant and are given for the survival predictor of treatment (mutant vs. *wildtype*) for Cox proportional hazard models in R. *p value for *age-1;cdc-25.1(RNAi)* mutants versus *cdc-25.1(RNAi)*.**Date of experimentation was observed to have a significant effect and was included in this model. Additional alleles for several genes included in this table and in these and/or interacting innate immune pathways are included in Table A.6

Chapter 3 - A transcriptomic and functional analysis of the interaction between *Caenorhabditis elegans* and *Stenotrophomonas maltophilia*

Introduction

The nematode *C. elegans* has been used as a model for the study of a number of nematode-bacterial pathogen interactions (Evans *et al.*, 2008a, Garsin *et al.*, 2001, Garsin *et al.*, 2003, Sifri *et al.*, 2002). We and others (Fouhy *et al.*, 2007) have discovered a pathogenic interaction between *C. elegans* and the Gram negative bacterium *S. maltophilia*. *S. maltophilia* is also toxic to the nematodes *Panagrellus redivivus* and *Bursaphelenchus xylophilus* (Huang *et al.*, 2009) and has been found in association with *Pristionchus pacificus* (Rae *et al.*, 2008). These bacteria are also emerging nosocomial pathogens that have been associated with a number of diseases and infections especially in immunocompromised patients (Denton *et al.*, 1998). We have found that *C. elegans* utilizes several evolutionarily conserved innate immune pathways (Gravato-Nobre *et al.*, 2005a, Kim *et al.*, 2005) for the response to *S. maltophilia* JCMS (Chapter 2). However, usually pathogen resistant and, thus, long-lived DAF-2/16 insulin-like signaling pathway mutants (Evans *et al.*, 2008a, Garsin *et al.*, 2003) are also susceptible to JCMS (Chapter 2). These results suggest that *S. maltophilia* JCMS evades the DAF-2/16 pathway and the effects of genes regulated by this pathway. Therefore, the major aim of this study is to identify genes that are uniquely involved in the nematode response to these bacteria.

Many candidate innate immunity genes have been identified through the comparison of nematode expression profiles on different bacteria. Such transcriptomic studies typically identify hundreds of genes with some functional commonality. These typically include ion channel activity, sugar and lipid binding, proteolysis and lysozyme activity (Coolon *et al.*, 2009, Irazoqui *et al.*, 2010b, Troemel *et al.*, 2006, Visvikis *et al.*, 2014, Wong *et al.*, 2007). These functions are

often nested in more broad functional categories such as metabolism and binding (Coolon *et al.*, 2009, Wong *et al.*, 2007). As expected for a nematode that encounters a diverse set of microbes, several studies have found that some functions are unique and shared between bacterial environments. For example, genes involved in proteolysis, stress response, insulin signaling and cell death are commonly differently expressed in nematodes exposed to *E. faecalis*, *E. carotovora* and *P. luminescens*, while only infection with *E. faecalis* is associated with a down-regulation of hormone receptors (reviewed in Wong *et al.*, 2007). Another study demonstrated that the *C. elegans* transcriptional response to *S. aureus*, *P. aeruginosa* and *M. nematophilum* was similar but had a substantial amount of unique genes (Irazoqui *et al.*, 2010b). In that study, some of the overlapping genes had functions in intracellular detoxification and iron sequestration, while genes that were unique to two bacterial environments such as *S. aureus* and *P. aeruginosa*, had transferase, protease and lipase activity (Irazoqui *et al.*, 2010b). These studies provide evidence for a pathogen response that involves both shared and unique functions.

Despite the numerous putative innate immune response gene candidates, only a handful has been functionally analyzed. For example, both the caenopore saposin-like protein encoding gene *spp-12* (Hoeckendorf *et al.*, 2012) and the antibacterial factor peptide encoding gene *abf-2* have antimicrobial activity (Kato *et al.*, 2002). In agreement with these data, knock-down of genes in either the saposin-like or antibacterial factor protein family cause a decrease in survival and/or an increase in bacterial load in the nematode fed bacterial pathogens (Alegado *et al.*, 2008, Hoeckendorf *et al.*, 2012, Roeder *et al.*, 2010). Several *C. elegans* C-type lectin encoding genes (Irazoqui *et al.*, 2010b), genes involved in general stress response (Portal-Celhay *et al.*, 2012b, Singh *et al.*, 2006) and lysozyme encoding genes are also needed for resistance to bacterial pathogens (Alper *et al.*, 2007, Mallo *et al.*, 2002, Murphy *et al.*, 2003, O'Rourke *et al.*,

2006, Portal-Celhay *et al.*, 2012b). Additionally, genes that are involved in the response to damage inflicted by bacterial infection such as the endo- and exocytosis regulators *rab-5* and *rab-11* (Los *et al.*, 2011), and those with a demonstrated role in autophagy (i.e. *lgg-1*, *vps-34* and *unc-51*)(Visvikis *et al.*, 2014) are also involved in bacterial resistance. Thus, the nematode response to bacterial pathogens involves proteins with antimicrobial activity and those involved in the response to the cellular stress inflicted by the pathogen. Therefore, any genes encoding proteins involved in these functions could be involved in the *C. elegans*-*S. maltophilia* interaction.

Here, we used transcriptomics to identify a list of genes that were differentially expressed in response to *S. maltophilia* strains JCMS and K279a and *E. coli* OP50. As expected, this list contained hundreds of gene candidates that were associated with functions that are putatively involved in the nematode-bacterial interaction. To interpret the list of gene candidates, we used WormNet v2 to generate a network model of differentially expressed genes and ranked genes according to the number of interactions they had within the network. The use of gene networks is becoming increasing popular and several different models have been validated (Huttenhower *et al.*, 2009, Lee *et al.*, 2010a, Lee *et al.*, 2008, Lee *et al.*, 2010b). For example, WormNet and AraNet, *C. elegans* and *A. thaliana* probabilistic gene network models, generated function linkages between genes with similar mutant phenotypes significantly more than was expected by chance (Lee *et al.*, 2010a, Lee *et al.*, 2008). WormNet has also been used to identify and validate gene functions by building a network of probabilistic functions around a seed set of genes with known phenotypes (Lee *et al.*, 2008). A similar approach was used to identify human proteins involved in macroautophagy through the query of a functional map with known autophagy

proteins (Huttenhower *et al.*, 2009). Thus, probabilistic network connections have proven predictive power and can aid in the identification of genes associated with similar traits.

Within this probabilistic network model, certain genes were more central than others and were deemed hubs. We hypothesized that these hubs were essential to the bacterial response due to their differential expression and centrality in the network. In agreement with our hypothesis, most of the genes tested had a phenotype on one or more of the bacterial environments in which they were differentially expressed. Almost all of these genes have no documented role in innate immune response which supports the merit of using a probabilistic network model to prioritize gene candidates for functional validation. All of the survival mutant phenotypes except for *dod-22* were specific to the bacterial environment tested which supports the notion of a less generalized *C. elegans* innate immune response. The survival phenotypes exhibited by mutants of *dod-22* also agree with previous data from our lab suggesting that genes regulated by DAF-2/16 signaling are involved in the response to *E. coli* but not *S. maltophilia* JCMS (Chapter 2). Taken together, these data provide more evidence for a *C. elegans* innate immune response that is specific to bacterial environment. This specificity requires genes with functions that were previously shown and hypothesized to be involved in response to bacterial pathogens.

Materials and Methods

Nematode strains

The following *C. elegans* strains containing the designated alleles were obtained from the *Caenorhabditis* Genetics Center (C.G.C.): N2, LG I: *kcnl-2(ok2818)*, LG II: *acr-7(tm863)*, *mpk-2 (ok219)*, LG III: C48B4.1 *(ok2619)*, *numr-1(ok2239)*, LG IV: *dod-22 (ok1918)*, *clec-67 (ok2770)*, *lys-6 (ok2075)*, *tctn-1(ok3021)*, LG V: *cpr-4 (ok3413)*, *gcy-14(pe1102)*, *srw-145(ok495)*, LG X: *acs-17 (ok1562)*, *lgc-11(tm627)*. Of the listed strains, *mpk-2*, C48B4.1*, dod-22*, *clec-67*, *lys-6*, *cpr-4* and were identified as gene candidates for functional validation and

were outcrossed four times. *acs-17* was also a gene candidate and the strain containing *acs-17(ok1562)* was only successfully outcrossed twice. Following each outcross, segregates were screened via PCR to obtain nematodes that were homozygous for the deletion allele at the desired locus. The inner and outer primer sequences used for screening are available from the C.G.C. and WormBase. N2 was used as the wild-type strain for outcrossing and survival analysis. This strain is kept frozen and thawed yearly for experimentation.

Bacterial strains and growth

S. maltophilia JCMS was isolated by our laboratory from a culture of *Mesorhabditis sp.* found in soils from Konza Prairie, near Manhattan, KS. The isolation was part of an effort to characterize bacteria associated with native nematodes from Tallgrass prairie soils as described in Chapter 2. *E. coli* OP50 was obtained from the *Caenorhabditis* Genetics Center and *S. maltophilia* K279a from R. Ryan (University College Cork). All bacterial strains were frozen at - 80˚C upon retrieval and were thawed regularly for use in experimentation. *S. maltophilia* strains are naturally Ampicillin resistant and, were streaked for colony isolation from frozen stock on Luria Broth (LB) agar containing 100µg/mL Ampicillin to selectively prevent growth of other bacterial contaminants. *E. coli* OP50 was streaked on LB agar for colony isolation. For each bacterial strain, liquid LB was inoculated and shaken overnight at 32˚C. Bacterial lawns used for survival were seeded on nematode growth medium (NGM) with bacterial culture at log/lag phase and grown overnight at room temperature.

Nematode survival assays

Nematodes were reared and synchronized as L4s at 20˚C on *E. coli* OP50 lawns. For survival analysis, 10 to 15 L4s were picked onto three to six replicate lawns of the treatment or control bacteria and maintained at 25˚C. The number of surviving nematodes was recorded daily and death was determined by lack of motion in response to prodding with a platinum wire pick.

Nematodes were picked to new bacterial lawns for the first five to six days after the start of the experiment to separate them from their progeny. Dead nematodes were removed upon discovery. Sample sizes (N = number of nematodes) varied due to the removal of replicates because of contamination and the removal of specimens that died via means other than the specified bacterial treatment. Such means include desiccation that occurs when nematodes leave the bacterial lawn and die at the plate edge. The presence of contamination was infrequent and was determined by observing bacterial lawn morphology. Kaplan-Meier estimates of survival over time and survival curve statistics using Cox proportional hazard tests were performed in R (Vienne, Austria: R Foundation for Statistical Computing). Survival curves can be statistically compared using the log-rank and Cox proportion hazard tests. Cox proportion hazard models were used to test the effect of independent variables such as genotype and bacteria on the hazard, a dependent variable defined as the probability of dying at a given time (Goel et al., 2010). The model used for analysis is indicated in the legend of the relevant tables. Models were evaluated by testing for a non-zero slope and visualizing the Schoenfeld residuals (UCLA: Statistical Consulting Group). A non-zero slope is an indication of proportional hazard assumption violation and models were fit to the data aiming to meet that assumption.

Bulk nematode RNA extraction

Synchronized wild-type nematodes were reared at 20˚C on *E. coli* OP50 from egg to L4. L4s were then washed off the rearing plates with M9 buffer and placed onto several lawns of *S. maltophilia* JCMS, K279a or OP50. After 24 hours of feeding on the treatment bacteria at 25˚C, young adult nematodes were collected in M9 buffer and lysed in TRIzol® reagent (Life Technologies). Only non-contaminated, un-starved nematode populations were used. This bulk extraction was considered one biological replicate and was repeated four or three times for each bacterial treatment for the microarray experiment or RT qPCR. RNA extraction and DNAse

treatment were performed using the PureLink RNA Mini Kit (Invitrogen) and on-column PureLink® DNase Treatment (Invitrogen), respectively. RNA quality was checked by visualizing 28S and 18S rRNA bands using gel electrophoresis and checking 260/280 and 260/230 absorbance ratios using a NanoDrop^{TM} 8000 Spectrophotometer. RNA extraction was performed similarly for downstream applications.

Reverse transcription quantitative polymerase chain reaction (RT qPCR)

Intact RNA was used for cDNA synthesis using a SuperScript® VILO cDNA Synthesis Kit (Invitrogen). RT qPCR was performed using the CFX96 Touch^{TM} Real-Time PCR Detection System (BIO RAD). Each amplification reaction was performed in triplicate and three biological replicates of pooled bulk nematode RNA extraction were done for each bacterial nematode combination. We chose genes that would validate several expression categories as pictured in Figure 3.1. The reference gene *csq-1* was used because this gene was not significantly differentially expressed between the bacterial treatments in this study. The efficiency of each primer pair was determined using a standard curve on each biological replicate of cDNA collected on JCMS, K279a and OP50. The efficiencies of the target and *csq-1* were determined to be approximately equal (Applied Biosystems) and were assumed to be 100% during ΔC_T quantification. Primer sequences for *csq-1* (5'- AACTGAGGTTCTGACCGAGAAG - 3' and 5'- TACTGGTCA AGCTCTGAGTCGTC - 3'), F53B2.8 (5'- GAAGTCGAGAGGAGCAGAAACGAGCC - 3'and 5'- CGGGGTGGTCTTGGGGCTGG - 3'), W03F9.4 (5'- AAACTCTTGTGTCTCTGCTCATC G - 3' and 5'- CGCTGTCGTTGCATAGCTTGGCTT - 3'), *ilys-3* C45G7.3 (5'-AGCCGCGTGG AAGAGGTGC - 3' and 5'- TGCATCCTTGTGGCCCTCCG - 3') and F08G2.5 (5'- TCTTCCT CGTCCTCTTCTTCCG - 3' and 5'- ATTGCGGTATGGTTCCCACG - 3') were designed using Geneious ®6.1.8 and checked for specificity using NCBI BLAST (nucleotide collection nr/nt

database). Differential expression was determined by comparing the $2^{-\Delta CT}$ values for biological replicates of the target gene on JCMS or K279a and OP50 in wild-type nematodes (Schmittgen *et al.*, 2008). Statistical significance was determined with a Student's t test assuming equal variance.

Microarray target preparation for hybridization

cDNA was synthesized using the SMARTer PCR cDNA synthesis kit (Clontech), amplified and optimized using the Advantage 2 PCR Kit (Clontech). Total RNA (250ng) was reverse transcribed using a modified oligo dT primer and SMARTScribe reverse transcriptase, followed by 2nd strand cDNA synthesis. PCR cycling parameters for 2nd strand synthesis were optimized to ensure that the generated dsDNA remained in the exponential phase of amplification. The phase of amplification was evaluated by observing the ds cDNA smear of each sample using gel electrophoresis. Four biological replicates of pooled bulk nematode RNA extractions were used for each bacterial nematode combination. Double-stranded cDNA was purified using the PureLink™ Quick PCR Purification Kit (Invitrogen) and ethanol precipitation. DNA quality was checked by checking 260/280 and 260/230 absorbance ratios using a NanoDrop™ 8000 Spectrophotometer. Purified cDNA was sent to NimbleGen Gene Expression Services at Roche NimbleGen Inc. for fragmentation and hybridization on one *C. elegans* Gene Expression 12x135K chip containing 12 single color arrays (one array per biological replicate). Each array contained a total of 136,883 probes (5 to 6 probes per gene) representing the entire *C. elegans* transcriptome (23,196 genes).

Microarray analysis

Summarized and baseline transformed files (NimbleGen) from all 12 arrays (4 per bacterial treatment) were uploaded into GeneSpring 12 (Agilent Technologies) and normalized using quantile normalization. Quality control results from principal components analysis, a

correlation table, correlation coefficients and box plots were used to evaluate the similarity among biological replicates within each treatment (data not shown). One biological replicate for nematodes exposed to *E. coli* OP50 was determined to be an outlier and removed from the analysis of differential expression. Following outlier removal, the biological replicates were grouped by treatment. Bacterial treatments were then compared within GeneSpring using a moderated T test (Baldi *et al.*, 2001) and a Benjamini-Hochberg multiple testing correction with a 1.5 fold change cut off. Genes with a 1.5-fold or greater change and a p value < 0.05 were considered significant.

Gene ontology annotation and enrichment analysis

All differentially expressed transcripts were annotated using the WormMart tool biomart version 0.7 dataset WS220-bugFix in WormBase Version: WS247. Transcripts that were unannotated in WormMart were individually checked manually in WormBase Version: WS247. All terms called for each transcript were used or consolidated into a summative GO term within each of the following GO categories: Biological process, Molecular function and Cellular component. Some transcripts were only annotated with one GO ontology category and were counted as unannotated for other categories. Significant enrichment was determined separately using DAVID Bioinformatics Resources 6.7 and the enriched GO terms were manually matched to the WormBase GO terms. Briefly, the functional annotation tool in DAVID was queried with the WormBase ID numbers of all 438 differentially expressed transcripts with the entire *C. elegans* genome-wide genes set as background. Clusters of categorized genes were grouped with medium stringency and assigned an EASE score. The EASE score is the geometric mean of all enrichment p values for each annotated GO term in the cluster, a higher EASE score indicates that the genes in that cluster are involved in more enriched terms (Huang *et al.*, 2008). Within each cluster, each GO term had an associated p value (modified Fisher's exact test) and a

multiple testing corrected p value (Benjamini). GO terms with significant ($p < 0.05$) corrected p values were considered enriched. However, the threshold of significance was lowered ($p < 0.1$) when determining enrichment for the gene network as this reduced gene list has less statistical power.

Accessions

The full-length *S. maltophilia* JCMS 16S rRNA gene sequence was deposited in GenBank with accession number KF724885.

Results

C. elegans differential expression profiles

Stenotrophomonas maltophilia JCMS was isolated in our laboratory and is more virulent to *C. elegans* than the clinical *S. maltophilia* isolate K279a and the standard laboratory food source *E. coli* OP50 (Chapter 2). In considering the virulence factors employed by JCMS, previous studies suggest that the accumulation of living bacteria in the intestine plays a large role in nematode pathogenesis. *S. maltophilia* JCMS also causes a significantly higher bacterial load after 24 hours of bacterial exposure as compared to other *S. maltophilia* strains and the standard laboratory food *E. coli* OP50 (Chapter 2, Figure 2.2A). We also reasoned that 24 hours of bacterial exposure was an intermediate time point between initial pathogen recognition and a decline in innate immune response with aging (Youngman *et al.*, 2011). This hypothesis is supported by a subset of transcriptomic data from a large-scale study that revealed an enrichment of putative pathogen recognition genes on JCMS vs. OP50 after 6-8 hours of bacterial exposure (unpublished data). To gain insight on how *C. elegans* combats *S. maltophilia* JCMS, we conducted a transcriptomic study in which *wildtype* nematodes were exposed to JCMS, K279a and OP50. Gene expression was assessed using microarrays for all bacterial treatment groups. We identified 438 significantly differentially expressed transcripts representing 395 unique genes

using a moderated t test with a false discovery rate of 5% (Table A.7). The differentially expressed transcripts were categorized as follows: 425 between K279a and JCMS, 29 between OP50 and JCMS and 3 between OP50 and K279a (Figure 3.1). Generally, the distribution of genes reflects a response to bacterial pathogenic effect as most genes are differentially expressed between JCMS and K279a. However, one might have also expected more differential expression between JCMS and OP50 due to differences in pathogenicity and bacterial species. On the other hand, the few differentially expressed genes between OP50 and K279a is less striking. We have observed K279a to be less virulent than OP50 and that both bacteria do not cause a substantial amount of bacterial load (Chapter 2). Thus, the nematode expression profiles on JCMS, K279a and OP50 reveal that *C. elegans* is primarily responding to pathogenicity and that bacteria are not being differentiated because of their species.

RT qPCR validation

Although, the comparison between JCMS and K279a yielded a large number of pathogenicity related gene candidates, we found the low number of significantly differentially expressed genes in the JCMS vs. OP50 peculiar. Furthermore, validation of the observed expression trends was essential since these data were to be used in the selection of gene candidates for functional analysis. To this end, we chose several genes that fell into one or two comparisons for validation. The genes and comparisons are as follows: F08G2.5 up-regulated on JCMS vs. K279a, *ilys-3* down-regulated on JCMS vs. K279a, F20G2.5 up-regulated on JCMS vs. OP50, F53B2.8 up-regulated on JCMS vs. K279a and OP50 and W03F9.4 down-regulated on OP50 vs. K279a and JCMS. The significance and regulation pattern of all genes except F20G2.5 were validated using RT qPCR (Figure 3.2). The expression trend for F20G2.5 on OP50 and JCMS did concur with the array data (data not shown). However, this gene was deemed outside the detection range for several OP50 templates and was not included in Figure 3.2. Additionally,

F08G2.5 and C45G7.3 were also significantly differentially expressed between OP50 and JCMS or K279a, which was not shown in the microarray experiment. Intriguingly, a subset of transcriptomic data retreived from a large-scale study (unpublished data) on various nematode species exposed to several types of bacteria contains two genes (W03F9.4 and F53B2.8) that overlap with the present study for JCMS vs. OP50 differential expression.

Genes that are differentially expressed between OP50, K279a or JCMS are primarily involved in ion transport and redox processes.

In order to characterize our list of differentially expressed genes, we used the gene ontology (GO) terms available in WormBase (Table A.8). Briefly, GO enables the functional interpretation of a list of genes by providing a consistent vocabulary of gene product characteristics. All terms were sorted by GO category (biological process, molecular function and cellular component) and the most common terms are visualized in Figure 3.3. Within each GO category, 35 - 26% of the genes were unannotated. Generally, the annotated genes are involved in functions that have been previously implicated in nematode-pathogen interactions (Coolon *et al.*, 2009, Irazoqui *et al.*, 2010b, Troemel *et al.*, 2006, Wong *et al.*, 2007). These terms include but are not limited to defense and stress response, lysozyme activity, metabolism, transport and development. In this study, metabolism is primarily characterized by lipid (31%) and carbohydrate (21%) metabolic processes. Some of the more interesting metabolism-related GO terms include: proteolysis (10%), enterobactin biosynthetic (6%) and peptidoglycan catabolic (3%). A good portion (50%) of the signaling transduction is dedicated to G-protein coupled receptor protein signaling and 62.5% of the other transport group was denoted as transmembrane transport. The other catalytic activity group included a number of enzymes including peroxidase, GTPase, helicase, exonuclease, hydrolase and protein kinase.

GO term enrichment was determined using the functional annotation tool in DAVID Bioinformatics Resources 6.7 and only functional annotation clusters with significant (FDR < 0.05) terms are included in Table A.9. Of the 438 differentially expressed transcripts, almost all genes are associated with GO terms that are significantly enriched. Ion transport, oxidation and reduction were enriched for biological processes. Types of ion transport that were enriched include metal ion, monovalent inorganic cation, cation and potassium. Ion binding (iron and cation), channel activity, and monooxygenase activity were enriched for molecular function. The following channel activity terms were enriched: potassium, passive transmembrane, substrate-specific, metal ion transmembrane, voltage-gated channel and extracellular ligand gated ion channel. The molecular function term "transferase activity" and summative term "other binding" were not significantly enriched; specifically, the transfer of glycosyl and hexosyl groups and tetrapyrrole and heme binding were over-represented. Integral or intrinsic to membrane, membrane and voltage-gated potassium channel complex were enriched cellular components. Of note, terms that are specific to the nematode-bacterial interaction such as defense response to bacterium, cilium assembly (Apfeld *et al.*, 1999, Lee *et al.*, 2012) and receptor mediated endocytosis (Samuelson *et al.*, 2007) are included in the gene list but were not enriched. Thus, the list of differentially expressed genes are involved in an array of functions including ion transport, oxidation and reduction, have channel activity and specific transferase activity and substrate binding.

The differentially expressed gene network

Most of the differentially expressed genes were included in the list of GO terms that were significantly enriched. Thus, another method was needed to determine which genes were good candidates for functional validation. WormNet v2 is a probabilistic functional gene network tool that employs a modified Bayesian integration of data from several different organisms to

measure the probability (log-likelihood score) of protein coding gene interactions (Lee *et al.*, 2008). WormNet v2 contains 999,367 functional linkages between 15,139 genes which represents 75.4% coverage of the *C. elegans* protein-coding loci and has previously been used in hypothesis building (Lee *et al.*, 2010b). We queried WormNet v2 with our entire list of differentially expressed genes and found 118 with putative interactions (Figure 3.4, Table 3.1). Table 3.1 lists all of the genes in the network with their associated rank and log-likelihood score that is based on the predictive power of the associated interaction evidence codes (Lee *et al.*, 2008). The number of genes connections range from 21to 1 with the predictive coherence of query genes being 0.896. The predictive coherence is indicated by an area under the receiver operating characteristic curve (AUC) with 0.5 indicating random performance and 1 perfect performance (Lee *et al.*, 2010a). The method provides a measure of true-positives compared with false positives through the ranking of the query genes within the network (Lee *et al.*, 2010b). An AUC value of 0.896 indicates high predictive power and the connections within the network are on average well supported. This predictive power is reduced when genes were manually organized by linkage group and submitted to WormNet, indicating that the differentially expressed genes are not interacting with genes in the same chromosomal region (data not shown). Furthermore, the separate analysis of up- and down-regulated genes and the 29 differently expressed genes between JCMS and OP50 also reduced power and did not give additional insights. Thus, we reasoned that it was best to use all differentially expressed genes within one network.

To determine the GO ontology of the network, the list of 118 genes included in the gene network was sorted by GO category and the most prevalent terms are visualized in Figure 3.5. Although, the list of gene candidates has been significantly reduced, the network GO terms are

similar to those in Figure 3.3. The most prevalent and shared GO terms include: metabolic process, growth and reproduction, catalytic activity, binding, channel activity and integral to membrane. Some of the less prevalent retained GO terms that may be linked to innate immunity include defense and stress response, proteolysis and transport. Similar ranges of the gene are still unannotated, 34 - 20% for the network versus 35 - 26% for all differentially expressed genes. Lastly, the GO terms ion transport for biological process and integral or intrinsic to membrane for cellular component remained significantly enriched for the gene network (Figure 3.5 and Table A.10). Thus, we have successfully reduced the list of gene candidates from 395 to 118 without losing the functions and processes that likely encompass the nematode-bacterial response.

Gene network analysis and functional validation

The use of gene networks to identify gene candidates for functional validation is becoming more common (Berger *et al.*, 2012, Huttenhower *et al.*, 2009, Lee *et al.*, 2010a). Like others (Özgür *et al.*, 2008, Özgür *et al.*, 2010), we reasoned that genes with high network connectivity (hubs) would be involved in the response associated with the original generation of the gene list used to build the network. This network centrality hypothesis is based on the idea that more central hubs are pleiotropic and essential for organismal survival (Fisher, 1930, Hahn *et al.*, 2005). In our case, the list of gene candidates was generated by the comparison of expression on different bacterial environments. Thus, we hypothesized that gene hubs would be involved in the nematode-bacterial response. Using the data generated from WormNet v2, genes were ranked by the number of predicted interactions (Table 3.1). The top 20 most connected gene hubs were: *pqn-98*, *cpr-4*, *dod-22*, F28A12.4, *acs-17*, F19B2.5, R03G5.5, B0024.4, *mpk-2*, T22F3.11, *ugt-22,* C14C6.2, C48B4.1, *clec-67*, F08G2.5, *lys-6*, T05F1.11, *ftn-1*, *lys-5* and C35E7.2. In order to functionally validate these genes, we needed to obtain loss of function

alleles to assess the nematode phenotype in the absence of the gene product. Of these candidate gene hubs, we chose to validate those with available deletion alleles. These genes were: *cpr-4* (cysteine protease related), *dod-22* (down-stream of *daf-16*), *acs-17* (fatty acid CoA synthetase), *mpk-2* (mitogen activated protein kinase), *lys-6* (lysozyme), *clec-67* (C-type lectin) and acyl-CoA oxidase C48B4.1. Of the these candidates, only *dod-22* is known to be involved in bacterial resistance (Sahu *et al.*, 2012) and most are not associated with any phenotype according to WormBase. Thus, the discovery of any phenotype associated with the nematode-bacterial interaction is novel and aids in our understanding of *C. elegans* innate immunity and in some cases, nematode biology.

In order to assess the involvement of each gene candidate, we performed survival analysis for each mutant versus *wildtype* on *S. maltophilia* K279a and JCMS. We also included survival analysis on *E. coli* OP50 which is the normal *C. elegans* lab food and served as a non-pathogenic control. K279a is also less pathogenic and allowed the comparison of avirulent and virulent *S. maltophilia* strains. All of the gene candidates were only significantly differentially expressed between JCMS and K279a. Thus, we hypothesized that these gene hubs would have survival phenotypes on one or both of these bacteria. Mutations in *cpr-4*, *mpk-2*, *lys-6*, *clec-67* and C48B4.1 caused hyper-susceptibility to JCMS (Figure 3.6 and Table 3.2). None of these mutants had a phenotype on K279a and only *dod-22* mutants were resistant to these bacteria. Mutations in *lys-6*, *cpr-4*, *acs-17* and *dod-22* caused resistance to OP50.These data indicate that *acs-17*, *cpr-4*, *mpk-2*, *lys-6*, *clec-67* and C48B4.1 have unique roles on one or more of the tested bacteria. The resistance of *dod-22* mutants to K279a and OP50 revealed a similar role on these bacteria while, this gene is not involved in the response to JCMS. In sum, six of seven mutants had phenotypes on JCMS or K279a (Table 3.2); validating the network centrality hypothesis. We

also tested the opposing hypothesis: disconnected genes are not functionally relevant and therefore, would not have a survival phenotype on JCMS or K279a. The genes selected for testing this hypothesis were outside of the network, had deletion alleles available and had expression patterns that were similar to the tested gene candidates. Surprisingly, three of seven gene mutants had significant survival phenotypes on JCMS or K279a (Table A.11). These data suggest that significant differential expression alone is also predictive in the identification of gene candidates. However, more survival phenotypes were observed within the connected and central candidate gene list making network centrality a greater functional predictor.

The degree to which the mutation of each candidate gene affects survival was inferred from the value of the mutant to *wildtype* hazard ratio (from the associated Cox proportional hazard model, see Materials and Methods). Values near one suggest lack of involvement, values greater than one indicate that the mutation shortens lifespan and values less than one indicate that the mutation increases survival. Thus, the hazard ratio is an indication of involvement on a given bacteria with greater deviations from one indicating greater involvement. On *S. maltophilia* JCMS, all the mutants with phenotypes are hyper-susceptible and the hazard ratios range from 2.1 to 1.5 (Table 3.2). *cpr-4* and *clec-67* mutants had the highest hazards and were two times as likely to die as *wildtype*. The hazard ratio for *dod-22* mutants on *S. maltophilia* K279a was 0.63 which is similar to the hazard ratio (0.61) on *E. coli* OP50, indicating a similar role. The hazard ratios for mutants on *E. coli* OP50 range from 0.61 to 0.16. Mutants of *lys-6* (0.65), *cpr-4* (0.64) and *dod-22* (0.61) all have almost identical ratios while, *acs-17* mutants are the most resistant and have the lowest risk of death.

Discussion

The expression analysis of nematodes fed *E. coli* OP50, *S. maltophilia* JCMS and K279a yielded a list of 438 differentially expressed transcripts (Figure 3.1). Like other studies that

transcriptionally compare bacterial environments (Coolon *et al.*, 2009, Irazoqui *et al.*, 2010b, Troemel *et al.*, 2006, Visvikis *et al.*, 2014), most of these genes are involved in metabolism, transport, binding, growth, reproduction and encode membrane proteins (Figure 3.3). In order to determine which functions and processes (GO term categories) were important for the nematode-bacterial interaction, we performed gene ontology enrichment analysis. The gene ontology terms that were significantly enriched for the list of differentially expressed genes were ion transport (metal ion, monovalent inorganic cation, cation and potassium), oxidation and reduction, tetrapyrrole and heme binding, channel activity, transferase activity, monooyxgenase activity and voltage-gated potassium channel complex. Although informative, the enriched GO terms were associated with most of the differentially expressed genes and did not allow for the nomination of promising candidate genes. Thus, an alternative method was used to determine which genes to functionally validate.

The use of gene networks for hypothesis building and functional validation is ever increasing (Berger *et al.*, 2012, Huttenhower *et al.*, 2009, Lee *et al.*, 2008). Furthermore, the availability of WormNet v2 provided the opportunity to utilize a probabilistic network model that was developed for the identification of novel genes associated with a phenotype of interest in *C. elegans* (Lee *et al.*, 2010b). WormNet v2 initially reduced our candidate gene list from 395 to the 118 genes that had probabilistic connections according to the network model (Figure 3.4). This network model was annotated with GO terms and several enriched terms that were reminiscent of the annotations associated with the entire list of gene candidates (Figure 3.3, 3.5 and Table A.9, A.10). The list of 118 candidate genes in the network was further narrowed to seven via the sorting of genes by the number of connections followed by the availability of loss of function alleles. Like others (Özgür *et al.*, 2008, Özgür *et al.*, 2010), we hypothesized that

genes that were central to the network of differentially expressed genes, would be required for the bacterial environments associated with their differential expression. Six of the seven evaluated genes had mutant phenotypes on JCMS or K279a, supporting our hypothesis (Figure 3.6, Table 3.2). Furthermore, five of the six genes had no documented innate immunity associated phenotype which further merits the use of gene network topology to identify candidate genes.

Here we show that the *C. elegans* response to *S. maltophilia* JCMS involves *lys-6*, *cpr-4*, *mpk-2*, *clec-67* and C48B4.1 (Figure 3.6 and Table 3.2). C48B4.1 encodes an ortholog of human acyl-CoA oxidase 1 and has no known function in innate immunity. The mitogen activated protein kinase (MAPK) gene *mpk-2* also has no demonstrated role in innate immunity but is regulated by the bacterial pathogens *S. aureus* and *P. aeruginosa* (Irazoqui *et al.*, 2010b). Another MAPK gene *mpk-1* is required to combat *M. nematophilum* infection (O'Rourke *et al.*, 2006), thus, a protective role for *mpk-2* upon *S. maltophilia* ingestion was expected. Also as predicted due to previous studies with similar genes (Irazoqui *et al.*, 2010b, Portal-Celhay *et al.*, 2012b), *clec-67* and *lys-6* were required for the response to *S. maltophilia* JCMS. We find that *clec-67* mutants had one of the highest hazard ratios on these bacteria (Table 3.2). If lectins are in fact involved in pathogen recognition as postulated (Nicholas *et al.*, 2004), this increased nematode hazard may be due to a failed elicitation of immune response. In agreement, *clec-67* is not required for response to the avirulent *S. maltophilia* K279a and *E. coli* OP50 (Figure 3.6B and Table 3.2) and *clec-67* was up-regulated on JCMS vs. K279a (Table A.7) and *S. enterica* (Kerry *et al.*, 2006). Intriguingly, the lysozyme *lys-6* was needed for *S. maltophilia* resistance but had one of the smallest hazard ratios (Table 3.2). This data suggests that the destruction of bacteria is needed but is not as imperative as other nematode functions. On the other hand, the

cysteine protease encoding gene *cpr-4* has the largest role of the tested genes on JCMS (Table 3.2). Although, intuitively involved in the nematode-bacterial interaction, *cpr-4* does not have a demonstrated role in innate immunity. However, protease activity has previously been linked to the nematode-bacterial pathogen response (reviewed in Wong *et al.*, 2007) and another cysteine protease related gene *cpr-2* is regulated by *S. aureus* infection (Irazoqui *et al.*, 2008). Furthermore, *cpr-4* is also expressed in the nematode during all life stages which further supports a major role (Larminie *et al.*, 1996).Thus, protease activity, sugar binding and to a lesser extent, fatty acid metabolism and/or oxidation and reduction, MAPK signaling and lysozyme activity are all involved in combating JCMS. Furthermore, *mpk-2*, *clec-67* and C48B4.1 only have a phenotype on JCMS, indicating that these genes play an *S. maltophilia* strain specific role.

The lysozyme *lys-6* and cysteine protease *cpr-4* are not specific to bacterial environment as these genes are also involved in the response to *E. coli* OP50 (Figure 3.6A,D and Table 3.2). However, the resistance of *lys-6*, *cpr-4* and *acs-17* mutants to *E. coli* indicates that the products of these genes are detrimental on these bacteria. The hazard ratios for *cpr-4* and *lys-6* mutants are similar to each other and have smaller deviations from one indicating a smaller role on these bacteria (Table 3.2). Mutants of the fatty acid CoA synthetase *acs-17* are uniquely resistant to OP50 and the hazard ratio for these mutants deviates the most from one compared to the other resistant mutants. This greater role could have to do with the involvement of fatty acid metabolism in the nematode-bacterial response and an overlapping function in nematode longevity (Murphy *et al.*, 2003). In summary, the production of protease, lysozyme and fatty acid CoA synthetase are detrimental to wild-type nematodes on *E. coli*. As discussed above, these processes, although, fatty acid metabolism involves different genes (C48B4.1 on JCMS and *acs-17* on OP50) are required for combating bacterial pathogens. Thus, the detrimental regulation of

these functions in wild-type nematodes on *E. coli* is linked to the general non-pathogenic nature of these bacteria.

The CUB domain containing protein DOD-22 (downstream of *daf-16*) is induced by gram negative pathogens (Alper *et al.*, 2007) and required for the response to *Vibrio cholerae* (Sahu *et al.*, 2012). In agreement with these data, we have found that *dod-22* is significantly induced on JCMS vs. K279a (Table A.7) suggesting that *dod-22* is required for JCMS response. However, *dod-22* is also regulated by *C. elegans* DAF-2/16 signaling (Murphy *et al.*, 2003) and we have demonstrated that this pathway is not involved in *S. maltophilia* JCMS response (Chapter 2). As expected given these data, the survival of *dod-22* mutants was not significantly different from wild-type on JCMS (Figure 3.6C and Table 3.2), indicating that like DAF-2/16 signaling components (Chapter 2, Table 2.1) this gene is dispensable on these bacteria. Furthermore, *dod-22* mutants had significantly extended lifespan on K279a and OP50 and the mutant to wild-type hazard ratios were very similar on these bacteria. These findings agree with a well-supported role for DAF-2/16 signaling on OP50 (Chapter 2, Table 2.1) and the resistance exhibited by *daf-2* mutants on K279a (Table A.13). Taken together, these data further support the JCMS specific evasion of the DAF-2/16 pathway and a role for DAF-2/16 signaling to effector genes such as *dod-22* on K279a and OP50. Furthermore, although, *dod-22* is regulated by DAF-2/16 signaling on JCMS (Chapter 2, Figure 2.7A), the up-regulation on JCMS versus K279a suggests that on these bacteria other immune pathways also regulate *dod-22*. Consistent with this hypothesis, *dod-22* is regulated by the p38 MAPK pathway (Alper *et al.*, 2007) that we have found to be involved in the response to JCMS (Chapter 2, Table 2.1). In summary, these data support a role for specific and overlapping gene effectors in the *C. elegans* response to bacteria. Here, the overlapping genes had different roles that corresponded with bacterial

pathogenicity regardless of bacterial strain. Thus, the nematode innate immune response is specific to bacterial pathogenicity rather than bacterial species.

Figures

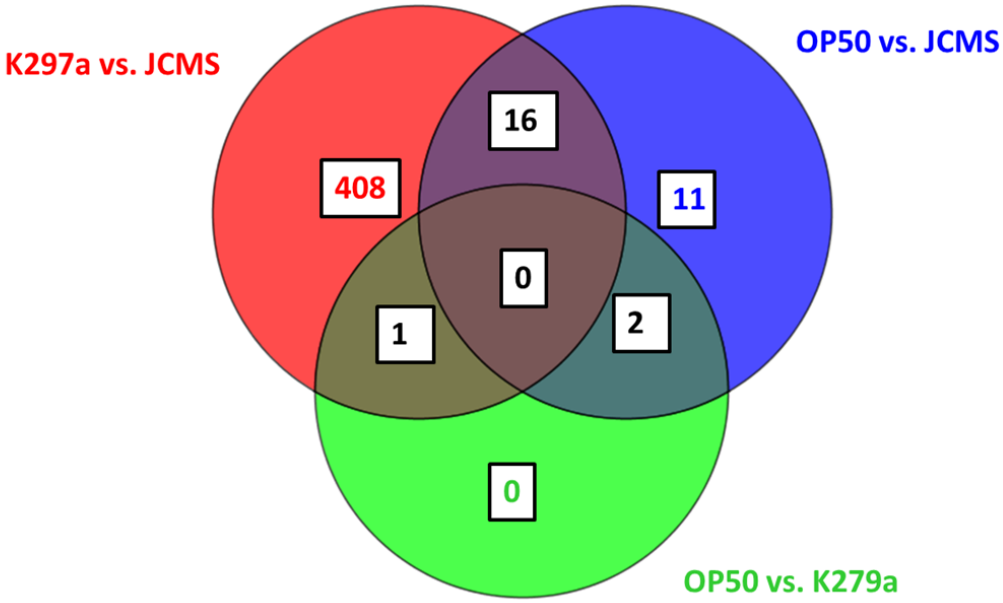


Figure 3.1 All significantly differentially expressed transcripts for wild-type nematodes fed *E. coli* OP50, *S. maltophilia* JCMS or K279a.

Differential expression was determined on all pairwise comparisons of *S. maltophilia* JCMS, *S. maltophilia* K279a and *E. coli* OP50. OP50 or K279a were the baseline treatment for each comparison. Statistical significance was determined using a moderated T test and a Benjamini-Hochberg multiple testing correction with a 1.5 fold change cut off. The corrected p values are listed for each transcript in Table A.7. A transcript was considered significant if the corrected p value was less than 0.05. There were 438 significantly differentially expressed transcripts representing 395 unique genes. All differentially expressed transcripts are included in this Venn diagram.

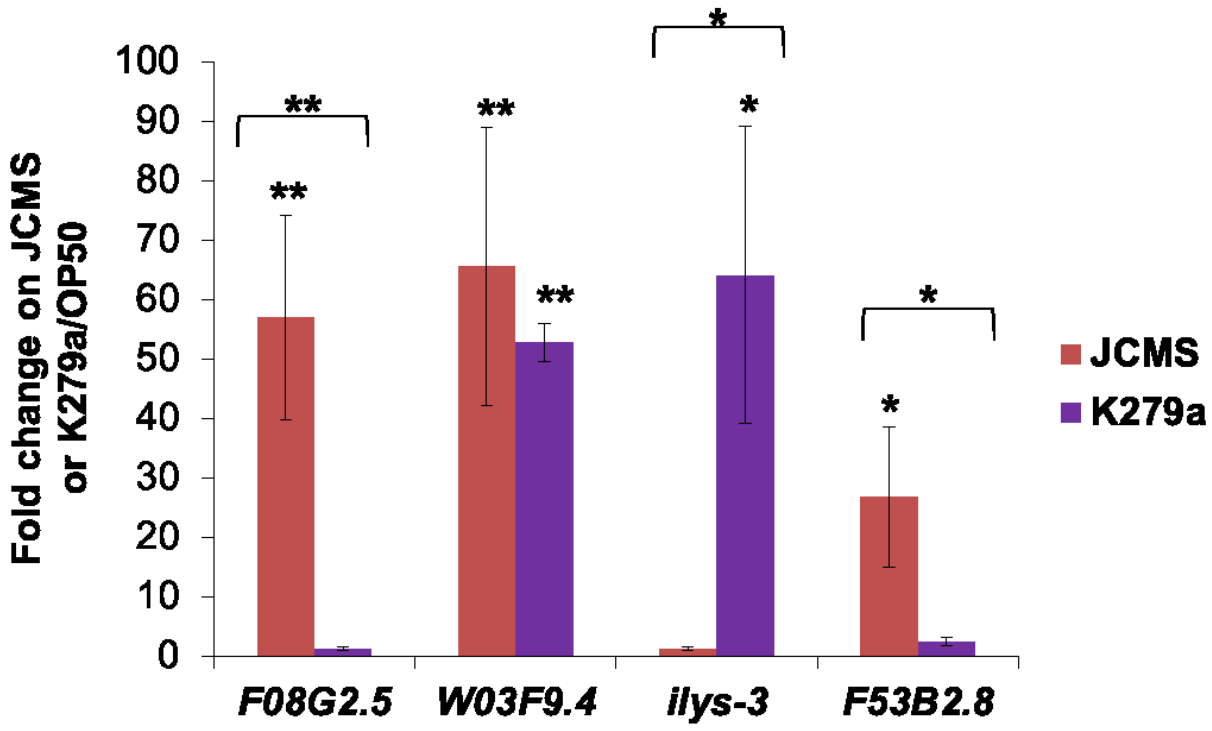
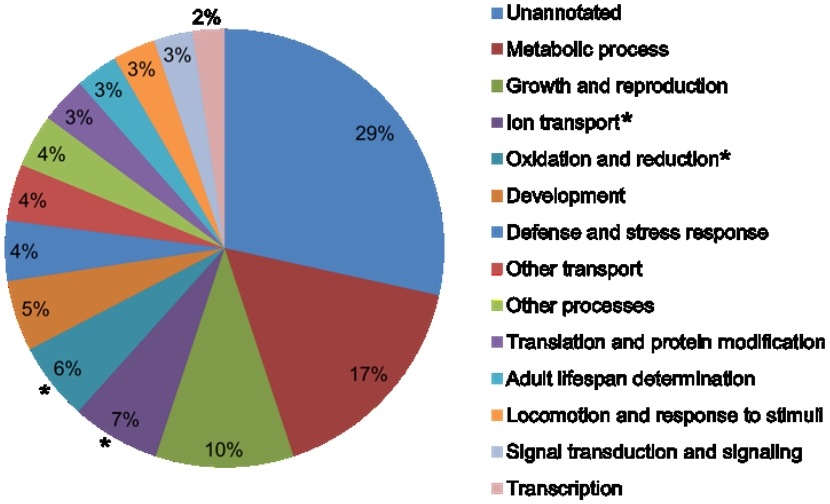


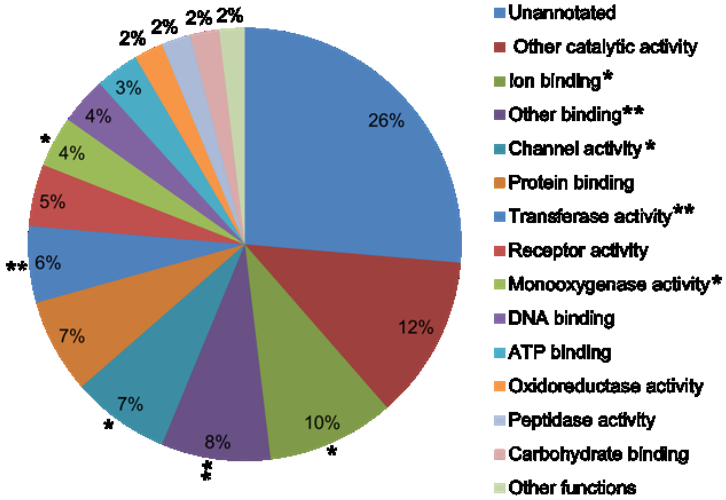
Figure 3.2 RT qPCR of several significantly differentially expressed genes validates the microarray dataset.

Expression of F08G2.5, W03F9.4, *ilys-3* and F53B2.8 in wild-type nematodes on *S. maltophilia* JCMS (red) or K279a (purple). Fold change is shown in reference to expression in the control sample (OP50). Statistical significance ($p < 0.05$** or 0.1*) was determined with a Student's t test assuming equal variance. Asterisk(s) above the error bars: expression on JCMS or K279a was significantly different from on OP50. Asterisk(s) above a bracket: expression on JCMS was significantly different from on K279a. The comparisons validated from the microarray experiment (Table A.7) are as follows: F08G2.5 up-regulated on JCMS vs. K279a, *ilys-3* down-regulated on JCMS vs. K279a, F53B2.8 up-regulated on JCMS vs. K279a and OP50 and, W03F9.4 down-regulated on OP50 vs. K279a and JCMS.

A. Biological process



B. Molecular function



C. Cellular component

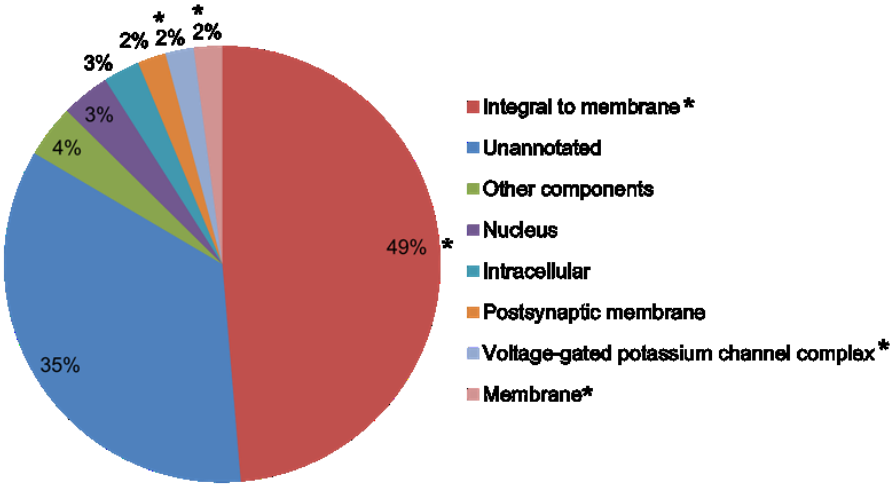


Figure 3.3 Gene ontology of all significantly differentially expressed transcripts for wild-type nematodes fed *E. coli* OP50, *S. maltophilia* JCMS or K279a.

All terms called for each transcript were used or consolidated into a summative GO term within each GO ontology category: A) Biological process, B) Molecular function and C) Cellular component. Terms are listed and ordered in the pie chart by frequency of occurrence. All differentially expressed transcripts were annotated using the WormMart tool biomart version 0.7 dataset WS220-bugFix in WormBase Version: WS247. Transcripts that were unannotated in WormMart were individually checked manually in WormBase Version: WS247. GO terms marked with an * were significantly ($p < 0.05$) enriched in the dataset compared to the frequency of said term in the *C. elegans* genome. Significant enrichment was determined using DAVID Bioinformatics Resources 6.7. All significantly enriched GO terms are listed in Table A.9 and all GO terms are listed in Table A.8. ** The molecular function terms "transferase activity" and "other binding" were not significantly enriched; only the transfer of glycosyl and hexosyl groups and tetrapyrrole and heme binding were over-represented.

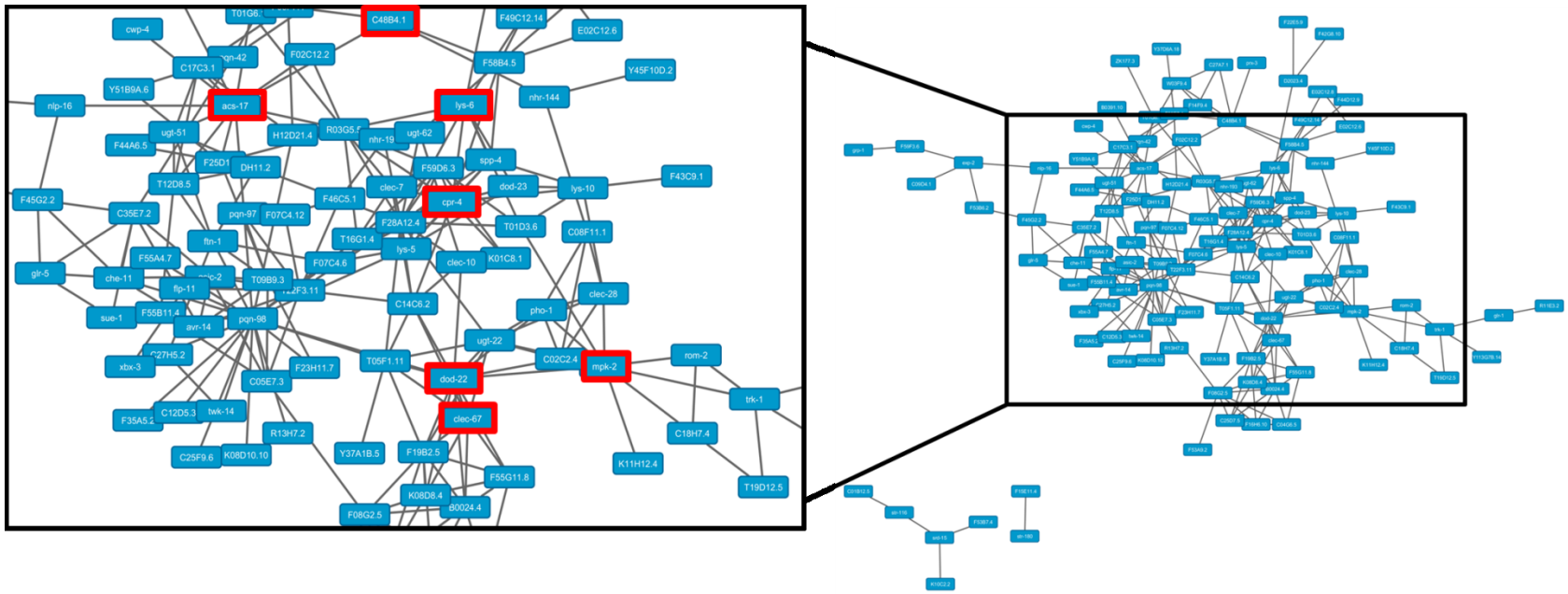
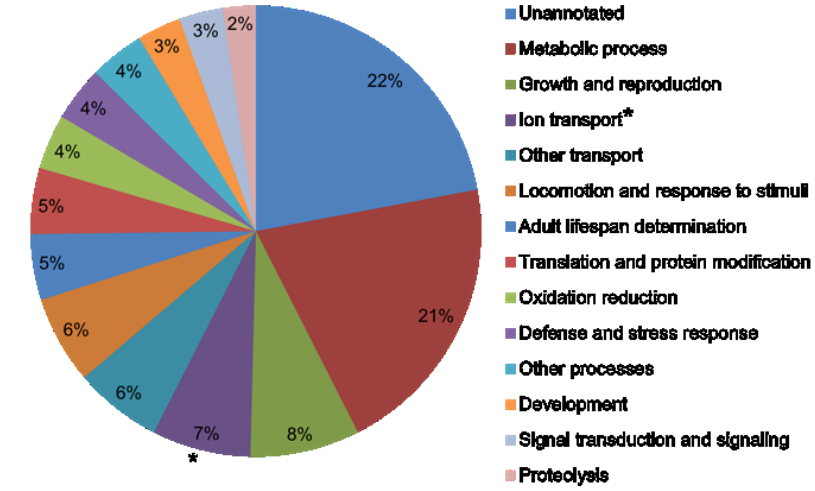


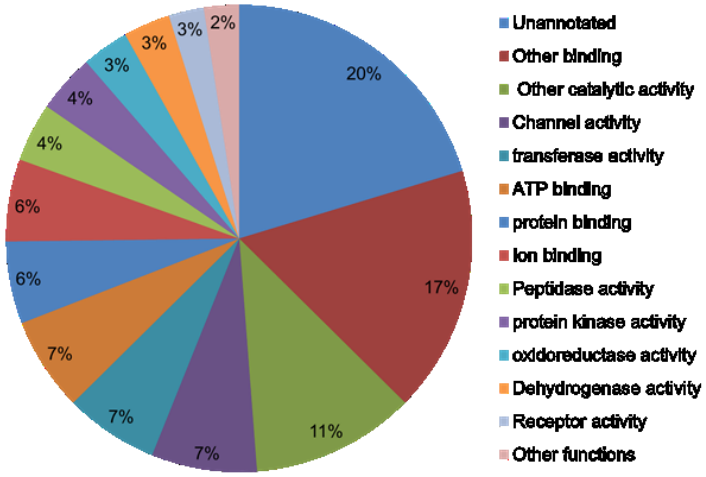
Figure 3.4 WormNet v2 network of differentially expressed genes on *S. maltophilia* JCMS, K279a or *E. coli* OP50.

The image above was generated in Cytoscape 3.1.1 using the probabilistic interaction information generated in WormNet v2 (Lee *et al.*, 2008, Lee *et al.*, 2010b). The probabilistic functional gene network model includes 118 of 395 unique differentially expressed genes (Table 3.1). The area under the receiver operating characteristic (ROC) curve (AUC) value for the network was 0.896. An AUC value of 0.896 indicates high predictive power and the connections within the network are on average well supported. Each blue box in the network represents a gene and the grey lines connecting genes are putative functional interactions. The zoomed in portion of the network shows the centrality of the gene hubs (boxed in red) chosen for validation.

A. Biological process



B. Molecular function



C. Cellular component

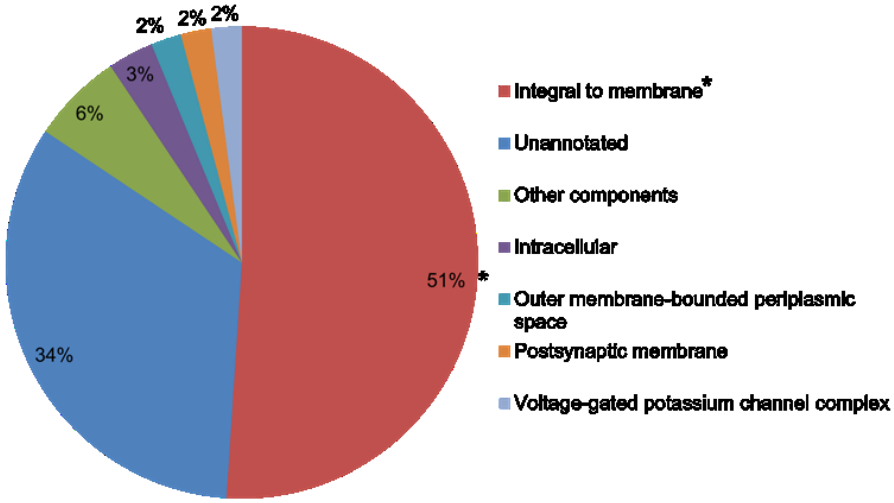


Figure 3.5 Gene ontology and enrichment of the differentially expressed gene network.

All terms called for each gene were used or consolidated into a summative GO term within each GO ontology category: A) Biological process, B) Molecular function and C) Cellular component. Terms are listed and ordered in the pie chart by frequency of occurrence. All 118 of the differentially expressed genes incorporated into the WormNet v2 gene network model were annotated using the WormMart tool biomart version 0.7 dataset WS220-bugFix in WormBase Version: WS247. Genes that were unannotated in WormMart were individually checked manually in WormBase Version: WS247. GO terms marked with an * were significantly ($p < 0.1$) enriched in the dataset compared to the frequency of the term in the *C. elegans* genome.

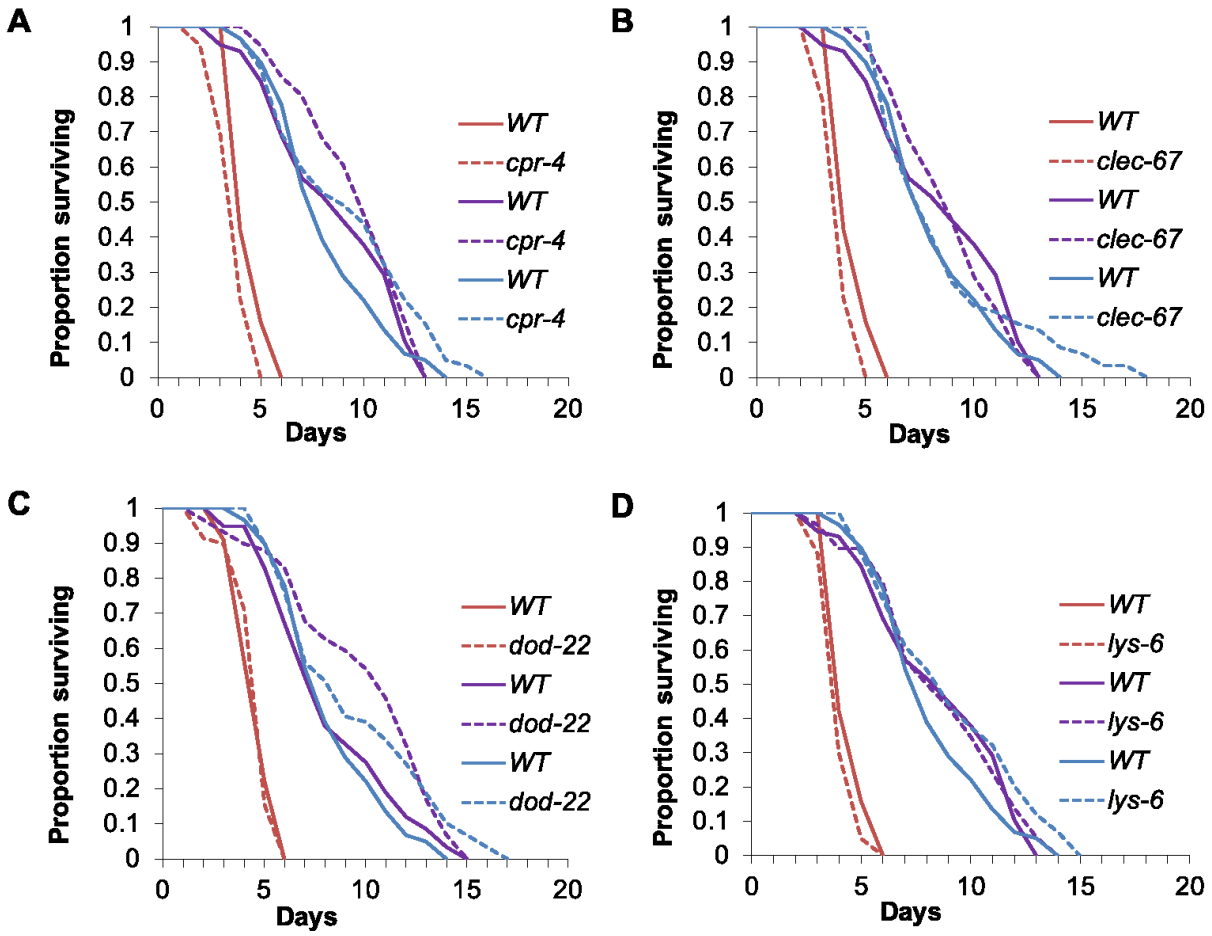


Figure 3.6 Survival of wild-type nematodes and array candidate mutants on *E. coli* OP50, *S. maltophilia* JCMS or K279a.

Survival of wild-type nematodes (solid lines) and select gene hub mutants (dashed lines) on *S. maltophilia* JCMS (red), K279a (purple) and *E. coli* OP50 (blue). A) *cpr-4(ok3413)*, B) *clec-67(ok2770),* C) *dod-22 (ok1918)* and D) *lys-6(ok2075)*. Results plotted are the proportion of surviving worms using Kaplan-Meier estimates for at least three replicate samples (10 to 15 nematodes per replicate) of the same nematode population. p values from the application of Cox proportional hazards models and sample sizes of each population are included in Table 3.2. Mutants of *cpr-4*, *clec-67*, *mpk-2* and *lys-6* were all significantly susceptible to JCMS while, *lys-6* and *cpr-4* mutants were significantly resistant to OP50. None of the mutants in this figure had a significant survival phenotype on K279a.

Tables

Table 3.1 WormNet v2 network of differentially expressed genes on *S. maltophilia* JCMS, K279a or *E. coli* OP50.

Gene name	Sequence name	Rank	Score	C	Linked genes
pqn-98	ZK488.7	23	2.05	21	C05E7.3 C12D5.3 C25F9.6 *che-11 acs-17 ftn-1 sue-1* F07C4.6 F23H11.7 F35A5.2 F55A4.7 *dod-22 lys-5* H12D21.4 *twk-14 flp-11* K08D10.10 *xbx-3* R13H7.2 T05F1.11 T16G1.4
cpr-4	F44C4.3	37	1.76	14	C14C6.2 F07C4.6 *clec-7 lys-10* F28A12.4 *dod-23 lys-5 lys-6* F58B4.5 F59D6.3 K01C8.1 R03G5.5 *spp-4* T16G1.4
dod-22	F55G11.5	2	2.89	13	B0024.4 C02C2.4 *clec-10 mpk-2 ugt-22* C14C6.2 *pho-1* F19B2.5 F55G11.8 *clec-67* K08D8.4 T05F1.11 *pqn-98*
F28A12.4	F28A12.4	21	2.09	13	*clec-10 ugt-22* C14C6.2 DH11.2 *cpr-4 lys-6* F59D6.3 *ugt-62* R03G5.5 T01D3.6 T05F1.11 *spp-4* T22F3.11
acs-17	C46F4.2	8	2.56	12	B0391.10 *ugt-51* C17C3.1 C48B4.1 F02C12.2 F25D1.5 *cwp-4* R03G5.5 T01G6.10 T12D8.5 *nlp-16 pqn-98*
F19B2.5	F19B2.5	1	2.89	10	B0024.4 *ugt-22* C25D7.5 F08G2.5 F16H6.10 *dod-22* F55G11.8 *clec-67* K08D8.4 T05F1.11
R03G5.5	R03G5.5	52	1.51	10	*acs-17* F02C12.2 *clec-7* F25D1.5 F28A12.4 *cpr-4 lys-6* F59D6.3 T01G6.10 T16G1.4
B0024.4	B0024.4	12	2.49	9	C04G6.5 C25D7.5 F08G2.5 F16H6.10 F19B2.5 *dod-22* F55G11.8 *clec-67* K08D8.4
mpk-2	C04G6.1	24	1.97	9	*ugt-22* C18H7.4 *rom-2 trk-1 pho-1 clec-28 dod-22* K11H12.4 T01D3.6
T22F3.11	T22F3.11	3	2.79	9	*avr-14* C05E7.3 C14C6.2 F07C4.12 F28A12.4 F46C5.1 *flp-11 asic-2 pqn-97*
ugt-22	C08F11.8	30	1.86	9	C02C2.4 *mpk-2 pho-1* F19B2.5 F28A12.4 *clec-28 dod-22 clec-67* T05F1.11
C14C6.2	C14C6.2	35	1.78	8	*clec-10 clec-7* F28A12.4 *cpr-4 dod-22 clec-67* T05F1.11 T22F3.11
C48B4.1	C48B4.1	32	1.83	8	*prx-3* C27A7.1 *acs-17* F09F7.4 F14F9.4 F58B4.5 *nhr-144* W03F9.4

clec-67	F56D6.2	18	2.22	8	B0024.4 *ugt-22* C14C6.2 F19B2.5 *dod-22* F55G11.8 K08D8.4 T05F1.11
F08G2.5	F08G2.5	9	2.56	8	B0024.4 C04G6.5 C05E7.3 C25D7.5 F16H6.10 F19B2.5 F53A9.2 K08D8.4
lys-6	F58B3.3	36	1.78	8	D2023.4 F28A12.4 *cpr-4* F49C12.14 *dod-23* R03G5.5 T01D3.6 *spp-4*
T05F1.11	T05F1.11	55	1.49	8	*ugt-22* C14C6.2 F19B2.5 F28A12.4 *dod-22 clec-67* Y37A1B.5 *pqn-98*
ftn-1	C54F6.14	40	1.7	7	*ugt-51* C27H5.2 DH11.2 F07C4.6 T09B9.3 T12D8.5 *pqn-98*
lys-5	F58B3.2	49	1.55	7	F07C4.6 *cpr-4 dod-23 nhr-193* F59D6.3 *spp-4 pqn-98*
C35E7.2	C35E7.2	4	2.71	7	*avr-14 che-11 sue-1* F45G2.2 *pqn-42 asic-2 glr-5*
K08D8.4	K08D8.4	11	2.51	7	B0024.4 C25D7.5 F08G2.5 F19B2.5 *dod-22* F55G11.8 *clec-67*
C17C3.1	C17C3.1	14	2.44	7	*ugt-51 acs-17* DH11.2 F09F7.4 H12D21.4 T12D8.5 Y51B9A.6
T09B9.3	T09B9.3	31	1.85	7	*avr-14* C05E7.3 *ftn-1* F23H11.7 F25D1.5 *asic-2 pqn-97*
F58B4.5	F58B4.5	39	1.73	7	C48B4.1 E02C12.6 E02C12.8 *cpr-4* F44D12.9 *ugt-62 spp-4*
F59D6.3	F59D6.3	56	1.48	7	F28A12.4 *cpr-4 lys-5* K01C8.1 R03G5.5 T01D3.6 T16G1.4
asic-2	T28F4.2	28	1.92	6	*avr-14* C05E7.3 *che-11* C35E7.2 T09B9.3 T22F3.11
che-11	C27A7.4	5	2.67	6	*avr-14* C35E7.2 F45G2.2 *asic-2 glr-5 pqn-98*
clec-28	F49A5.5	20	2.17	6	C02C2.4 *mpk-2* C08F11.1 *ugt-22 pho-1 lys-10*
lys-10	F17E9.11	58	1.45	6	F43C9.1 *cpr-4 clec-28 dod-23* T01D3.6 *spp-4*
F55G11.8	F55G11.8	7	2.6	6	B0024.4 C04G6.5 F19B2.5 *dod-22 clec-67* K08D8.4
C05E7.3	C05E7.3	41	1.7	6	F08G2.5 F55B11.4 T09B9.3 T22F3.11 *asic-2 pqn-98*
DH11.2	DH11.2	42	1.69	6	*ugt-51* C17C3.1 *ftn-1* F28A12.4 F44A6.5 T12D8.5
T12D8.5	T12D8.5	54	1.5	6	*ugt-51* C17C3.1 *acs-17 ftn-1* DH11.2 F55B11.4
pho-1	EGAP2.3	26	1.97	6	C02C2.4 *mpk-2* C08F11.1 *ugt-22 clec-28 dod-22*
spp-4	T08A9.8	43	1.69	6	*lys-10* F28A12.4 *cpr-4 lys-5 lys-6* F58B4.5
avr-14	B0207.12	22	2.06	5	*che-11* C35E7.2 T09B9.3 T22F3.11 *asic-2*

F16H6.10	F16H6.10	13	2.47	5	B0024.4 C04G6.5 C25D7.5 F08G2.5 F19B2.5
C25D7.5	C25D7.5	17	2.27	5	B0024.4 F08G2.5 F16H6.10 F19B2.5 K08D8.4
F45G2.2	F45G2.2	25	1.97	5	*che-11* C35E7.2 F53B6.2 *nlp-16 glr-5*
C02C2.4	C02C2.4	53	1.5	5	C08F11.1 *ugt-22 pho-1 clec-28 dod-22*
T01D3.6	T01D3.6	67	1.37	5	*mpk-2 lys-10* F28A12.4 *lys-6* F59D6.3
T16G1.4	T16G1.4	69	1.32	5	*clec-7 cpr-4* F59D6.3 R03G5.5 *pqn-98*
trk-1	D1073.1	46	1.61	5	*mpk-2 glr-1 rom-2* T19D12.5 Y113G7B.14
ugt-51	C03A7.11	19	2.22	5	C17C3.1 *acs-17 ftn-1* DH11.2 T12D8.5
clec-10	C03H5.1	50	1.55	4	C14C6.2 F28A12.4 *dod-22 ugt-62*
clec-7	F10G2.3	74	1.25	4	C14C6.2 *cpr-4* R03G5.5 T16G1.4
dod-23	F49E12.2	68	1.32	4	*lys-10 cpr-4 lys-5 lys-6*
exp-2	F12F3.1	6	2.63	4	C09D4.1 F53B6.2 F59F3.6 *nlp-16*
glr-5	ZC196.7	61	1.44	4	*che-11* C35E7.2 *sue-1* F45G2.2
F09F7.4	F09F7.4	16	2.28	4	C17C3.1 C48B4.1 H12D21.4 Y37D8A.18
C08F11.1	C08F11.1	27	1.96	4	C02C2.4 *pho-1 clec-28 nhr-144*
F25D1.5	F25D1.5	34	1.79	4	*acs-17* R03G5.5 T09B9.3 Y51B9A.6
F14F9.4	F14F9.4	38	1.75	4	C27A7.1 C48B4.1 *pqn-42* W03F9.4
W03F9.4	W03F9.4	44	1.66	4	C27A7.1 C48B4.1 F14F9.4 *pqn-42*
C04G6.5	C04G6.5	47	1.58	4	B0024.4 F08G2.5 F16H6.10 F55G11.8
F07C4.6	F07C4.6	59	1.45	4	*ftn-1 cpr-4 lys-5 pqn-98*
H12D21.4	H12D21.4	66	1.37	4	C17C3.1 F09F7.4 *ugt-62 pqn-98*
ugt-62	M88.1	57	1.47	4	*clec-10* F28A12.4 F58B4.5 H12D21.4
flp-11	K02G10.4	81	1.18	3	*xbx-3* T22F3.11 *pqn-98*
F46C5.1	F46C5.1	15	2.31	3	F07C4.12 *nhr-193* T22F3.11
C27A7.1	C27A7.1	45	1.65	3	C48B4.1 F14F9.4 W03F9.4
D2023.4	D2023.4	71	1.29	3	F22E5.9 F42G8.10 *lys-6*
T01G6.10	T01G6.10	79	1.22	3	*acs-17* R03G5.5 ZK177.3
C18H7.4	C18H7.4	80	1.22	3	*mpk-2 rom-2* T19D12.5
nhr-144	F59E11.12	72	1.28	3	C08F11.1 C48B4.1 Y45F10D.2
nlp-16	T13A10.5	29	1.88	3	*acs-17 exp-2* F45G2.2
pqn-42	F53G2.4	60	1.44	3	C35E7.2 F14F9.4 W03F9.4
rom-2	C48B4.2	76	1.22	3	*mpk-2* C18H7.4 *trk-1*
srd-15	C04E6.10	83	1.12	3	*str-116* F53B7.4 K10C2.2
sue-1	F07A5.5	63	1.41	3	C35E7.2 *glr-5 pqn-98*
glr-1	C06E1.4	95	0.94	2	*trk-1* R11E3.2
F59F3.6	F59F3.6	10	2.52	2	*exp-2 grp-1*
Y51B9A.6	Y51B9A.6	48	1.57	2	C17C3.1 F25D1.5

E02C12.8	E02C12.8	62	1.41	2	E02C12.6 F58B4.5
E02C12.6	E02C12.6	65	1.38	2	E02C12.8 F58B4.5
C27H5.2	C27H5.2	70	1.3	2	*ftn-1 twk-14*
F02C12.2	F02C12.2	78	1.22	2	*acs-17* R03G5.5
F23H11.7	F23H11.7	82	1.17	2	T09B9.3 *pqn-98*
F07C4.12	F07C4.12	84	1.11	2	F46C5.1 T22F3.11
F55B11.4	F55B11.4	85	1.1	2	C05E7.3 T12D8.5
T19D12.5	T19D12.5	86	1.05	2	C18H7.4 *trk-1*
K01C8.1	K01C8.1	87	1.02	2	*cpr-4* F59D6.3
F53B6.2	F53B6.2	90	0.99	2	*exp-2* F45G2.2
nhr-193	F57G8.6	89	1	2	F46C5.1 *lys-5*
pqn-97	ZK488.10	77	1.22	2	T09B9.3 T22F3.11
str-116	F07B10.2	88	1.02	2	C01B12.5 *srd-15*
twk-14	K01D12.4	73	1.25	2	C27H5.2 *pqn-98*
xbx-3	M04D8.6	64	1.4	2	*flp-11 pqn-98*
cwp-4	K11D12.1	51	1.51	1	*acs-17*
grp-1	K06H7.4	75	1.25	1	F59F3.6
Y37D8A.18	Y37D8A.18	33	1.79	1	F09F7.4
F22E5.9	F22E5.9	91	0.99	1	D2023.4
C09D4.1	C09D4.1	92	0.99	1	*exp-2*
R13H7.2	R13H7.2	93	0.95	1	*pqn-98*
Y45F10D.2	Y45F10D.2	94	0.95	1	*nhr-144*
F55A4.7	F55A4.7	96	0.93	1	*pqn-98*
C12D5.3	C12D5.3	97	0.89	1	*pqn-98*
F49C12.14	F49C12.14	98	0.88	1	*lys-6*
F43C9.1	F43C9.1	99	0.87	1	*lys-10*
F44D12.9	F44D12.9	100	0.85	1	F58B4.5
B0391.10	B0391.10	101	0.84	1	*acs-17*
F44A6.5	F44A6.5	102	0.82	1	DH11.2
K08D10.10	K08D10.10	103	0.82	1	*pqn-98*
C25F9.6	C25F9.6	104	0.81	1	*pqn-98*
F42G8.10	F42G8.10	105	0.81	1	D2023.4
C01B12.5	C01B12.5	106	0.81	1	*str-116*
K10C2.2	K10C2.2	107	0.79	1	*srd-15*
F15E11.4	F15E11.4	109	0.79	1	*str-180*
F53B7.4	F53B7.4	110	0.79	1	*srd-15*
F35A5.2	F35A5.2	111	0.78	1	*pqn-98*
F53A9.2	F53A9.2	112	0.78	1	F08G2.5
K11H12.4	K11H12.4	113	0.78	1	*mpk-2*
ZK177.3	ZK177.3	114	0.78	1	T01G6.10
R11E3.2	R11E3.2	116	0.76	1	*glr-1*

Y113G7B.14	Y113G7B.14	117	0.67	1	*trk-1*
Y37A1B.5	Y37A1B.5	118	0.48	1	T05F1.11
prx-3	C15H9.8	115	0.77	1	C48B4.1
str-180	T10H9.6	108	0.79	1	F15E11.4

WormNet v2 was queried with all 395 unique significantly differentially expressed genes shown in Figure 3.1 and Table A.7. 118 of the 395 differentially expressed genes had putative connections and are listed by the number of connections in the probabilistic functional gene network model. The WormNet rank and score are listed for each gene. C = Number of genes that are connected. WormNet linkages are probability based on a modified Bayesian integration of likelihood scores from individual datasets. Likelihood score and rank (based on score) are included for each gene. Genes that were predicted to have a functional linkage to the listed gene hub are in the corresponding linked genes column.

Table 3.2 Survival of wild-type nematodes versus gene candidate mutants on *S. maltophilia* JCMS, K279a or *E. coli* OP50.

***S. maltophilia* JCMS**					
Genotype	**M**	**SE**	**N**	**Hazard Ratio**	**p value**
wildtype (WT)	4.6	0.105	56	N/A	N/A
lys-6(ok2075)	4.2	0.092	60	1.455	0.0453
mpk-2(ok219)	4.3	0.082	58	1.495	0.043
clec-67(ok2770)	4.0	0.086	58	2.016	4.5E-04
cpr-4(ok3413)	3.9	0.108	58	2.123	1.6E-04
acs-17(ok1562)	4.5	0.146	59	1.0	0.962
dod-22(ok1918)	4.7	0.134	59	1.02	0.919
C48B4.1*(ok2619)*	4.1	0.116	59	1.75	0.0032
***S. maltophilia* K279a**					
Genotype	**M**	**SE**	**N**	**Hazard Ratio**	**p value**
wildtype (WT)	8.7	0.4	58	N/A	N/A
lys-6(ok2075)	8.8	0.39	58	0.887	0.527
mpk-2(ok219)	8.1	0.37	56	1.26	0.216
clec-67(ok2770)	9.0	0.31	56	1.07	0.727
cpr-4(ok3413)	9.8	0.33	56	0.788	0.204
acs-17(ok1562)	10	0.29	53	0.738	0.118
dod-22(ok1918)	10	0.47	59	0.63	0.0132
C48B4.1*(ok2619)*	10	0.36	58	0.713	0.0734
***E. coli* OP50**					
Genotype	**M**	**SE**	**N**	**Hazard Ratio**	**p value**
wildtype (WT)	8.3	0.33	59	N/A	N/A
lys-6(ok2075)	9.3	0.41	59	0.65	0.027
mpk-2(ok219)	8.6	0.37	59	0.9	0.561
clec-67(ok2770)	8.8	0.4	59	0.83	0.317
cpr-4(ok3413)	9.3	0.44	59	0.64	0.0208
acs-17(ok1562)	13.0	0.38	56	0.16	3.6E-14
dod-22(ok1918)	9.5	0.45	59	0.61	0.013
C48B4.1*(ok2619)*	8.7	0.42	59	0.77	0.184

p values are given for the survival predictor of treatment (mutant nematode genotype) for Cox proportional hazard models in R. Hazard ratios represent the hazard of the treatment divided by the control (wild-type) of the same bacteria. A hazard is the probability that a nematode at a given time dies. p values less than 0.05 were considered significant. Number of nematodes tested= N. M = mean survival units (days).

Chapter 4 - The identification of *S. maltophilia* virulence factors

Introduction

The bacterium *Stenotrophomonas maltophilia* is Gram negative, ubiquitously found and an emerging nosocomial opportunistic pathogen (Brooke, 2012, Denton *et al.*, 1998, Looney *et al.*, 2009). In nature, these bacteria are found in plant rhizospheres, oil brines, a variety of soil types and a number of water sources including rivers, sewage and wells (reviewed in Denton *et al.*, 1998). *S. maltophilia* infection can be community-acquired (Falagas *et al.*, 2009), but a more recent study revealed that most cases were hospital-acquired with some being health-care associated (Garazi *et al.*, 2012). Like other nosocomial pathogens, *S. maltophilia* are resistant to antibiotics and have been associated with a number of diseases and infections (reviewed in Brooke, 2012 and Denton *et al.*, 1998). Mortality rates vary and range from 14 to 69% in patients with bacteremia (Jang *et al.*, 1992, Victor *et al.*, 1994). *S. maltophilia* also accounts for a small percentage of nosocomial pneumonias (A'Court *et al.*, 1992), the pathogenesis of Crohn's disease and ulcerative colitis (Parent *et al.*, 1978, Parent *et al.*, 1976) and has been found to colonize 30% of patients with cystic fibrosis (Steinkamp *et al.*, 2005). Other common infections/diseases associated with *S. maltophilia* include biliary sepsis, endocarditis, urinary tract, soft tissue, eye, chronic obstructive pulmonary disease, meningitis and patients with obstructive lung cancer (reviewed in Brooke, 2012).

Despite the increasing biomedical importance of *S. maltophilia*, there is still a lot to be learned about their mode of action. As expected for an ever-present pathogen, *S. maltophilia* encodes numerous putative virulence factors. For example, the bacterium produces pili that are implicated in bacterial cell ahseion to epithelial and abiotic surfaces (De Oliveira-Garcia *et al.*, 2003). Other factors include peptidoglycan synthetase, haemagglutinin, LPS O antigen and genes encoding type I, II, IV, V and arginine transporter (TAT) secretion systems (reviewed in Ryan *et*

al., 2009). *S. maltophilia* also produce a range of enzymes that likely play a role in virulence (Bottone *et al.*, 1986), including proteases, elastases, hyaluronidases, DNases, RNases, fibrinolysin, lipases and chitinases (reviewed in Denton *et al.*, 1998 and Ryan *et al.*, 2009). Additionally, *S. maltophilia* can efficiently capture siderophores (iron sequesteration compounds) produced by other microorganisms (Jurkevitch *et al.*, 1992), a process that may be linked to siderophore virulence factor regulation (Lamont *et al.*, 2002). *S. maltophilia* can also potentially defend against protozoa as these bacteria encode refractile inclusion bodies (R bodies) (Ryan *et al.*, 2009) that are toxic to freshwater *Paramecium* (Heruth *et al.*, 1994). Lastly, the cytotoxic effect of exposure to *S. maltophilia* has been demonstrated *in vivo* against several human cell lines (Figueirêdo *et al.*, 2006). Thus, *S. maltophilia* likely produces a deleterious agent that aids in human infection.

Although, *S. maltophilia* has a number of candidate virulence factors, few have been directly shown to be involved in pathogenicity. We and others have found that *S. maltophilia* K279a kills *C. elegans* (Chapter 2 and Fouhy *et al.*, 2007). Briefly, Fouhy et al. found that *S. maltophilia* K279a is lethal to *C. elegans* and required the action of a *rpf*/DSF (diffusible signaling factor)-quorum sensing system (Fouhy *et al.*, 2007). This system is differentially required for virulence in *S. maltophilia* as several strains such as JV3 that contain elements of the *rpf* gene cluster do not require *rpfF* for several different virulence-related phenotypes (Huedo *et al.*, 2014). *S. maltophilia* also requires the Xps type II secretion system to induce death of human lung epithelial cells (Karaba *et al.*, 2013). Both systems are multi-step processes that regulate a number of virulence factors including bacterial secretions (Fouhy *et al.*, 2007, He *et al.*, 2006, Karaba *et al.*, 2013), most of which have yet to be validated individually in this system. However, several bacterial secretions have been implicated in animal pathogenesis. *S.*

maltophilia G2 has toxic activity against the free-living nematode *Panagrellus redivivus* and the pine wood pathogenic nematode *Bursaphelenchus xylophilus* (Huang *et al.*, 2009). This nematotoxic activity is mediated by a serine protease that has the ability to degrade human proteins (Hagemann *et al.*, 2006). Furthermore, the bi-functional mutase SpgM is required for virulence in a rat lung infection model (McKay *et al.*, 2003). Taken together, these studies support the continued evaluation of quorum sensing, secretion systems and secretions in the discovery of *S. maltophilia* virulence factors.

C. elegans is an excellent model for the study of pathogen-host interactions due to the ease of genetic manipulation, natural co-existence with pathogens and its emergence as an innate immunity model for the study of other microbial pathogens (reviewed in Chapter 1). *S. maltophilia* JCMS was isolated in our laboratory, colonizes the gut of *C. elegans* and requires living bacteria to be virulent (Chapter 2). JCMS also evades the *C. elegans* DAF-2/16 pathway but the nematode response to JCMS involves several other conserved innate immune pathways (Chapter 2). Here we have used the nematode model to identify virulence factors for *S. maltophilia* JCMS. We have also included work with the *S. maltophilia* isolate JV3 as a virulent control and to explore bacterial virulence factor specificity. Generally, the Xps type II secretion system is required for *S. maltophilia* virulence while, the DSF (diffusible signaling factor)–quorum-sensing system has a strain specific role. On *S. maltophilia* JCMS, several bacterial secretion encoding genes are required for wild-type nematode survival. Furthermore, the production these secretions help JCMS evade the *C. elegans* DAF-2/16 innate immune pathway.

Materials and methods

Nematode strains

The following *C. elegans* strains containing the following alleles were obtained from the *Caenorhabditis* Genetics Center (C.G.C.): LG III: *daf-2(e1370)* and *daf-2(e1368)*. N2 was also

obtained from the C.G.C. and used as the wild-type strain for survival analysis. This strain is kept frozen and thawed yearly for experimentation.

Bacterial strains and growth

S. maltophilia JCMS was isolated by our laboratory from a culture of *Mesorhabditis sp.* found in soils from Konza Prairie, near Manhattan, KS. The isolation was part of an effort to characterize bacteria associated with native nematodes from Tallgrass prairie soils as described in Chapter 2 of this dissertation. *E. coli* OP50 was obtained from the *Caenorhabditis* Genetics Center, *S. maltophilia* JV3 from J. Tiedje (Michigan State University) and *S. maltophilia* FW from F. White (Kansas State University). All bacterial strains were frozen at - 80˚C upon retrieval and were thawed regularly for use in experimentation. *S. maltophilia* strains are naturally Ampicillin resistant and, were streaked for colony isolation from frozen stock on Luria Broth (LB) agar containing 100μg/mL Ampicillin to selectively prevent growth of other bacterial contaminants. *E. coli* OP50 was streaked on LB agar for colony isolation. For each bacterial strain, liquid LB was inoculated and shaken overnight at 32˚C. Bacterial lawns used for survival were seeded on nematode growth medium (NGM) with bacterial culture at log/lag phase and grown overnight at room temperature.

Bacterial mutagenesis

Candidate virulence factors were selected based on knowledge of virulence factors in other closely related systems, i.e. *Xanthomonas* (Büttner *et al.*, 2010) and previous studies with other *S. maltophilia* strains (Fouhy *et al.*, 2007, Huang *et al.*, 2009, Karaba *et al.*, 2013). The pZPtet-Km vector was generated via the addition of a tetracycline marker gene through the *Xba*I restriction enzyme site in the pKNOCK-Km vector (Alexeyev, 1999). The target genes were PCR amplified by specific primers (Table 4.1) and cloned into the pZPtet-Km vector by *Xho*I and *Kpn*I restriction enzyme sites. The resulting vectors with partial fragments of target genes

were transformed into *E. coli* S17-1 *pir* strain for bacterial conjugation with *S. maltophilia* JV3 or JCMS. The mixture of S17-1 *pir* and JCMS or JV3 was plated on LB agar, grown for 24 hours at 28˚C and transferred to LB plates with 45ug/mL Tetracycline and 10 ug/mL Norfloxacin for selection of specific mutants at 28˚C (Zhang *et al.*, 2000b). Genomic DNA of each candidate mutant was extracted for validation via PCR. The Tet2-Val primer (5'-GGGCTGACTTCAGG TGCTAC-3') is specific to the pZPtet-Km vector and the gene specific primers were located up or down-stream of the cloned fragments. The same primers were used for knock out of *rpfF* and *xps* in JV3 and JCMS. The JCMS and JV3 *ΔrpfF* mutants were generated by first cloning *rpfF* into a TOPO vector (Invitrogen). The partial *rpfF* sequence was then cloned into the pZPtet-Km vector for conjugation by *Xho*I and *Kpn*I restriction digestion of the cloned gene. Multiple deletion alleles were induced for experimental validation of the following genes: *rpfF* (three JCMS and JV3 isolates), *xps* (two JCMS and JV3 isolates) and *arac* (two JCMS isolates). These additional alleles are denoted by number in the appropriate tables.

Nematode survival assays

Nematodes were reared and synchronized as L4s at 20˚C on *E. coli* OP50 lawns. For survival analysis, 10 to 15 L4s were picked onto three to six replicate lawns of the treatment or control bacteria and maintained at 25˚C. The number of surviving nematodes was recorded daily and death was determined by lack of motion in response to prodding with a platinum wire pick. Nematodes were picked to new bacterial lawns for the first five to six days after the start of the experiment to separate them from their progeny. Dead nematodes were removed upon discovery. Sample sizes (N = number of nematodes) varied due to the removal of replicates because of contamination and the removal of specimens that died via means other than the specified bacterial treatment. Such means include desiccation that occurs when nematodes leave the bacterial lawn and die at the plate edge. The presence of contamination was infrequent and

was determined by observing bacterial lawn morphology. Kaplan-Meier estimates of survival over time and survival curve statistics using Cox proportional hazard tests were performed in R (Vienne, Austria: R Foundation for Statistical Computing). Survival curves can be statistically compared using the log-rank and Cox proportion hazard tests. Cox proportion hazard models were used to test the effect of independent variables such as, genotype and bacteria on the hazard, a dependent variable defined as the probability of dying at a given time (Goel *et al.*, 2010). The model used for analysis is indicated in the legends of the relevant tables. Models were evaluated by testing for a non-zero slope and visualizing the Schoenfeld residuals (UCLA: Statistical Consulting Group). A non-zero slope is an indication of proportional hazard assumption violation and models were fit to the data aiming to meet that assumption.

Accessions

The full-length *S. maltophilia* JCMS 16S rRNA gene sequence was deposited in GenBank with accession number KF724885.

Results

rpfF and *xps* are *S. maltophilia* JCMS virulence factors

Through the course of our studies, we have found that *S. maltophilia* JCMS and JV3 are the most virulent *S. maltophilia* strains tested in our hands (Figure 4.1 and Table A.13). Of the virulent *S. maltophilia* isolates, we chose to focus primarily on the nematode interaction with JCMS due to the demonstrated evasion of the *C. elegans* DAF-2/16 innate immune pathway (Chapter 2). *S. maltophilia* isolate JV3 was used as a positive control and to explore bacterial virulence factor specificity. Given that the *rpfF* gene has been detected in a number of *S. maltophilia* isolates (Huedo *et al.*, 2014) and is required for the virulence of *S. maltophilia* K279a (Fouhy *et al.*, 2007), we hypothesized that this gene was also present and involved in the virulence of *S. maltophilia* JCMS. As expected, knock out of *rpfF* in JCMS significantly

extended wild-type nematode survival (Figure 4.1A, Table 4.2) and the ability to hydrolyze casein (Figure 4.2A). Thus, the *rpf*/DSF (diffusible signaling factor)-quorum sensing system is required for JCMS virulence and the production of the proteolytic enzyme casease. As suggested via sequence data in a previous study (Huedo *et al.*, 2014), *S. maltophilia* JV3 did not require *rpfF* for virulence in the nematode model (Figure 4.1A, Table 4.2). Furthermore, the production of casease in JV3 *ΔrpfF* mutants was only slightly affected (Figure 4.2B) and the growth phenotype of JCMS and JV3 *ΔrpfF* mutants in liquid nematode growth medium (NGM) was vastly different (Figure 4.2C, D). Thus, deletion of *rpfF* in JCMS has pleiotropic effects and the role of quorum sensing in *S. maltophilia* virulence is strain specific.

The role of *rpfF* in JCMS virulence becomes even more evident when comparing the *ΔrpfF* mutant to *wildtype* hazard ratio. Briefly, a hazard is the probability that an individual nematode dies at a given time on a bacterial treatment. A ratio close to one indicates an equal hazard for the compared treatments i.e. bacterial mutant versus wild-type bacteria. A ratio that deviates greatly from one indicates a large difference between the treatment and control. Furthermore, these deviations from one can be compared in order to assess the amount of involvement or role a mutated gene has in the death of the nematode. The JCMS *ΔrpfF2* mutant isolate to *wildtype* hazard ratio was 0.04 (Table 4.2), which deviates greatly from one and supports a large role for the *rpf*/DSF (diffusible signaling factor)-quorum sensing system in JCMS virulence (Figure 4.2A).

The type II secretion outer-membrane protein Xps and an AraC family transcription regulator have been previously implicated in bacterial virulence (Frota *et al.*, 2004, Iwobi *et al.*, 2003, Tyson *et al.*, 2013) and were promising putative *S. maltophilia* virulence factors due to their interaction with many genes involved in virulence (Gallegos *et al.*, 1997, Rossier *et al.*,

2004, Yang *et al.*, 2011). Surprisingly, the evaluation of multiple *Δarac* mutant isolates revealed that AraC was not required for *S. maltophilia* JCMS virulence (Table 4.2). Thus, AraC is not a JCMS virulence factor and the regulation of bacterial genes downstream of AraC is not necessary for *S. maltophilia* JCMS pathogenicity. The Xps type II secretion system has previously been implicated in *S. maltophilia* K279a virulence (Karaba *et al.*, 2013). Thus, we hypothesized that *xps* was required for *S. maltophilia* JV3 and JCMS virulence. As anticipated, loss of the *xps* gene in *S. maltophilia* JCMS and JV3 significantly extended wild-type nematode survival for all *Δxps* mutants tested (Figure 4.1B, Table 4.2). The JCMS *Δxps1* mutant isolate to *wildtype* hazard ratio was 0.085 versus 0.409 for the JV3 *Δxps1* mutant isolate ratio, indicating a greater role for the Xps type II secretion system in JCMS virulence.

We also choose to induce several additional mutant alleles in JCMS in order to survey the involvement of bacterial secretions in *S. maltophilia* virulence. The candidate genes and their encoded secretions are as follows: *cs* (cyclolysin secretion ATP-binding protein), *p773* (extracellular protease), *p1176* (protease), *xdi* (isoaspartyl aminopeptidase) and *pi1y1* (type IV fimbrial biogenesis protein). Briefly, the bifunctional cyclolysin has adenylate cyclase and haemolysin activities that are both deemed *Bordetella pertussis* virulence factors (Weiss *et al.*, 1986) and aminopeptidase activity is required for *P. aeruginosa* infection (Luckett *et al.*, 2012). Proteolytic activity is well known to be involved in bacterial virulence (reviewed in Lebrun *et al.*, 2009) and type IV fimbriae is a *Dichelobacter nodosus* virulence factor and is required for protease secretion (Han *et al.*, 2007, Kennan *et al.*, 2001). Additionally, protease activity has also been implicated in *S. maltophilia* nematode pathogenesis (Huang *et al.*, 2009). Given the validation of protease, cyclolysin, fimbrial biogenesis protein and aminopeptidase as virulence factors in other systems, we hypothesized that these factors would also be required for *S.*

maltophilia JCMS virulence. To our surprise, JCMS *Δcs*, *Δp773*, *Δp1176*, and *Δxdi* mutants were significantly more virulent to wild-type nematodes and Δ*pi1y1* mutants had the same virulence as wild-type bacteria (Figure 4.1C and Table 4.2). The hazard ratios for these hyper-virulent JCMS mutants ranged from 3.98 to 2.67 with *Δp1176* and *Δp773* mutants being the most and least detrimental to nematodes. Of note, we have tested the crude protease activity of JCMS and other *S. maltophilia* strains and this activity does not correlate with pathogenicity (Figure A.6). Taken together, these data provide evidence that *cs*, *p773*, *p1176* and *xdi* do not encode JCMS factors that potentiate virulence.

Several factors are required for *S. maltophilia* JCMS evasion of DAF-2/16 signaling

In the course of our studies, we have discovered that *S. maltophilia* JCMS is virulent to normally pathogen resistant *daf-2* mutants (Chapter 2) while, *daf-2* mutants are resistant to *S. maltophilia* K279a, R551-3, JV3 and FW (Table A.13). The longevity of *daf-2* mutants was different for each *S. maltophilia* isolate, with nematode lifespan being shorter on the more virulent strains. Additionally, bacterial virulence is known to change when bacteria are grown on fast-killing (PGS) (Tan *et al.*, 1999a) or enriched medium (BHI)(Garsin *et al.*, 2001). Generally, *daf-2* mutants were long-lived on most bacteria regardless of growth medium (Table A.13). Of note, *daf-2* mutants were also not long-lived on JCMS when these bacteria were grown on PGS medium but were significantly long-lived on BHI (Table A.13). This result is likely attributed to the observed difference in *daf-2* mutant survival when these and other bacteria were grown on BHI medium (Table A.12). In sum, *daf-2* mutants are long-lived on all tested *S. maltophilia* strains except JCMS and the longevity of these mutants depends on strain virulence and growth medium.

Since we observed that only JCMS was virulent to *daf-2* mutants, we assessed the effects of each JCMS mutant isolate in a *daf-2* background. As with wild-type nematodes (Figure 4.1B

and Table 4.2), the *xps* gene was required for *S. maltophilia* JCMS and JV3 virulence (Table 4.3). On the other hand, *rpfF* is required for virulence to *daf-2* nematodes for both JCMS and JV3. The *Δxps* and *ΔrpfF* mutant isolate hazard ratios for JCMS and JV3 were as follows: JCMS *ΔrpfF2* 0.027, JV3 *ΔrpfF2* 0.342, JCMS *Δxps1* 0.24 and JV3 *Δxps1* 0.485 (Table 4.3). As for wild-type nematodes fed *Δxps* mutant isolates, the JCMS *Δxps* and *ΔrpfF* mutant isolate hazard ratios deviate more from one than on JV3 mutants. These results suggest that the Xps type II secretion and *rpf*/DSF (diffusible signaling factor)-quorum sensing systems play a greater role in JCMS than in JV3 induced *daf-2* mutant killing. Furthermore, the requirement for quorum sensing in JV3 virulence changes with nematode genetic background. Additionally, and also not observed for wild-type nematodes, JCMS *arac* is required for *daf-2* mutant nematode virulence. Thus, the role of AraC regulated genes in JCMS also changes with nematode genetic background. Lastly, all of the JCMS mutants that were hyper-virulent to wild-type nematodes were also hyper-virulent to *daf-2* mutants. Intriguingly, almost all mutant to wild-type JCMS and JV3 hazard ratios for *daf-2* mutants deviated more from one than for wild-type nematodes. For example, the hazard ratio for JCMS *Δp1176* mutants was 3.98 in wild-type nematodes and 8.78 in *daf-2* mutants (Table 4.2 and 4.3). Generally, the hyper-virulent JCMS mutant hazard ratios ranged from 2.1 to 8.87 for *daf-2* mutants compared to the 2.67 to 3.98 range for wild-type nematodes. The hazard ratio for loss of *rpfF2* in JCMS was 0.027 for *daf-2* mutants compared to 0.04 for wild-type nematodes. Conversely, the hazard ratio for loss of *xps1* in JCMS and JV3 was 0.24 and 0.485 for *daf-2* mutants compared to 0.085 and 0.409 in wild-type nematodes. Thus, in JCMS, quorum sensing, AraC transcriptional regulation and several bacterial secretions (cyclolysin secretion ATP-binding protein, proteases and aminopeptidase) play a greater role in *daf-2* mutants and Xps type II secretion has a greater role in wild-type nematodes. This statement

also holds true for quorum sensing and Xps type II secretion in *S. maltophilia* JV3 suggesting that this nematode genetic background dependent change in role is conserved across *S. maltophilia* strains.

To determine whether *daf-2* mutant longevity was affected by bacterial mutant background, we compared the survival of *daf-2* mutants and wild-type nematodes on each bacterial mutant. As seen for wild-type JCMS (Chapter 2), *daf-2* mutants and wild-type nematodes had the same survival on all *ΔrpfF* mutant isolates (Figure 4.3A, Table 4.4). Thus, quorum sensing in JCMS is not involved in the JCMS induced loss of *daf-2* mutant resistance. The Xps type II secretion system and AraC are also not involved. In JV3, knock-out of *rpfF* and *xps* did not affect *daf-2* resistance and the *daf-2* mutant to wild-type nematode hazard ratios were comparable between wild-type bacteria and JV3 *ΔrpfF* and *Δxps* isolates (Figure 4.3B, Table 4.4, A.13). Thus, the longevity exhibited by *daf-2* mutants on JV3 does not involve quorum sensing and Xps type II secretion. However, a significant increase in *daf-2* mutant survival was observed on JCMS *Δp1176*, *Δcs*, *Δxdi*, *Δp773* and *Δpi1y1* mutants (Figure 4.3C, Table 4.4). Therefore, cyclolysin secretion ATP-binding protein, proteases, aminopeptidase and type IV fimbrial biogenesis protein are all involved in JCMS evasion of the DAF-2/16 pathway. The *daf-2* mutant to wild-type nematode hazard ratio on JCMS for knockout of these secretion encoding genes ranged from 0.546 to 0.347 with loss of *p773*, a protease encoding gene, having the largest role.

Discussion

Quorum sensing is a cell communication process that allows bacteria to coordinate population behavior. Elements of this system are associated with virulence in several bacterial-nematode interactions (Gallagher *et al.*, 2001, Garsin *et al.*, 2001, Tan *et al.*, 1999b). Quorum sensing in *S. maltophilia* is known to depend on the DSF (diffusible signal factor) (Huang et al., 2007) which is regulated by the enoyl coenzyme A hydratase RpfF (Barber et al., 1997). We

observed that *S. maltophilia* JCMS *ΔrpfF* mutants were significantly less virulent to wild-type nematodes (Figure 4.1A, Table 4.2). JCMS *ΔrpfF* mutants also had an aggregative growth phenotype that was distinct from wild-type bacteria and a reduced production of protease (Figure 4.2A,C). Thus, although we did not find *S. maltophilia* K279a to be pathogenic (Chapter 2), RpfF was previously demonstrated to be a virulence factor for *S. maltophilia* K279a (Fouhy *et al.*, 2007), and here we found that is it a virulence factor for JCMS with loss of function causing pleiotropic effects. On the other hand, we have found that *S. maltophilia* JV3 does not require *rpfF* for virulence in wild-type nematodes or protease secretion (Figure 4.1A, Figure 4.2B and Table 4.2). Taken together, these findings and previous work (Huedo et al., 2014) support a strain specific role for DSF-quorum sensing in *S. maltophilia* pathogenicity.

The type II secretion (T2S) outer membrane pore forming protein Xps was also previously implicated in *S. maltophilia* pathogenesis (Karaba *et al.*, 2013). Here we show that *xps* is a virulence factor for *S. maltophilia* JCMS and JV3 in the nematode model (Figure 4.1B, Table 4.2). Furthermore, the *Δxps* mutant isolate to wild-type bacteria hazard ratio deviates more from one for wild-type nematodes on JCMS than JV3, indicating that Xps plays a larger role in JCMS virulence. In *S. maltophilia* K279a, The Xps T2S regulates several proteins including some with proteolytic activity that are hypothesized to be virulence factors (Karaba *et al.*, 2013). Intriguingly, the protease encoding genes *p773* and *p1176* are not involved in JCMS virulence (Figure 4.1C and Table 4.2). Additionally, the removal of the bacterial secretion encoding genes *cs* and *xdi* also do not attenuate JCMS virulence. Furthermore, the JCMS mutant to *wildtype* hazard ratios revealed that loss of the bacterial gene *p1176* was the most detrimental to nematodes followed by *cs*, *xdi* and *p773* (Table 4.2). Thus, *cs*, *xdi*, *p1176* and *p773* are involved

in the JCMS-nematode interaction but further investigation is required to determine the mechanism behind the requirement of these genes for wild-type nematode survival.

We have discovered that *S. maltophilia* JCMS uniquely evades the effects that produce *daf-2* mutant longevity in nematodes (Chapter 2). Other *S. maltophilia* strains are not virulent to *daf-2* mutants and *daf-2* mutants have different levels of resistance to these strains that correlate with bacterial virulence (Table A.13). Here we investigated the effect of several bacterial mutations on *C. elegans daf-2* mutant longevity in order to gain insight on what genes are required for JCMS-specific DAF-2/16 pathway evasion. RpfF and/or Xps conferred JCMS and JV3 virulence is independent of *C. elegans daf-2* (Figure 4.3A, B and Table 4.4). In support of this conclusion, the *Δxps* mutant isolate to wild-type JV3 and JCMS hazard ratio was greater for wild-type nematodes than for *daf-2* mutants (Table 4.2 and 4.3). However, *rpfF* has a greater role in *daf-2* mutants than in wild-type nematodes on both *S. maltophilia* strains. Additionally, loss of the AraC transcriptional regulator in JCMS only negatively affected *daf-2* mutant nematodes (Tables 4.2 and 4.3) but, *arac* was not involved in JCMS induced loss of *daf-2* resistance (Table 4.4). Perhaps, this set of observations is linked to the roles of DAF-2/16 signaling in aging, longevity and innate immunity (Evans *et al.*, 2008a, Garsin *et al.*, 2003, Gems *et al.*, 1998). However, the data support a bacteria and nematode gene by gene interaction and additional research is needed to confirm and determine why certain *S. maltophilia* virulence factors are unique to *daf-2* mutants.

In JCMS, the bacterial genes *cs*, *p773*, *p1176* and *xdi* also played a greater role in a *daf-2* mutant background. Additionally, JCMS *Δp1176*, *Δcs*, *Δxdi* and *Δp773* mutants all confer *daf-2* resistance (Figure 4.3C, Table 4.4). Loss of JCMS *pi1y1* did not cause a significant difference in survival for *daf-2* mutants or wild-type nematodes (Table 4.2 and 4.3). However, *daf-2* mutants

are also significantly long lived on JCMS *Δpi1y1* mutants (Table 4.4). Thus, the tested protease, cyclolysin, aminopeptidase and a type IV fimbrial biogenesis protein encoding genes are not virulence factors but, help JCMS evade the *C. elegans* DAF-2/16 pathway. The potential involvement of these genes in *C. elegans* innate immune response evasion reveals an additional class of *S. maltophilia* factors that are involved in the bacterial-nematode interaction and not directly in pathogenicity.

Figures

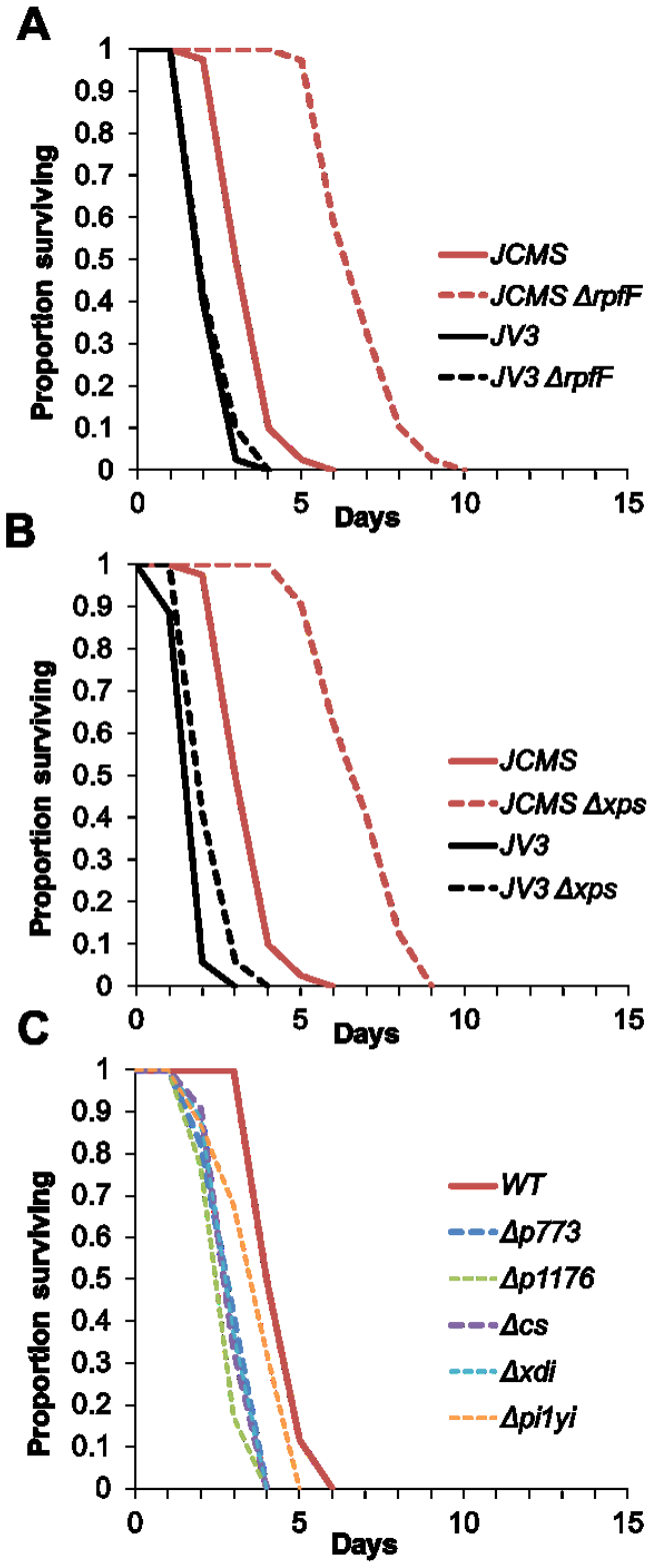

A
Proportion surviving
Days
JCMS
JCMS ΔrpfF
JV3
JV3 ΔrpfF
B
Proportion surviving
Days
JCMS
JCMS Δxps
JV3
JV3 Δxps
C
Proportion surviving
Days
WT
Δp773
Δp1176
Δcs
Δxdi
Δpi1yi

Figure 4.1 *rpfF* and *xps* are *S. maltophilia* JCMS virulence factors.

Survival of wild-type nematodes fed *S. maltophilia* JCMS (solid red lines), JV3 (solid black lines) *wildtype* or mutant bacteria (dashed lines). A) JCMS *ΔrpfF2* (red dashed lines), JV3 *ΔrpfF2* (black dashes lines) mutants. B) JCMS *Δxps1* (red dashed lines) and JV3 *Δxps1* (black dashed lines) mutants. C) JCMS *Δp773* (blue), *Δp1176* (green), *Δcs* (purple), *Δxdi* (light blue), *Δpi1y1* (orange) mutants. Results plotted are the proportion of surviving worms using Kaplan-Meier estimates for at least three replicate samples (10 to 15 nematodes per replicate) of the same nematode population. p values from the application of Cox proportional hazards models and sample sizes of each population are included in Table 4.2. Deletion of JCMS *rpfF* and *xps* significantly extends nematode survival while, deletion of JCMS *p773*, *p1176*, *cs* and *xdi* shortens survival. JCMS *pi1yi* and JV3 *rpfF* mutants do not affect nematode survival and deletion of JV3 *xps* extends survival.

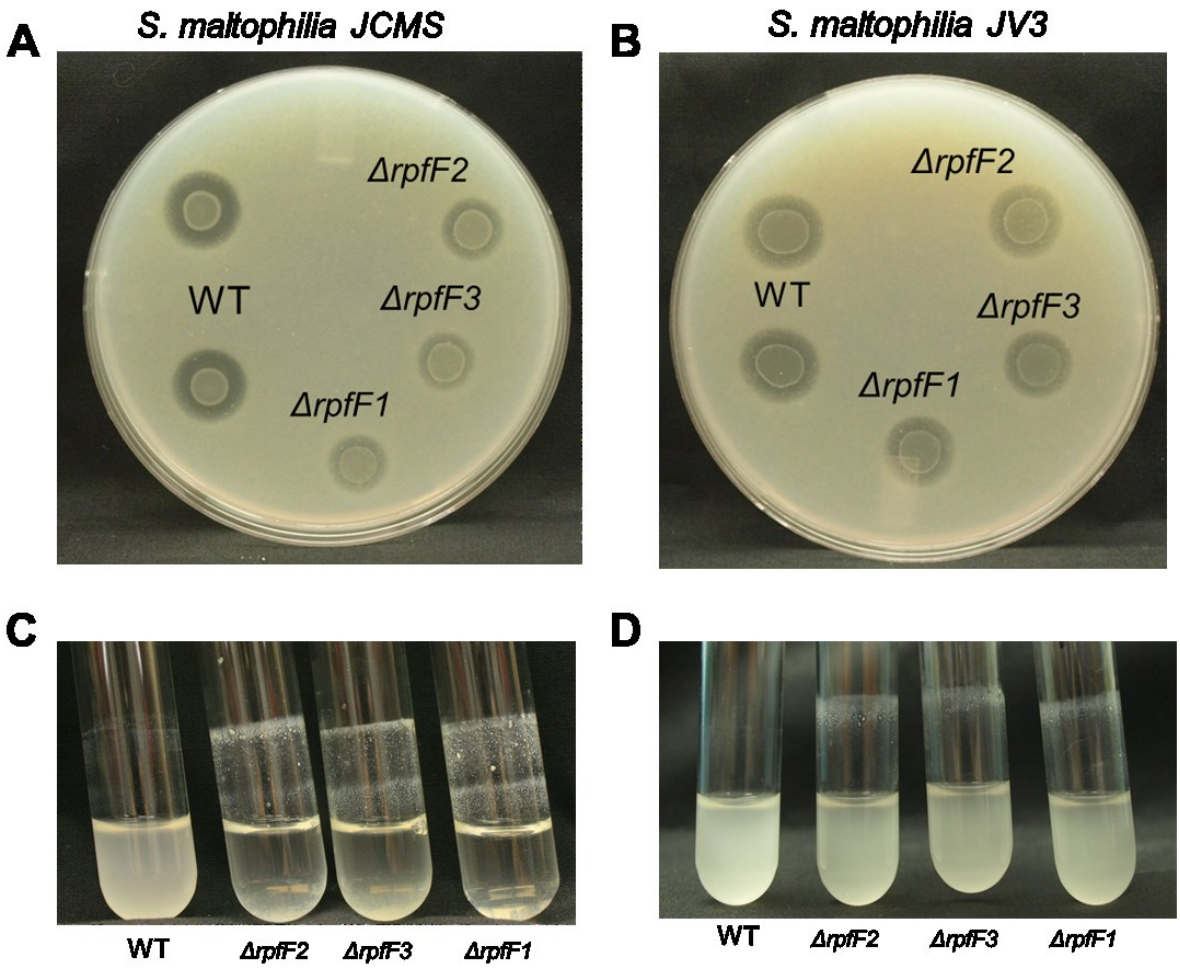


Figure 4.2 *S. maltophilia* JCMS *ΔrpfF* mutants have pleiotropic effects.

S. maltophilia JCMS (A and C) and JV3 (B and D) were inoculated and grown for 24 hours on skim milk agar plates (A and B) or grown in liquid nematode growth medium (NGM) (C-D). Two replicates of each plate and inoculum were completed. JCMS *ΔrpfF* mutants have reduced casease activity and an aggregative growth phenotype in liquid NGM. JV3 *rpfF* does not control casease production or substantially affect growth.

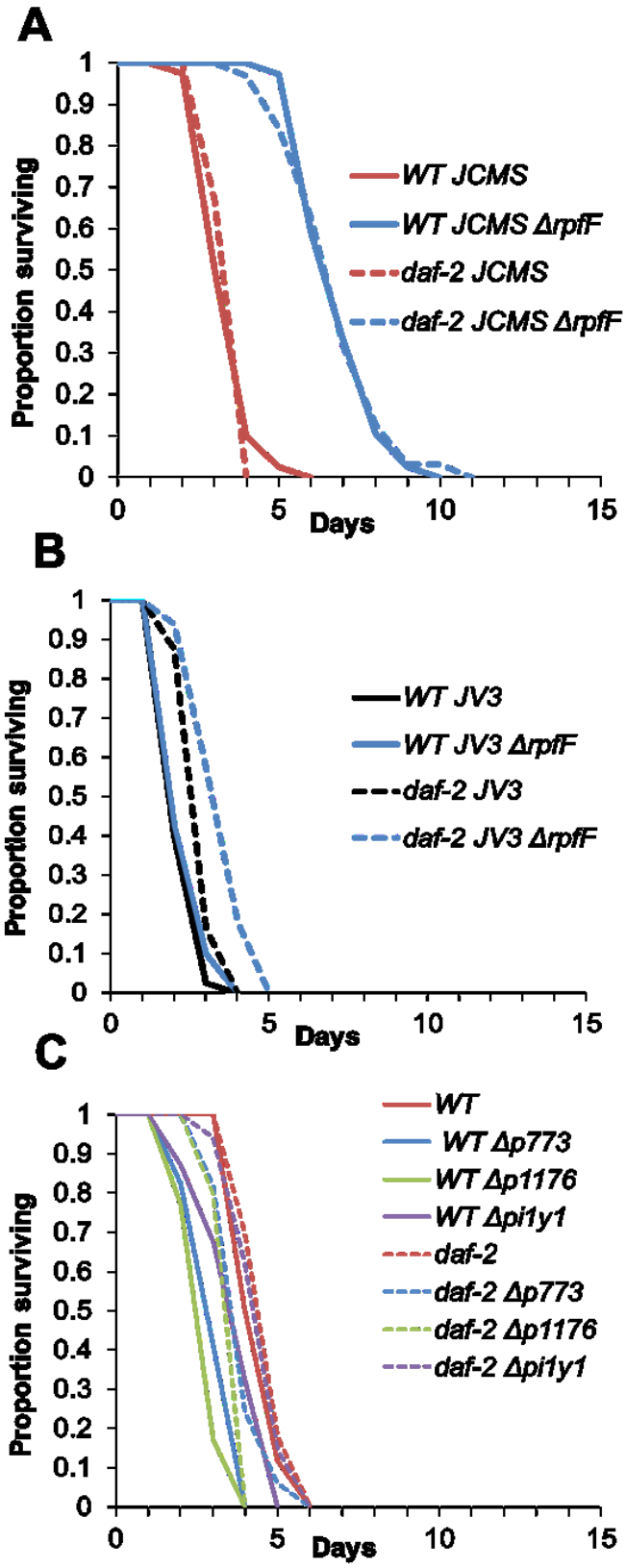

A
Proportion surviving
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
5
10
15
Days
WT JCMS
WT JCMS ΔrpfF
daf-2 JCMS
daf-2 JCMS ΔrpfF
B
Proportion surviving
Days
WT JV3
WT JV3 ΔrpfF
daf-2 JV3
daf-2 JV3 ΔrpfF
C
Proportion surviving
Days
WT
WT Δp773
WT Δp1176
WT Δpi1y1
daf-2
daf-2 Δp773
daf-2 Δp1176
daf-2 Δpi1y1

Figure 4.3 *S. maltophilia* JCMS *p773*, *p1176* and *pi1y1* promote DAF-2/16 evasion.

A) Survival of wild-type nematodes (solid lines) or *daf-2* mutants (dashed lines) on *S. maltophilia* JCMS wild-type (red) or *ΔrpfF2* mutants (blue). B) Survival of wild-type nematodes (solid lines) or *daf-2* mutants (dashed lines) on *S. maltophilia* JV3 wild-type (black) or *ΔrpfF2* mutants (blue). C) Survival of wild-type nematodes (solid lines) or *daf-2* mutants (dashed lines) on *S. maltophilia* JCMS wild-type (red), *Δp773* mutants (blue), *Δp1176* mutants (green) and *Δpi1y1* mutants (purple). Results plotted are the proportion of surviving worms using Kaplan-Meier estimates for at least three replicate samples (10 to 15 nematodes per replicate) of the same nematode population. p values from the application of Cox proportional hazards models and sample sizes of each population are included in Table 4.4. JCMS and JV3 *rpfF* does not affect *daf-2* longevity but, feeding on JCMS *p773*, *p1176* and *pi1yi* confers *daf-2* mutant resistance.

Tables

Table 4.1 Bacterial mutagenesis primers.

Target gene	Gene description	Forward primer	Reverse primer	Out primer
cs	Cyclolysin secretion ATP-binding protein	5'-aataggtaccttg atggcaagctttcactg-3'	5'-atatctcgaggc ccactttggtgtcgtaac-3'	5'-ctatgtgaatgctggc ttcg-3'
pi1y1	Type IV fimbrial biogenesis protein	5'-aataggtaccccat caacaagctcagcaac -3'	5'- atatctcgagcag cgaattgcacttgtcac- 3'	5' - cgatccagaactttg ccaac -3'
arac	Transcriptional regulator, AraC family	5'-aataggtacctca gtgagttcaccctgagc- 3'	5'-atatctcgaggca tggagagatagcggttc-3'	5'-atcagttgcggtt tccattg -3'
xdi	Isoaspartyl aminopeptidase	5'- aataggtaccagg acgatccgaccttcaac- 3'	5'-atatctcgagatt ggcctcttccttcagc- 3'	5'-ctgtccctgccat tgctt- 3'
p773	Extracellular protease	5'-aataggtaccgta ctgcgttcaatgccaag- 3'	5'-atatctcgagccg ttgtaggacgcataggt-3'	5'-cgatctcaatgcca acatcc- 3'
p1176	Protease	5'-aataggtaccacc taccaggatgcgatcag-3'	5'-atatctcgaggttt ccttcagcagggtacg-3'	5'-gtggctacctctc cgacatc- 3'
xps	Type II secretion outermembrane pore forming protein	5'-aataggtaccgcct gttctcctacgagctg -3'	5'-atatctcgagacc agttcacgccgtacttc -3'	5'-tcttcgatgtcgact ggttg-3'
rpfF	Enoyl coenzyme A hydratase	5'-caccgcacctggccgag aag -3'	5' -ccatggtgcgcagcg aacgg -3'	5' -tgacctggacctgtt cacc -3'

List of *S. maltophilia* gene targets for mutagenesis and their corresponding primers.

Table 4.2 *rpfF* and *xps* are *S. maltophilia* JCMS virulence factors in wild-type nematodes.

***S. maltophilia* JCMS**

Control	M	SE	N	Treatment	M	SE	N	Hazard ratio	p value
JCMS WT	3.6	0.12	39	JCMS *ΔrpfF1*	7.3	0.308	34	0.053	9.02E-11
JCMS WT	3.6	0.12	39	JCMS *ΔrpfF2*	6.7	0.107	37	0.04	2.22E-16
JCMS WT	3.6	0.12	39	JCMS *ΔrpfF3*	7.9	0.19	35	0.018	2.01E-12
JCMS WT	4.6	0.12	34	JCMS *Δxps1*	6.7	0.15	32	0.085	5.90E-11
JCMS WT	4.6	0.12	34	JCMS *Δxps2*	6.4	0.22	33	0.119	3.36E-09
JCMS WT	4.6	0.12	34	JCMS *Δarac2*	4.2	0.09	33	1.86	0.0163
JCMS WT	4.6	0.12	34	JCMS *Δarac3*	4.4	0.11	33	1.41	0.18
JCMS WT	3.9	0.096	30	JCMS *Δcs*	3.2	0.105	33	2.92	0.000157
JCMS WT	3.9	0.096	30	JCMS *Δp773*	3.2	0.13	34	2.67	0.000443
JCMS WT	3.9	0.096	30	JCMS *Δp1176*	2.9	0.107	35	3.98	1.52E-06
JCMS WT	3.9	0.096	30	JCMS *Δpi1y1*	3.9	0.18	31	0.934	0.791
JCMS WT	3.9	0.096	30	JCMS *Δxdi*	3.3	0.11	30	2.78	0.000244

***S. maltophilia* JV3**

Control	M	SE	N	Treatment	M	SE	N	Hazard ratio	p value
JV3 WT	2.4	0.078	39	JV3 *ΔrpfF1*	2.6	0.091	35	0.687	0.116
JV3 WT	2.4	0.078	39	JV3 *ΔrpfF2*	2.5	0.088	39	0.792	0.318
JV3 WT	2.4	0.078	39	JV3 *ΔrpfF3*	2.8	0.079	39	0.526	0.0064
JV3 WT	1.9	0.069	35	JV3 *Δxps1*	2.4	0.093	34	0.409	0.0006
JV3 WT	1.9	0.069	35	JV3 *Δxps2*	2.3	0.11	35	0.449	0.0021

Control: wild-type nematodes fed wild-type *S. maltophilia* JCMS and JV3. Treatment: wild-type nematodes fed various *S. maltophilia* JCMS and JV3 mutants. Numbers following each gene name indicate independently generated deletion alleles. p values are given for the survival predictor of treatment (bacterial mutation) for Cox proportional hazard models in R. p values less than 0.05 were considered significant. Hazard ratios represent the hazard of the treatment divided by the control bacteria of the same row. Number of nematodes tested = N. Mean = mean survival units (days).

Table 4.3 *S. maltophilia* JCMS and JV3 require *rpfF* and *xps* for virulence in a *daf-2* background.

***S. maltophilia* JCMS**

Control	M	SE	N	Treatment	M	SE	N	Hazard ratio	p value
JCMS WT	3.9	0.104	27	JCMS *ΔrpfF1*	7.8	1.8	35	0.0125	1.9E-08
JCMS WT	3.9	0.104	27	JCMS *ΔrpfF2*	6.5	0.13	30	0.027	9.66E-10
JCMS WT	3.9	0.104	27	JCMS *ΔrpfF3*	7.6	0.28	37	0.0264	2.37E-11
JCMS WT	4.9	0.12	33	JCMS *Δxps1*	6	0.14	31	0.24	1.06E-06
JCMS WT	4.9	0.12	33	JCMS *Δxps2*	6.1	0.16	33	0.242	1.7E-06
JCMS WT	4.9	0.12	33	JCMS *Δarac2*	4.2	0.1	33	2.69	0.000253
JCMS WT	4.9	0.12	33	JCMS *Δarac3*	4	0.01	35	3.33	9.83E-06
JCMS WT	4.9	0.12	33	JCMS *Δcs*	3.8	0.073	34	8.87	1.48E-08
JCMS WT	4.9	0.12	33	JCMS *Δp773*	4.1	0.13	33	2.1	0.00329
JCMS WT	4.9	0.12	33	JCMS *Δp1176*	3.8	0.068	35	8.78	1.58E-08
JCMS WT	4.9	0.12	33	JCMS *Δpi1y1*	4.7	0.14	34	1.14	0.585
JCMS WT	4.9	0.12	33	JCMS *Δxdi*	3.8	0.067	33	8.63	2.5E-08

***S. maltophilia* JV3**

Control	M	SE	N	Treatment	M	SE	N	Hazard ratio	p value
JV3 WT	3.04	0.12	23	JV3 *ΔrpfF1*	3.4	0.15	20	0.5806	0.0818
JV3 WT	3.04	0.12	23	JV3 *ΔrpfF2*	3.7	0.11	31	0.342	0.00041
JV3 WT	3.04	0.12	23	JV3 *ΔrpfF3*	3.6	0.102	32	0.422	0.00314
JV3 WT	2.4	0.11	42	JV3 *Δxps1*	3.1	0.084	42	0.485	0.00107
JV3 WT	2.4	0.11	42	JV3 *Δxps2*	3.2	0.084	41	0.458	0.00046

Control: *daf-2(e1368)* mutant nematodes fed wild-type *S. maltophilia* JCMS and JV3. Treatment: *daf-2(e1368)* mutant nematodes fed various *S. maltophilia* JCMS and JV3 mutants. Numbers following each gene name indicate independently generated deletion alleles. p values are given for the survival predictor of treatment (bacterial mutation) for Cox proportional hazard models in R. p values less than 0.05 were considered significant. Hazard ratios represent the hazard of the treatment divided by the control bacteria of the same row. Number of nematodes tested = N. M = mean survival units (days).

Table 4.4 *S. maltophilia* JCMS mutant background affects the longevity of *daf-2* mutants.

***S. maltophilia* JCMS.**

Control	M	SE	N	Treatment	M	SE	N	Hazard ratio	p value
WT JCMS *ΔrpfF1*	7.3	0.308	34	*daf-2* JCMS *ΔrpfF1*	7.8	1.8	35	0.7045	0.148
WT JCMS *ΔrpfF2*	6.7	0.107	37	*daf-2* JCMS *ΔrpfF2*	6.5	0.13	30	0.963	0.879
WT JCMS *ΔrpfF3*	7.9	0.19	35	*daf-2* JCMS *ΔrpfF3*	7.6	0.28	37	1.016	0.948
WT JCMS *Δxps1*	6.7	0.15	32	*daf-2* JCMS *Δxps1*	6.0	0.14	31	2.65	0.00047
WT JCMS *Δxps2*	6.4	0.22	33	*daf-2* JCMS *Δxps2*	6.1	0.16	33	1.4	0.18
WT JCMS *Δarac2*	4.2	0.09	33	*daf-2* JCMS *Δarac2*	4.2	0.1	33	0.955	0.848
WT JCMS *Δarac3*	4.4	0.11	33	*daf-2* JCMS *Δarac3*	4.0	0.01	35	1.74	0.0251
WT JCMS *Δcs*	3.2	0.105	33	*daf-2* JCMS *Δcs*	3.8	0.073	34	0.543	0.0133
WT JCMS *Δp773*	3.2	0.13	34	*daf-2* JCMS *Δp773*	4.1	0.13	33	0.347	0.00011
WT JCMS *Δp1176*	2.9	0.107	35	*daf-2* JCMS *Δp1176*	3.8	0.068	35	0.351	2.44E-05
WT JCMS *Δpi1y1*	3.9	0.18	31	*daf-2* JCMS *Δpi1y1*	4.7	0.14	34	0.453	0.00271
WT JCMS *Δxdi*	3.3	0.11	30	*daf-2* JCMS *Δxdi*	3.8	0.067	33	0.546	0.0134

***S. maltophilia* JV3**

Control	M	SE	N	Treatment	M	SE	N	Hazard ratio	p value
WT JV3 *ΔrpfF1*	2.6	0.091	35	*daf-2* JV3 *ΔrpfF1*	3.4	0.15	20	0.356	0.00058
WT JV3 *ΔrpfF2*	2.5	0.088	39	*daf-2* JV3 *ΔrpfF2*	3.7	0.11	31	0.2203	5.63E-08
WT JV3 *ΔrpfF3*	2.8	0.079	39	*daf-2* JV3 *ΔrpfF3*	3.6	0.102	32	0.309	1.10E-05
WT JV3 *Δxps1*	2.4	0.093	34	*daf-2* JV3 *Δxps1*	3.1	0.084	42	0.464	0.00105
WT JV3 *Δxps2*	2.3	0.11	35	*daf-2* JV3 *Δxps2*	3.2	0.084	41	0.426	0.00029

Control: wild-type nematodes fed mutant *S. maltophilia* JCMS and JV3 isolates. Treatment: *daf-2(e1368)* mutant nematodes fed mutant *S. maltophilia* JCMS and JV3 isolates. Numbers following each gene name indicate independently generated deletion alleles. p values are given for the survival predictor of treatment (nematode mutant genotype) for cox proportional hazard models in R. p values less than 0.05 were considered significant. Hazard ratios represent the hazard of the treatment divided by the control of the same row. A hazard is the probability that a nematode at a given time dies. Number of nematodes tested = N. M = mean survival units (days).

Chapter 5 - Summary and future work

Our lab is interested in the environmental factors that shape bacterivorous nematode communities. These factors include both abiotic factors and biotic factors such as the surrounding microbial community. This study has focused on the prey/pathogen-predator/host relationship between the model nematode *C. elegans* and the opportunistic bacteria *S. maltophilia*. When comparing multiple *S. maltophilia* isolates, neither the environmental isolates R551-3 and FW, nor clinical isolate K279a were as pathogenic to *C. elegans* as a local isolate JCMS. Intriguingly, we have also found that the mode of action is distinct when comparing pathogenic *S. maltophilia* strains. Like *P. aeruginosa* (Tan *et al.*, 1999a), R551-3 does not require proliferating cells to be pathogenic and appears to employ a toxin. On the other hand, JCMS induced death requires living cells and likely does not involve a toxin. The degree of *S. maltophilia* pathogenicity also correlates with whole nematode and intestinal bacterial accumulation. Furthermore, as observed with other bacterial pathogens that cause gut infection such as *P. aeruginosa* (Tan *et al.*, 1999a) and *S. marcescens* (Kurz *et al.*, 2003a) bacterial accumulation is accompanied by intestinal distention (reviewed in Marsh *et al.*, 2012b). Also, similar to other pathogens, *S. maltophilia* accumulates in the anterior portion of the intestine (Darby, 2005, Irazoqui *et al.*, 2010b, Spanier *et al.*, 2010). Thus, intestinal infection is a common mode of action for the pathogenic bacteria-nematode interaction while the requirement for living cells or a toxin is more specific.

As observed with the clinical isolate K279a, the type II secretion outer membrane pore forming protein Xps (Karaba *et al.*, 2013) and the enoyl coenzyme A hydratase RpfF (Fouhy *et al.*, 2007) are required for JCMS virulence. We have also found that Xps is required for virulence in *S. maltophilia* JV3. Although, we have not tested the requirement for *xps* in K279a mediated killing, these results suggest that action through the type II secretion system is

necessary for the pathogen-host side of the *C. elegans*-*S. maltophilia* interaction. and that pathogenic strains require *xps* for virulence. As inferred from the *rpfF* gene sequence data in a previous study (Huedo *et al.*, 2014), RpfF is not required for JV3 virulence. This study suggests that *S. maltophilia* isolates with the *rpfF* variant RpfF-1 produce a diffusible signaling factor that is involved in several virulence-related phenotypes (Huedo *et al.*, 2014). Future work should involve the sequencing of *rpfF* in JCMS to determine if this is the case. Nevertheless, these data suggest that RpfF and thus, quorum sensing plays a role in *S. maltophilia* virulence that is strain specific.

Our preliminary work surveying additional putative JCMS virulence factors reveals that future work should also involve the evaluation of bacterial secretions as factors required for host survival. Intriguingly, loss of the bacterial genes *cs*, *p773*, *p1176* and *xdi* in JCMS causes nematode hyper-susceptibility. Furthermore, these genes and *arac* have a greater role in *C. elegans daf-2* mutants. Taken together, these results indicate that *arac*, *cs*, *p773*, *p1176*, and *xdi* have a unique role in the nematode-bacterial interaction. This statement is further supported when comparing the survival of wild-type nematodes and *daf-2* mutants fed these bacterial mutants. Recall that *daf-2* mutants are significantly long-lived on JCMS *Δcs*, *Δp773*, *Δp1176*, *Δpi1y1* and *Δxdi* mutants. Thus, the gene products of *cs*, *p773*, *p1176*, *pi1y1* and *xdi* are required for JCMS evasion of *daf-2* mutant pathogen resistance. Supplementary work, including the evaluation of additional bacterial deletion mutant isolates, is required to determine why ATP-binding protein, protease, aminopeptidase and fimbrial biogenesis protein are involved in this evasion.

Here we have elucidated novel and existing genes and pathways that are required for the interaction of *Caenorhabditis elegans* with *Stenotrophomonas maltophilia*. Several pathways

that serve to protect *C. elegans* from various pathogenic bacteria have been discovered, including the p38 MAPK, UPR and Sma/Mab TGFβ-related pathways. Mutants that disrupt numerous components of these pathways are hypersensitive to both JCMS and OP50, suggesting that the functions of these genes are needed for a general bacterial response. Surprisingly, most DAF-2/IIS pathway mutants displayed shortened lifespans on *S. maltophilia* JCMS, which is striking as most have long lifespans on other bacterial pathogens. Additionally, several DAF-2/16 signaling effector genes are not significantly differentially expressed between JCMS and avirulent *E. coli* OP50. Thus, we conclude that the DAF-2/16 pathway plays a diminishing if any role in the *C. elegans* defense response to *S. maltophilia* JCMS. Furthermore, the role of DAF-2/16, p38 MAPK and TGFβ-like pathway components is specific to JCMS or OP50 and, this specificity was not observed for the UPR pathway. These findings warrant the future investigation of individual pathway component function in response to different bacteria.

We have used several different strategies to identify candidate genes and/or pathways that might explain this *S. maltophilia* JCMS specific evasion of the DAF-2/IIS defense pathway. One strategy involved the use of transcriptomics and a probabilistic gene network model to prioritize gene candidates. This prioritization allowed the identification of five genes (C48B4.1, *mpk-2*, *cpr-4*, *clec-67* and *lys-6)* with unique roles in JCMS response. The identification of novel bacteria specific immune effector genes suggests that there is still much to be discovered about how different, although related, bacterial pathogens elicit nematode immune response. Follow up with these genes could involve the engineering of transcriptional gfp fusions to determine where these genes are expressed in wild-type nematodes on related (JCMS and K279a) and divergent (K279a and OP50) bacteria. Additionally, the determination of nematode mutant bacterial localization and accumulation would allow the functional characterization (infection tolerance or

removal) of these genes (reviewed in Chapter 1 and Ayres *et al.*, 2012). This characterization could also be compared between bacterial environments to find functional specificity.

Here we have determined the *S. maltophilia* mode of action, *S. maltophilia* virulence factors, and genes that are required for the nematode innate immunity response. We have also identified bacterial genes that are not classified as *bona fide* "virulence factors" but, have a more indirect role in *S. maltophilia* JCMS pathogenicity. Additionally, only some bacterial virulence factors are conserved. A similar case was also observed for nematode innate immunity in that the requirement for certain nematode innate immune effector genes changed with bacterial environment. Moreover, we observed an intriguing bacterial genotype by nematode genotype interaction. Given this specificity, the characterization of the *C. elegans* interaction with different bacteria is required for the elucidation of a systematic overview of *C. elegans* prey and/or pathogen response. Furthermore, the next step in our understanding of nematode innate immunity is to examine the effects of combinations of bacteria, as some studies have begun to explore (Darby *et al.*, 2014, Kim *et al.*, 2012, Montalvo-Katz *et al.*, 2013). Only with these combined studies can we start to unveil the dynamic network of *C. elegans* response to environmental stress that is of evolutionary significance.

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Appendix A - Supplemental figures and tables

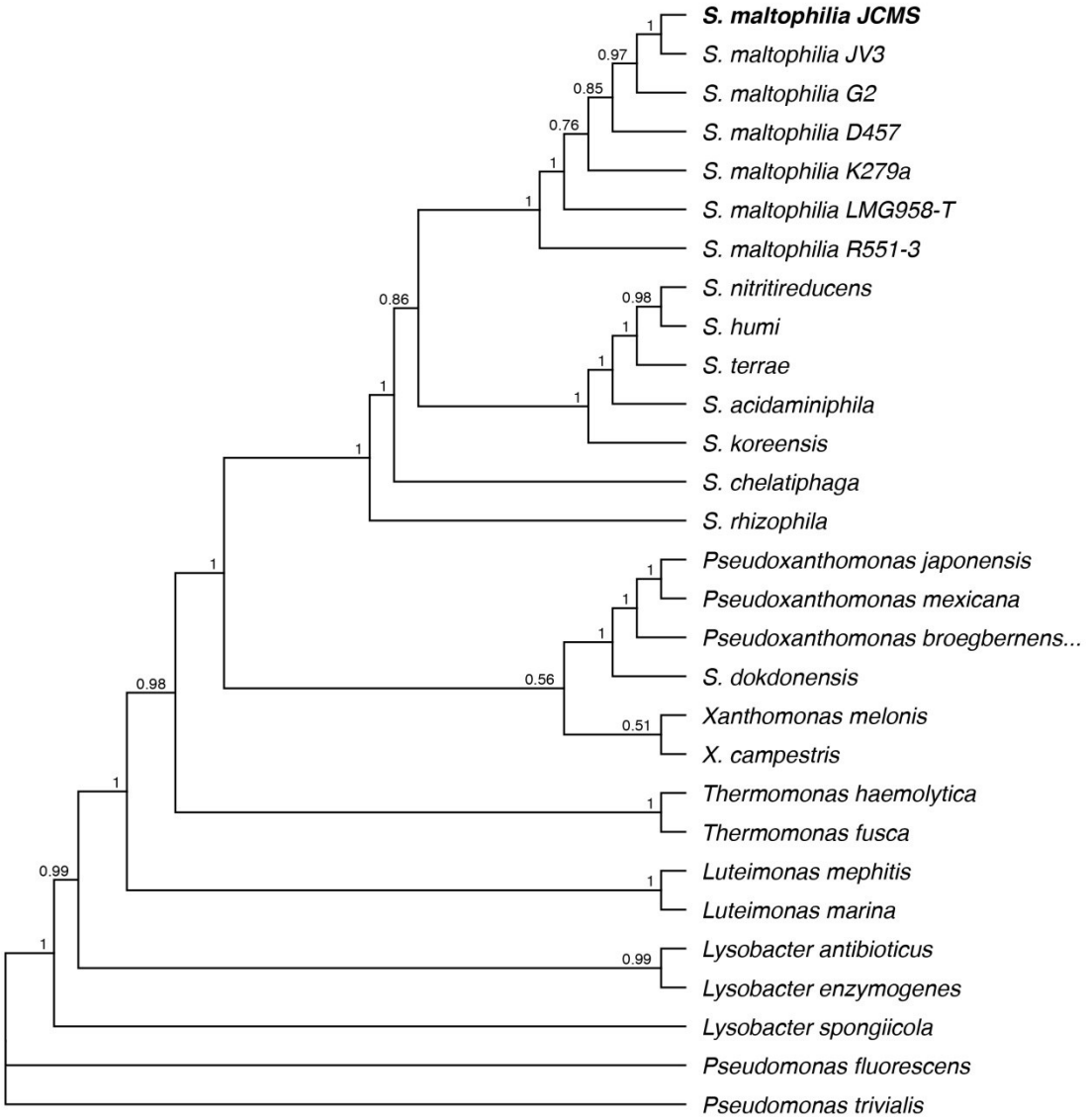

Figure A.1 Bayesian phylogenetic tree of bacterial 16S rRNA gene sequences.

Sequences included JCMS (KF724885), K279a, R551-3, JV3 (CP002986), G2 (EU927145) and those included in a previous study (Ryan *et al.*, 2009). *S. maltophilia* JCMS, R551-3 and K279a were involved in this study. Alignments and phylogenetic trees were generated using packages contained within the Geneious software package (Biomatters, Inc.). Full-length 16S rRNA sequences were aligned with ClustalW, and phylogenetic trees generated using MrBays (v.3.1.2.) (Huelsenbeck *et al.*, 2001) using default MrBayes parameters implemented within Geneious. We used a HKY85 sequence substitution model with a 4 category gamma-distributed rate variation across sites with *Pseudomonas fluorescens* as the out-group. The MCMC length was 1,100,000 with sampling every 200 generations using 4 heated chains. Posterior probabilities are indicated and branch lengths are proportional. JCMS is more closely related to K279a than to R551-3.

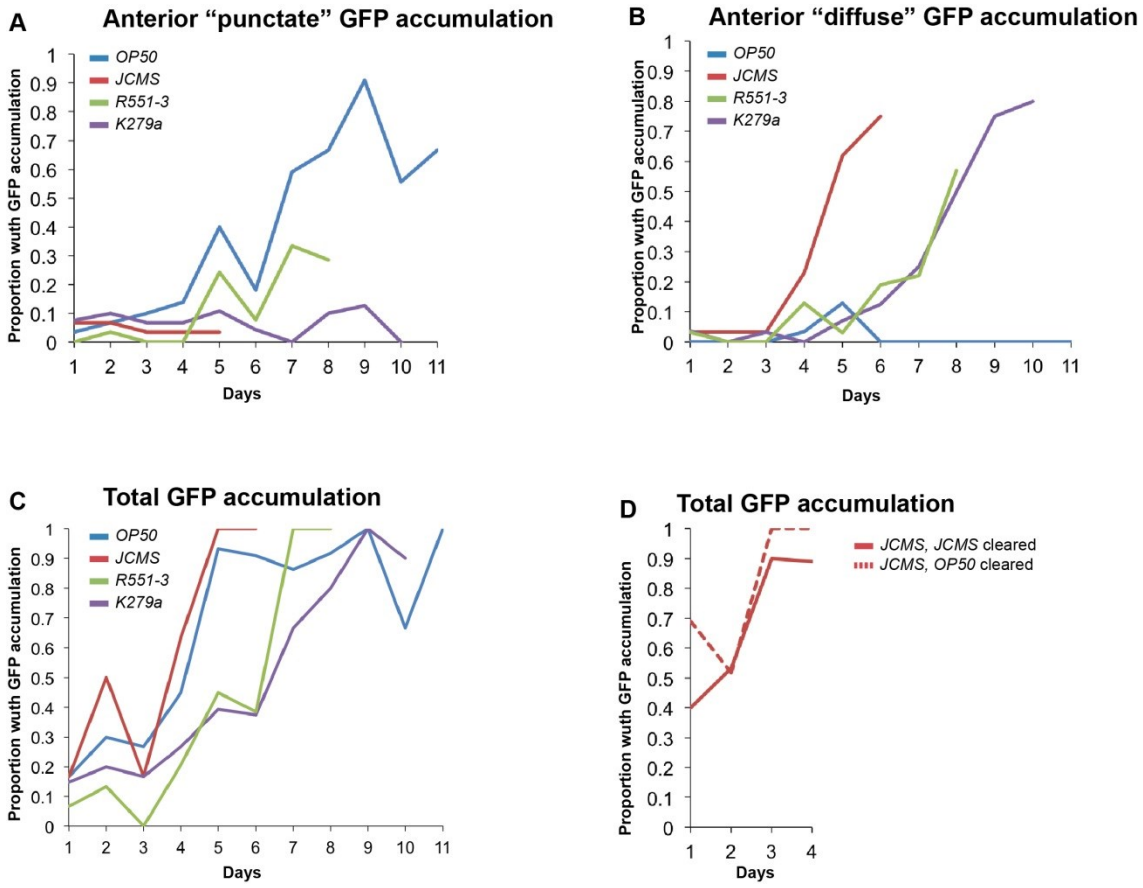

Figure A.2 *S. maltophilia* JCMS accumulates in the intestine in a diffuse pattern.

Nematodes were fed GFP versions of the various *S. maltophilia* strains. Intestinal accumulation of GFP-labeled bacteria was scored. Proportion of surviving nematodes after 1 hour of clearing on non-GFP bacteria of the same strain with (A) punctate GFP accumulation in the anterior portion of the intestine, (B) diffuse GFP accumulation in the anterior portion of the intestine and (C) total (anterior, middle and posterior) GFP accumulation for day 1 through 11. n = 153 for *S. maltophilia* JCMS, 189 for *S. maltophilia* R551-3, 209 for *S. maltophilia* K279a and 228 for *E. coli* OP50. D) Proportion of surviving nematodes fed JCMS-GFP after 1 hour of clearing on non-GFP OP50 (dashed line) or JCMS (solid line) with total GFP accumulation for day 1 - 4. n = 112 for clearing on OP50 and 117 for clearing on JCMS. *S. maltophilia* JCMS accumulated in the intestine to a greater extent when competing with *E. coli* OP50.

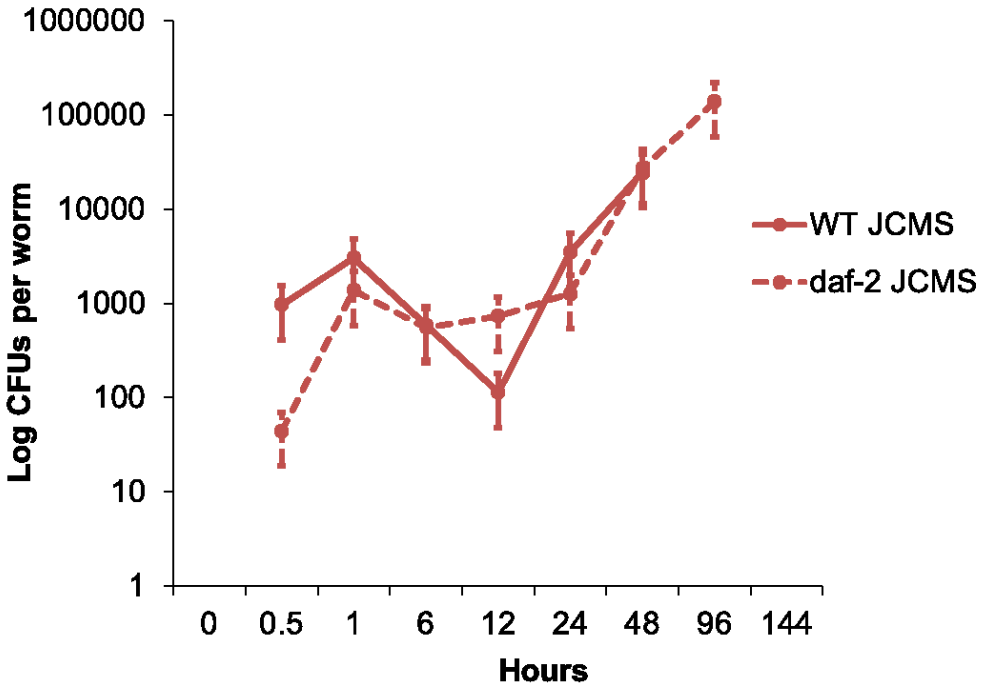

Figure A.3 Mean bacterial load of wild-type and *daf-2* mutant nematodes fed *S. maltophilia* JCMS.

Synchronized wild-type or *daf-2(e1368)* mutant nematodes were fed non-GFP bacteria from 0.5 to 144 hours on NGM plates at 25˚C. Triplicates of 10 nematodes were picked after 0.5, 1, 6, 12, 24, 48, 96 and 144 hours of exposure to *S. maltophilia* JCMS and fed non-GFP *E. coli* OP50 for 1 hour of clearing. Nematodes were then placed on un-seeded NGM doxycycline (120µg/mL) plates for washing: once with 25mM levamisole/M9 (LM) buffer, twice with LM buffer with doxycycline (120µg/mL) and twice with M9 buffer. Washed nematodes were then placed in a 1.7mL microcentrifuge tube containing 50µL of M9 buffer + 1% Triton^{TM} X-100, Sigma-Aldrich and homogenized using a pestle motor. Crushed nematodes were diluted and plated on LB agar containing 100µg/mL Ampicillin to select for growth of adherent strains. Mutants of *daf-2* and wild-type nematodes have a similar bacterial load over time.

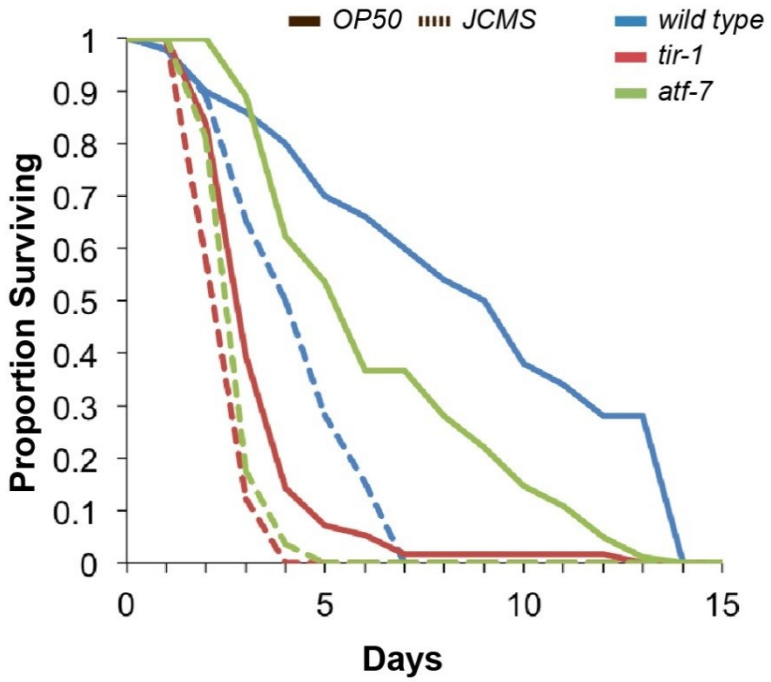

Figure A.4 Survival of select p38 MAPK immune pathway mutants.

Survival of wild-type nematodes (WT, blue), *tir-1(qd4)* (red) and *atf-7(qd137)* (green) mutants on *E. coli* OP50 (solid lines) or *S. maltophilia* JCMS (dashed lines). Results plotted are the proportion of surviving worms using Kaplan-Meier estimates for at least three replicate samples (10 to 15 per replicate) of the same nematode population. p values from the application of Cox proportional hazards models and sample sizes of each population are included in Table 2.1. Survival of *tir-1* and *atf-7* mutants was significantly shorter than *wildtype* on JCMS and OP50.

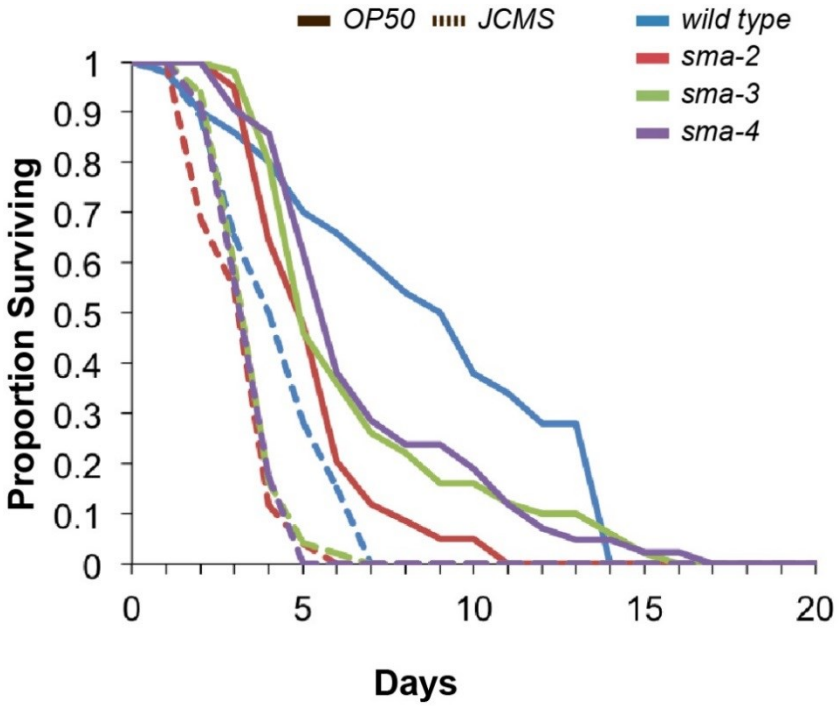

Figure A.5 Survival of select TGFβ-like immune pathway mutants.

Survival of wild-type nematodes (WT, blue), *sma-2(e502)* (red), *sma-3(e491)* (green) and *sma-4(e729)* (purple) mutants on *E. coli* OP50 (solid lines) or *S. maltophilia* JCMS (dashed lines). Results plotted are the proportion of surviving worms using Kaplan-Meier estimates for at least three replicate samples (10 to 15 nematodes per replicate) of the same nematode population. p values from the application of Cox proportional hazards models and sample sizes of each population are included in Table 2.1. Survival of *sma-2, sma-3* and *sma-4* mutants was significantly shorter than *wildtype* on JCMS and OP50.

Table A.1 Survival of nematodes fed *E. coli*, *P. aeruginosa*, *E. faecalis* and *S. maltophilia* strains.

Control	Mean	SE	N	Treatment	Mean	SE	N	Hazard ratio	p value
OP50	8.53	0.187	243	**JCMS**	4.69	0.0884	150	8.62	<2E-16
OP50	8.53	0.187	243	**R551-3**	6.73	0.294	49	2.27	4.7E-07
OP50	8.53	0.187	243	**K279a**	9.17	0.536	49	0.772	0.106
K279a	9.17	0.536	49	**JCMS**	4.69	0.0884	150	9.83	<2E-16
R551-3	6.73	0.294	49	**JCMS**	4.69	0.0884	150	4.11	6.1E-13
V583	4.11	0.181	63	**JCMS**	4.69	0.0884	150	0.752	0.0663
PA14	3.68	0.134	73	**JCMS**	4.69	0.0884	150	0.494	1.2E-06

Control or treatment bacteria: *E. faecalis* V583, *P. aeruginosa* PA14, *E. coli* OP50, *S. maltophilia* JCMS, R551-3 or K279a. Number of nematodes tested = N. p values were significant if less than 0.05 and are given for the survival predictor of bacterial treatment for Cox proportional hazards models in R.

Table A.2 Effect of different media on the survival of nematodes fed *S. maltophilia* strains and *P. aeruginosa* PA14.

Control	Mean	SE	N	Treatment	Mean	SE	N	Hazard ratio	p value
PA14 NGM	3.68	0.13	73	**PA14 PGS**	2.88	0.266	43	2.09	0.00154*
JCMS NGM	4.69	0.0884	150	**JCMS PGS**	5.6	0.358	30	0.442	0.00016
JCMS NGM	4.69	0.0884	150	**JCMS BHI**	5.71	0.305	29	0.389	1.86E-5
K279a NGM	9.17	0.536	49	**K279a PGS**	5.63	0.26	55	2.76	3.44E-6
K279a NGM	9.17	0.536	49	**K279a BHI**	5.76	0.304	54	3.18	5.2E-7
R551-3 NGM	6.7	0.27	47	**R551-3 PGS**	5.4	0.44	28	1.48	0.103
R551-3 NGM	6.7	0.27	47	**R551-3 BHI**	4.3	0.25	29	4.17	1.17E-07
OP50 NGM	8.31	0.164	240	**OP50 PGS**	5.1	0.35	27	4.65	8.8E-13
OP50 NGM	8.31	0.164	240	**OP50 BHI**	6.6	0.18	25	2.87	2.4E-06
PA14 NGM	3.68	0.13	73	**K279a NGM**	9.17	0.536	49	0.0511	1.1E-14
PA14 PGS	2.88	0.266	43	**K279a PGS**	5.63	0.26	55	0.215	1.7E-9
PA14 PGS	2.88	0.266	43	**JCMS PGS**	5.6	0.358	30	0.175	1.1E-7

Control or treatment bacteria: *P. aeruginosa* PA14, *E. coli* OP50 *S. maltophilia* JCMS, K279a or R551-3. Control or treatment growth medium: NGM = nematode growth medium, BHI = brain heart infusion medium (enriched media) or PGS = peptone-glucose-sorbitol (fast killing media). *Date of experimentation was observed to have a significant effect and was included in this model. Number of nematodes tested = N. p values were significant if less than 0.05 and are given for the survival predictor of bacterial treatment for Cox proportional hazards models in R.

Table A.3 Survival of nematodes fed non-GFP *E. coli*, *S. maltophilia* versus GFP strains.

Control	Mean	SE	N	Treatment	Mean	SE	N	Hazard ratio	p value
JCMS	4.94	0.15	50	**JCMS GFP**	4.88	0.135	50	1.23	0.318
R551-3	6.62	0.357	52	**R551-3 GFP**	6.82	0.323	44	0.95	0.805
K279a	8.87	0.418	47	**K279a GFP**	9.56	0.47	45	0.777	0.23
OP50	8.82	0.343	50	**OP50 GFP**	7.65	0.364	54	1.37	0.124

Number of nematodes tested = N. p values were significant if less than 0.05 and are given for the survival predictor of bacterial treatment (GFP integration) for Cox proportional hazards models in R.

Table A.4 Survival of nematodes fed heat and antibiotic treated *E. coli* and *S. maltophilia* versus non-treated strains.

Control	Mean	SE	N	Treatment	Mean	SE	N	Hazard ratio	p value
OP50	9.16	0.47	68	**OP50 AK**	13.9	0.688	60	0.302	3.82E-03
OP50	9.16	0.47	68	**OP50 HK**	7.7	0.414	61	5.1	0.000451
K279a	10.7	0.369	59	**K279a AK**	16.3	0.737	62	0.0665	4.23E-06
K279a	10.7	0.369	59	**K279a HK**	9.25	0.532	53	1.14	0.822
R551-3	6.65	0.219	74	**R551-3 AK**	4.46	0.501	39	4.91	5.49E-03
R551-3	6.65	0.219	74	**R551-3 HK**	5.79	0.372	56	1.32	0.48
JCMS	3.98	0.0986	60	**JCMS AK**	11.6	0.774	62	0.113	3.01E-05
JCMS	3.98	0.0986	60	**JCMS HK**	9.13	0.482	46	0.0308	3.25E-07

Control or treatment bacteria: Non-treated (control), heat or doxycycline treated (treatment) *E. coli* OP50, *S. maltophilia* JCMS, R551-3 or K279a. Number of nematodes tested = N. p values were significant if less than 0.05 and are given for the survival predictor of bacterial treatment for Cox proportional hazards models (all full factorial models) in R.

Table A.5 Survival of nematodes fed *E. coli* treated with OP50 secretions versus *S. maltophilia* secretions.

Control	Mean	SE	N	Treatment	Mean	SE	N	Hazard ratio	p value
OP50 secretions	9.01	0.601	47	**JCMS secretions**	8.23	0.438	57	0.591	0.401
OP50 secretions	9.01	0.601	47	**R551-3 secretions**	7.91	0.376	58	0.477	0.2296
OP50 secretions	9.01	0.601	47	**K279a secretions**	9.31	0.557	49	0.177	0.00905

Bacterial secretion control or treatment: *E. coli* OP50 (control), *S. maltophilia* JCMS, R551-3 or K279a (treatment). Number of nematodes tested = N. p values were significant if less than 0.05 and are given for the survival predictor of bacterial treatment for Cox proportional hazards models (all full factorial models) in R.

Table A.6 Survival of wild-type nematodes versus several defense pathway mutants on *S. maltophilia* and *E. coli.*

	***S. maltophilia* JCMS**					***E. coli* OP50**				
Genotype	**M**	**SE**	**N**	**Hazard Ratio**	**p value**	**M**	**SE**	**N**	**Hazard Ratio**	**p value**
***wildtype* (WT)**	4.69	0.088	150	N/A	N/A	8.47	0.18	244	N/A	N/A
akt-1 (mg306)	4.2	0.13	60	1.605	0.0022	12	0.28	55	0.208	< 2E-16
pdk-1 (mg142)	4.8	0.16	60	0.876	0.39	8.1	0.32	59	1.03	0.829
pdk-1 (sa680)	2.8	0.11	59	6.69	< 2E-16	3.0	0.15	57	21.7	< 2E-16
sgk-1 (ok538)	6.3	0.17	57	0.29	4.0E-13	8.04	0.29	55	1.25	0.142
kri-1 (ok1251)	4.1	0.12	60	1.75	3.2E-04	8.5	0.35	58	0.892	0.438
daf-18 (ok480)	4.6	0.22	60	0.875	0.4	7.1	0.31	57	1.93	2.3E-05
ire-1 (zc14)	3.04	0.15	25	5.73	7.1E-14	4.9	0.58	27	2.6	2.95E-06
nsy-1 (ag3)	2.7	0.076	60	13.5	< 2E-16	3.9	0.29	60	4.93	< 2E-16
atf-7 (gk715)	4.3	0.11	49	1.61	0.0045	7.9	0.51	47	0.865	0.376
agls219 atf-7 (qd22 qd130)	2.9	0.077	90	7.8	< 2E-16	6.3	0.24	108	2.25	3.07E-11
agls219 atf-7(qd22)	2.4	0.097	80	13	< 2E-16	7.9	0.45	51	1.04	0.798
agls219 transgene	3.0	0.061	70	10.5	< 2E-16	5.9	0.26	71	2.85	7.3E-06
daf-16 (mgDf50)	3.8	0.13	55	2.46	0.0045	7.1	0.26	55	1.79	1.6E-04

These survival experiments were completed to assess the involvement of several immune pathway components on *S. maltophilia* JCMS and *E. coli* OP50. Some of these genes are also discussed in Chapter 2 and there are additional alleles for those genes included here. p values are given for the survival predictor of treatment (mutant nematode genotype) for Cox proportional hazard models in R. Hazard ratios represent the hazard of the treatment divided by the control (wild-type) of the same bacteria. A hazard is the probability that a nematode at a given time dies. p values less than 0.05 were considered significant. Number of nematodes tested = N. Mean = mean survival units (days). Mutants of *akt-1(mg306)*, *kri-1(ok1251)* and *atf-7(gk715)* were specifically susceptible to JCMS while, *daf-18(ok480)*, *sgk-1(ok538)* mutants were specifically short lived on OP50. Mutants of *akt-1(mg306)* were resistant to OP50 and *sgk-1(ok538)* mutants were specifically resistant to JCMS. The susceptible phenotypes of *pdk-1(mg142)*, *pdk-1(sa680)*,

ire-1(zc14), *nsy-1(ag3)*, *agls219 atf-7 (qd22 qd130)*, *agls219 atf-7(qd22)* and *daf-16(mgDf50)* were non-specific.

Table A.7 All significantly differentially expressed transcripts for wild-type nematodes fed *E. coli* OP50, *S. maltophilia* JCMS or K279a.

Gene public name	Sequence name	p value corrected	FC	Regulation	Comparison
F10A3.1	F10A3.1	0.026743438	25.140755	up	JCMS vs. **OP50**
F10A3.1	F10A3.1	0.002704226	20.981218	up	JCMS vs. **K279a**
fbxa-163	C08E3.6	4.72E-04	20.227543	up	JCMS vs. **K279a**
fbxa-161	C08E3.4	8.08E-05	13.905435	up	JCMS vs. **K279a**
W03F9.4	W03F9.4	0.004437486	13.847095	up	K279a vs. **OP50**
fbxa-163	C08E3.6	0.024396664	13.503406	up	JCMS vs. **OP50**
F08G2.5	F08G2.5	0.002111807	13.103755	up	JCMS vs. **K279a**
spp-12	T22G5.7	0.013737984	12.969098	down	JCMS vs. **K279a**
tag-293	C03G6.13	0.030908348	12.935655	down	JCMS vs. **K279a**
T22F3.11	T22F3.11a	0.014975314	12.083424	up	JCMS vs. **K279a**
W03F9.4	W03F9.4	0.003406334	11.83796	up	JCMS vs. **OP50**
fbxa-162	C08E3.5	0.002704226	11.537813	up	JCMS vs. **K279a**
fbxa-161	C08E3.4	0.003406334	11.32513	up	JCMS vs. **OP50**
F15B9.6	F15B9.6	0.037475243	11.202448	up	JCMS vs. **OP50**
T22F3.11	T22F3.11b	0.017357128	10.966784	up	JCMS vs. **K279a**
Y41D4B.18	Y41D4B.18	0.014757021	9.250423	up	JCMS vs. **OP50**
Y58A7A.5	Y58A7A.5	0.0028043	9.21022	up	JCMS vs. **K279a**
F49H6.13	F49H6.13	0.002704226	8.887562	up	JCMS vs. **K279a**
K11H12.3	K11H12.3	0.007689229	8.219166	up	JCMS vs. **K279a**
F43C1.7	F43C1.7	0.026743438	8.053111	up	JCMS vs. **OP50**
F19B10.4	F19B10.4	0.002704226	7.5302453	up	JCMS vs. **K279a**
F15B9.6	F15B9.6	0.001906469	7.1408734	up	JCMS vs. **K279a**
Y58A7A.5	Y58A7A.5	0.009184291	7.104403	up	JCMS vs. **OP50**
K10D11.2	K10D11.2	0.0028043	7.1027737	up	JCMS vs. **K279a**
F43C1.7	F43C1.7	0.011217603	6.8470755	up	JCMS vs. **K279a**
Y38H6C.19	Y38H6C.19	0.007689229	6.5854115	down	JCMS vs. **K279a**
clec-70	Y46C8AL.3	0.028550781	6.354619	down	JCMS vs. **K279a**
K11H12.3	K11H12.3	0.02696083	6.324137	up	JCMS vs. **OP50**
ilys-3	C45G7.3	0.042742778	6.275182	down	JCMS vs. **K279a**
F19B10.4	F19B10.4	0.03679428	6.2738795	up	JCMS vs. **OP50**
cyp-13A6	T10B9.3	0.007689229	6.189299	down	JCMS vs. **K279a**
T08G5.1	T08G5.1	0.01324283	5.882459	down	JCMS vs. **OP50**
str-116	F07B10.2	0.002704226	5.854447	down	JCMS vs. **K279a**
cyp-13A12	F14F7.3	0.011563951	5.6554594	up	K279a vs. **OP50**
Y38H6C.21	Y38H6C.21	0.015425405	5.644647	down	JCMS vs. **K279a**
F20G2.5	F20G2.5	0.028171588	5.565966	up	JCMS vs. **OP50**
F46E10.1	F46E10.1a	0.007314117	5.5214744	down	JCMS vs. **OP50**
F22E5.6	F22E5.6	0.046115838	5.4138503	up	JCMS vs. **OP50**
F53A9.2	F53A9.2	0.044467654	5.236226	up	JCMS vs. **OP50**

F07C4.12	F07C4.12b	0.014975314	5.21325	up	JCMS vs. **K279a**
ftn-1	C54F6.14	0.048612747	5.137447	down	JCMS vs. **K279a**
ZK177.9	ZK177.9	0.009940833	5.1099415	up	JCMS vs. **K279a**
lbp-8	T22G5.6	0.047514576	5.0101504	down	JCMS vs. **K279a**
F08A10.1	F08A10.1e	0.03570299	5.001947	down	JCMS vs. **K279a**
Y51H4A.25	Y51H4A.25b	0.04017914	4.9572034	up	JCMS vs. **OP50**
C25F9.11	C25F9.11	0.030908348	4.9012184	up	JCMS vs. **K279a**
ugt-51	C03A7.11	0.007689229	4.8780174	down	JCMS vs. **K279a**
H12D21.4	H12D21.4	0.028550781	4.769112	down	JCMS vs. **K279a**
fbxa-158	C08E3.10b	0.028550781	4.7650046	up	JCMS vs. **K279a**
pqn-97	ZK488.10	0.013737984	4.7519946	up	JCMS vs. **K279a**
F08A10.1	F08A10.1b	0.031215737	4.7260656	down	JCMS vs. **K279a**
H34P18.1	H34P18.1	0.028550781	4.704518	down	JCMS vs. **K279a**
sdz-35	ZC239.12	0.028171588	4.691884	up	JCMS vs. **OP50**
cyp-13A7	T10B9.10	0.03808078	4.6299686	down	JCMS vs. **K279a**
C07G3.2	C07G3.2	0.03317524	4.6106873	up	JCMS vs. **K279a**
T02B5.1	T02B5.1	0.03131432	4.595823	down	JCMS vs. **K279a**
ugt-15	C44H9.1	0.001906469	4.5936995	down	JCMS vs. **K279a**
T24C4.3	T24C4.3	0.019358443	4.5530953	up	JCMS vs. **OP50**
fbxa-158	C08E3.10a	0.028550781	4.5367937	up	JCMS vs. **K279a**
cyp-14A4	R04D3.1	0.020570438	4.4660544	down	JCMS vs. **K279a**
B0024.4	B0024.4	0.007572418	4.427903	up	JCMS vs. **K279a**
F46C5.1	F46C5.1	0.041117292	4.4275136	up	JCMS vs. **K279a**
K04A8.5	K04A8.5	0.030055868	4.413593	down	JCMS vs. **K279a**
F46E10.1	F46E10.1b	0.01324283	4.3980374	down	JCMS vs. **OP50**
Y41D4B.18	Y41D4B.18	0.002704226	4.374552	up	JCMS vs. **K279a**
Y69A2AR.25	Y69A2AR.25	0.030570457	4.367409	up	JCMS vs. **K279a**
F07C4.12	F07C4.12a	0.014975314	4.3471575	up	JCMS vs. **K279a**
Y69A2AR.12	Y69A2AR.12	0.029480534	4.3247895	up	JCMS vs. **K279a**
pqe-1	F52C9.8f	0.013122048	4.205232	up	JCMS vs. **K279a**
R09H10.7	R09H10.7	0.010202582	4.1892204	down	JCMS vs. **K279a**
F08A10.1	F08A10.1c	0.040522408	4.1711435	down	JCMS vs. **K279a**
T16G1.4	T16G1.4	0.0028043	4.1483865	down	JCMS vs. **K279a**
str-204	F10D2.1	0.020291237	4.0855665	up	JCMS vs. **OP50**
F08A10.1	F08A10.1a	0.039219737	4.084542	down	JCMS vs. **K279a**
F46E10.1	F46E10.1c	0.01324283	4.059224	down	JCMS vs. **OP50**
Y58A7A.4	Y58A7A.4	0.041092202	4.0153866	up	JCMS vs. **K279a**
F08A10.1	F08A10.1d	0.0398986	3.995056	down	JCMS vs. **K279a**
Y69A2AR.13	Y69A2AR.13	0.024047945	3.967095	up	JCMS vs. **K279a**
C25G6.4	C25G6.4	0.010202582	3.9513028	down	JCMS vs. **K279a**
C53A3.1	C53A3.1	0.02931667	3.9228299	down	JCMS vs. **K279a**
lys-5	F58B3.2	0.024452075	3.9026153	down	JCMS vs. **K279a**
str-204	F10D2.1	0.01709641	3.8966427	up	JCMS vs. **K279a**

F45G2.2	F45G2.2a	0.04869883	3.8787715	down	JCMS vs. **K279a**
Y37H2B.1	Y37H2B.1	0.016251264	3.820403	up	JCMS vs. **K279a**
F49F1.6	F49F1.6	0.034673207	3.8176854	up	JCMS vs. **OP50**
F07C4.6	F07C4.6	0.04262033	3.8041437	down	JCMS vs. **K279a**
F35E12.4	F35E12.4	0.001906469	3.7671666	up	JCMS vs. **K279a**
srw-86	C25F9.7	0.041117292	3.7559125	up	JCMS vs. **K279a**
ZC404.11	ZC404.11	0.044269264	3.7544444	down	JCMS vs. **K279a**
C25F9.6	C25F9.6	0.03570299	3.7508907	up	JCMS vs. **K279a**
clec-45	F07C4.2	0.047187086	3.7342002	up	JCMS vs. **K279a**
pqn-98	ZK488.7	0.002704226	3.734168	up	JCMS vs. **K279a**
F14F9.4	F14F9.4	0.044564333	3.6392503	up	JCMS vs. **K279a**
C54D10.12	C54D10.12	0.021677643	3.633187	up	JCMS vs. **K279a**
srd-64	Y22D7AR.8	0.01885526	3.6139646	up	JCMS vs. **K279a**
T19D12.5	T19D12.5	0.030055868	3.6138763	up	JCMS vs. **K279a**
srsx-36	T26E4.15	0.045368545	3.5835717	down	JCMS vs. **K279a**
clec-174	Y46C8AL.2	0.010226341	3.580755	up	JCMS vs. **OP50**
Y37H2B.1	Y37H2B.1	0.018999398	3.564599	up	JCMS vs. **OP50**
cyp-13A12	F14F7.3	0.01324283	3.5312407	up	JCMS vs. **OP50**
lys-6	F58B3.3	0.007986588	3.5260484	down	JCMS vs. **K279a**
F35E12.9	F35E12.9a	0.002704226	3.4538333	up	JCMS vs. **K279a**
fbxa-88	F10A3.2	0.034811806	3.4087105	up	JCMS vs. **K279a**
Y51B9A.6	Y51B9A.6	0.039950997	3.407992	down	JCMS vs. **K279a**
cyp-14A2	K09A11.3	0.014975314	3.4046097	down	JCMS vs. **K279a**
F33H12.7	F33H12.7	0.028550781	3.4027917	up	JCMS vs. **K279a**
ugt-31	Y39G10AR.6	0.002704226	3.4026275	up	JCMS vs. **K279a**
R08E5.1	R08E5.1	0.042742778	3.3641481	down	JCMS vs. **K279a**
C01G10.15	C01G10.15	0.034811806	3.3505726	down	JCMS vs. **K279a**
T12D8.5	T12D8.5	0.014278974	3.3210993	down	JCMS vs. **K279a**
clec-218	W02D7.2	0.013737984	3.256298	down	JCMS vs. **K279a**
T24C4.3	T24C4.3	0.013472779	3.2401876	up	JCMS vs. **K279a**
ZK105.5	ZK105.5	0.047095913	3.228722	down	K279a vs. **OP50**
scrm-5	K08D10.8	0.04869883	3.2232726	up	JCMS vs. **K279a**
T24E12.5	T24E12.5	0.015425405	3.2043004	up	JCMS vs. **K279a**
nhr-112	Y70C5C.6b	0.026743438	3.2036278	up	JCMS vs. **OP50**
C09D4.1	C09D4.1a	0.020570438	3.1726167	down	JCMS vs. **K279a**
srh-279	F11A5.2	0.030908348	3.1431034	down	JCMS vs. **K279a**
Y43D4A.2	Y43D4A.2	0.0419092	3.1365645	up	JCMS vs. **K279a**
K11H12.10	K11H12.10	0.048500955	3.1239836	up	JCMS vs. **K279a**
F44D12.9	F44D12.9	0.013122048	3.121463	down	JCMS vs. **K279a**
F11C7.7	F11C7.7	0.028550781	3.1077058	down	JCMS vs. **K279a**
F19B2.5	F19B2.5	0.048808206	3.083435	up	JCMS vs. **K279a**
W09G12.9	W09G12.9	0.03570299	3.0323138	down	JCMS vs. **K279a**
tba-7	T28D6.2	0.01324283	3.0278692	up	JCMS vs. **OP50**

str-180	T10H9.6a	0.009940833	3.0118532	down	JCMS vs. **K279a**
F49H6.3	F49H6.3	0.031215737	3.0093915	up	JCMS vs. **K279a**
Y75B8A.39	Y75B8A.39	0.03570299	2.9993463	up	JCMS vs. **K279a**
W04C9.6	W04C9.6	0.036159135	2.995348	down	JCMS vs. **K279a**
cyp-34A9	B0213.15a	0.028550781	2.9687698	down	JCMS vs. **K279a**
C12D5.3	C12D5.3	0.007986588	2.9662726	down	JCMS vs. **K279a**
cyp-13B1	F02C12.5a	0.041117292	2.9463434	down	JCMS vs. **K279a**
ZK177.3	ZK177.3	0.031215737	2.9395697	up	JCMS vs. **K279a**
F09G8.5	F09G8.5	0.044269264	2.9316928	down	JCMS vs. **K279a**
F20B6.6	F20B6.6	0.04458514	2.9117959	up	JCMS vs. **K279a**
dsl-5	F58B3.8	0.032612246	2.9083457	down	JCMS vs. **K279a**
R02D5.6	R02D5.6	0.040522408	2.9010782	down	JCMS vs. **K279a**
cyp-34A9	B0213.15b	0.028550781	2.8906405	down	JCMS vs. **K279a**
F14H8.4	F14H8.4	0.04815337	2.8827844	down	JCMS vs. **K279a**
F53B2.8	F53B2.8	0.026743438	2.8747728	up	JCMS vs. **OP50**
cyp-13A4	T10B9.1	0.04877365	2.8737109	down	JCMS vs. **K279a**
C18H7.4	C18H7.4	0.041117292	2.8580022	up	JCMS vs. **K279a**
Y45F10D.2	Y45F10D.2	0.03570299	2.8388643	down	JCMS vs. **K279a**
dod-22	F55G11.5	0.030908348	2.834632	up	JCMS vs. **K279a**
nhr-193	F57G8.6	0.04994786	2.8307352	down	JCMS vs. **K279a**
cyp-33D3	Y17D7A.4	0.024452075	2.8238966	down	JCMS vs. **K279a**
E02C12.6	E02C12.6	0.047187086	2.8215961	down	JCMS vs. **K279a**
F44A6.5	F44A6.5	0.028550781	2.8189805	down	JCMS vs. **K279a**
clec-76	Y46C8AR.1	0.042778008	2.8150475	down	JCMS vs. **K279a**
F35E8.1	F35E8.1	0.023398524	2.8146644	up	JCMS vs. **K279a**
bcmo-1	Y46G5A.24	0.018479727	2.8027186	down	JCMS vs. **K279a**
E02C12.8	E02C12.8b	0.023416784	2.7923608	up	JCMS vs. **K279a**
cyp-35A5	K07C6.5	0.031215737	2.7789774	down	JCMS vs. **K279a**
numr-1	F08F8.5	0.030570457	2.7712927	up	JCMS vs. **K279a**
cwp-4	K11D12.1	0.034811806	2.7645028	down	JCMS vs. **K279a**
C18H7.11	C18H7.11	0.024047945	2.7574396	up	JCMS vs. **K279a**
F56B3.9	F56B3.9	0.040522408	2.7505083	up	JCMS vs. **K279a**
T05F1.11	T05F1.11	0.0398986	2.738262	down	JCMS vs. **K279a**
F43C11.8	F43C11.8	0.015864044	2.7224658	up	JCMS vs. **K279a**
str-162	E03H12.1	0.002704226	2.7008302	down	JCMS vs. **K279a**
nex-4	C37H5.1	0.043472152	2.695738	down	JCMS vs. **K279a**
F35E12.9	F35E12.9b	0.002704226	2.680187	up	JCMS vs. **K279a**
T05E12.3	T05E12.3	0.034811806	2.6747677	down	JCMS vs. **K279a**
C03H5.7	C03H5.7	0.030570457	2.6654096	down	JCMS vs. **K279a**
C18B2.2	C18B2.2	0.024452075	2.6467037	down	JCMS vs. **K279a**
F19C7.6	F19C7.6	0.030055868	2.6437697	up	JCMS vs. **K279a**
cyp-13A5	T10B9.2	0.048808206	2.622028	down	JCMS vs. **K279a**
T19D7.5	T19D7.5	0.03570299	2.6188521	down	JCMS vs. **K279a**

R09A1.3	R09A1.3	0.030055868	2.6171632	up	JCMS vs. **K279a**
F49F1.6	F49F1.6	0.030174859	2.6092024	up	JCMS vs. **K279a**
ZK1055.7	ZK1055.7	0.008675934	2.6062374	up	JCMS vs. **K279a**
clec-7	F10G2.3	0.006968097	2.6054037	down	JCMS vs. **K279a**
F19C7.5	F19C7.5	0.032612246	2.6032863	up	JCMS vs. **K279a**
Y73F8A.35	Y73F8A.35	0.045368545	2.5978796	down	JCMS vs. **K279a**
T09B9.3	T09B9.3	0.04869883	2.5972717	down	JCMS vs. **K279a**
Y82E9BL.18	Y82E9BL.18	0.02155503	2.5894032	up	JCMS vs. **K279a**
F55G11.8	F55G11.8	0.018187836	2.5874596	up	JCMS vs. **K279a**
clec-28	F49A5.5b	0.044467654	2.5838923	down	JCMS vs. **OP50**
Y69A2AR.5	Y69A2AR.5	0.028550781	2.5625331	down	JCMS vs. **K279a**
twk-9	ZK1251.8	0.028550781	2.5603218	down	JCMS vs. **K279a**
F58G6.9	F58G6.9a	0.028550781	2.5569139	down	JCMS vs. **K279a**
Y17D7B.2	Y17D7B.2	0.047187086	2.551688	up	JCMS vs. **K279a**
T08G11.3	T08G11.3	0.04345726	2.546843	down	JCMS vs. **K279a**
cyp-34A9	B0213.15c	0.029480534	2.528082	down	JCMS vs. **K279a**
ZK1240.6	ZK1240.6	0.032894265	2.5057452	up	JCMS vs. **K279a**
Y113G7B.14	Y113G7B.14	0.014101754	2.5021906	up	JCMS vs. **K279a**
Y119C1B.3	Y119C1B.3	0.013737984	2.489872	down	JCMS vs. **K279a**
C05E7.3	C05E7.3	0.026643677	2.485823	down	JCMS vs. **K279a**
pme-5	ZK1005.1a	0.013122048	2.4696016	up	JCMS vs. **K279a**
T05F1.9	T05F1.9	0.03490468	2.466899	up	JCMS vs. **K279a**
F58G6.9	F58G6.9b	0.044151295	2.46054	down	JCMS vs. **K279a**
nhr-167	C49F5.4	0.045368545	2.4588962	down	JCMS vs. **K279a**
K11H12.4	K11H12.4	0.049546637	2.4494026	up	JCMS vs. **K279a**
B0244.5	B0244.5	0.03483439	2.4392924	down	JCMS vs. **K279a**
F43C9.1	F43C9.1	0.028550781	2.4195716	down	JCMS vs. **K279a**
F53B7.2	F53B7.2	0.018985962	2.4081595	down	JCMS vs. **K279a**
clec-140	T05A7.2	0.014726291	2.4049413	up	JCMS vs. **K279a**
ubxn-3	F48A11.5a	0.028550781	2.395927	up	JCMS vs. **K279a**
lgc-11	F48E3.7	0.04934841	2.3889809	down	JCMS vs. **K279a**
R13H7.2	R13H7.2b	0.03570299	2.3889477	down	JCMS vs. **K279a**
Y50D7A.5	Y50D7A.5	0.03600195	2.3886034	up	JCMS vs. **K279a**
fbxa-30	ZC47.4	0.030908348	2.381512	up	JCMS vs. **K279a**
F58B4.5	F58B4.5	0.010202582	2.3720615	down	JCMS vs. **K279a**
F58G6.8	F58G6.8	0.024452075	2.362775	down	JCMS vs. **K279a**
T02B5.3	T02B5.3	0.046352427	2.357667	down	JCMS vs. **K279a**
C48B4.1	C48B4.1	0.03570299	2.3529356	down	JCMS vs. **K279a**
Y34F4.2	Y34F4.2b	0.014975314	2.3513885	down	JCMS vs. **K279a**
R03G5.5	R03G5.5b	0.022911746	2.3420885	down	JCMS vs. **K279a**
Y54G2A.5	Y54G2A.5b	0.040522408	2.332621	up	JCMS vs. **K279a**
nhr-112	Y70C5C.6b	0.02465265	2.3285453	up	JCMS vs. **K279a**
ubxn-3	F48A11.5b	0.026941897	2.3257072	up	JCMS vs. **K279a**

gcy-22	T03D8.5	0.03490468	2.3252292	down	JCMS vs. **K279a**
K10C2.2	K10C2.2	0.031215737	2.3200014	down	JCMS vs. **K279a**
che-12	B0024.8	0.029384451	2.314695	down	JCMS vs. **K279a**
R13H7.2	R13H7.2a	0.014975314	2.2967906	down	JCMS vs. **K279a**
T05A7.3	T05A7.3	0.01074132	2.292454	up	JCMS vs. **K279a**
aqp-9	K07A1.16	0.045058887	2.289289	down	JCMS vs. **K279a**
Y75B7B.2	Y75B7B.2	0.04090871	2.275684	up	JCMS vs. **K279a**
Y34F4.2	Y34F4.2a	0.018985962	2.2751365	down	JCMS vs. **K279a**
srw-145	R10D12.17	0.028550781	2.272631	down	JCMS vs. **K279a**
asm-2	ZK455.4	0.04387101	2.2653534	down	JCMS vs. **K279a**
odc-1	K11C4.4	0.013122048	2.2418237	up	JCMS vs. **K279a**
T08G5.7	T08G5.7	0.04994786	2.237912	up	JCMS vs. **K279a**
acs-17	C46F4.2	0.034534205	2.233329	down	JCMS vs. **K279a**
C25D7.5	C25D7.5	0.045808833	2.2268002	up	JCMS vs. **K279a**
Y43F8B.15	Y43F8B.15	0.030425193	2.223969	up	JCMS vs. **K279a**
F55G7.1	F55G7.1	0.040522408	2.2170167	up	JCMS vs. **K279a**
Y60A9.1	Y60A9.1	0.020579303	2.2081168	down	JCMS vs. **K279a**
Y40B1A.2	Y40B1A.2	0.031215737	2.207644	up	JCMS vs. **K279a**
nhr-144	F59E11.12b	0.04289596	2.2075257	down	JCMS vs. **K279a**
K08D10.10	K08D10.10	0.043472152	2.200963	up	JCMS vs. **K279a**
Y37A1B.5	Y37A1B.5	0.031215737	2.1980653	down	JCMS vs. **K279a**
gcy-14	ZC412.2	0.040522408	2.185077	down	JCMS vs. **K279a**
xbx-3	M04D8.6	0.043064047	2.177181	down	JCMS vs. **K279a**
pho-1	EGAP2.3	0.028550781	2.1660457	down	JCMS vs. **K279a**
sre-1	B0495.1	0.04086817	2.1596591	down	JCMS vs. **K279a**
Y82E9BR.13	Y82E9BR.13	0.028550781	2.1593437	up	JCMS vs. **K279a**
fbxa-218	Y49E10.17	0.033603776	2.155939	up	JCMS vs. **K279a**
C49G7.12	C49G7.12	0.013472779	2.153203	up	JCMS vs. **K279a**
F28A12.3	F28A12.3	0.048145175	2.150003	down	JCMS vs. **K279a**
E02C12.8	E02C12.8a	0.042742778	2.1472645	up	JCMS vs. **K279a**
eak-6	F10G8.4a	0.04815337	2.1457975	down	JCMS vs. **K279a**
R10E11.5	R10E11.5	0.042742778	2.1455803	down	JCMS vs. **K279a**
Y53G8AR.1	Y53G8AR.1	0.04533843	2.1452768	up	JCMS vs. **K279a**
M02F4.1	M02F4.1	0.03131432	2.141954	up	JCMS vs. **K279a**
lgc-38	F11H8.2	0.040651113	2.1359448	down	JCMS vs. **K279a**
glr-1	C06E1.4	0.030055868	2.1284325	down	JCMS vs. **K279a**
che-11	C27A7.4	0.04934841	2.126832	down	JCMS vs. **K279a**
C27A7.1	C27A7.1b	0.028550781	2.1200242	down	JCMS vs. **K279a**
R03G5.5	R03G5.5a	0.03932306	2.1077402	down	JCMS vs. **K279a**
C02C2.4	C02C2.4	0.0374213	2.10336	down	JCMS vs. **K279a**
M176.11	M176.11	0.048808206	2.1016767	down	JCMS vs. **K279a**
C27A7.1	C27A7.1a	0.030425193	2.091023	down	JCMS vs. **K279a**
R11E3.2	R11E3.2	0.04106163	2.0907052	down	JCMS vs. **K279a**

C02B4.4	C02B4.4	0.042742778	2.0884178	down	JCMS vs. **K279a**
cal-3	M02B7.6	0.044726003	2.0870142	down	JCMS vs. **K279a**
F10E7.2	F10E7.2	0.048198223	2.085245	up	JCMS vs. **K279a**
F54D12.4	F54D12.4	0.04345726	2.0761042	up	JCMS vs. **K279a**
F49C12.14	F49C12.14	0.034811806	2.0731337	down	JCMS vs. **K279a**
Y71A12B.10	Y71A12B.10	0.028550781	2.0668995	up	JCMS vs. **K279a**
clec-194	Y116A8A.8	0.04934841	2.0617044	down	JCMS vs. **K279a**
Y58A7A.3	Y58A7A.3	0.030055868	2.0593345	up	JCMS vs. **K279a**
fbxa-98	C08F11.5	0.028550781	2.03866	down	JCMS vs. **K279a**
pqn-42	F53G2.4a	0.03490468	2.0339327	up	JCMS vs. **K279a**
nas-25	F46C5.3	0.038542368	2.0307205	down	JCMS vs. **K279a**
Y19D10B.3	Y19D10B.3	0.048808206	2.0306332	down	JCMS vs. **K279a**
ZK105.5	ZK105.5	0.040522408	2.0290573	up	JCMS vs. **K279a**
C08F11.1	C08F11.1	0.0419092	2.0228615	down	JCMS vs. **K279a**
F55B11.4	F55B11.4	0.042742778	2.021856	up	JCMS vs. **K279a**
F19G12.4	F19G12.4	0.039219737	2.021552	up	JCMS vs. **K279a**
R06F6.7	R06F6.7	0.043428164	2.018966	down	JCMS vs. **K279a**
clec-67	F56D6.2	0.040522408	2.018523	up	JCMS vs. **K279a**
Y48G1BM.5	Y48G1BM.5	0.041303933	2.0149834	up	JCMS vs. **K279a**
C25G6.1	C25G6.1	0.01822858	2.0137663	down	JCMS vs. **K279a**
F02C12.2	F02C12.2	0.028550781	2.0071678	down	JCMS vs. **K279a**
M117.4	M117.4	0.04274996	2.0032787	up	JCMS vs. **K279a**
ZK1240.9	ZK1240.9	0.04827613	2.002712	up	JCMS vs. **K279a**
F36D1.6	F36D1.6	0.032612246	2.001769	up	JCMS vs. **K279a**
ZK1055.6	ZK1055.6a	0.028550781	1.9955378	up	JCMS vs. **K279a**
C41C4.3	C41C4.3	0.039950997	1.9947385	down	JCMS vs. **K279a**
C14C6.2	C14C6.2	0.044564333	1.9943364	down	JCMS vs. **K279a**
F46B6.2	F46B6.2	0.028550781	1.9931067	down	JCMS vs. **K279a**
rom-2	C48B4.2	0.04090871	1.9911656	down	JCMS vs. **K279a**
E04A4.6	E04A4.6	0.024452075	1.9901129	up	JCMS vs. **K279a**
T08B2.3	T08B2.3	0.034811806	1.9898045	up	JCMS vs. **K279a**
F27C8.2	F27C8.2	0.030055868	1.9856819	down	JCMS vs. **K279a**
F59D6.3	F59D6.3	0.048808206	1.9812044	down	JCMS vs. **K279a**
srsx-29	C51E3.4	0.03570299	1.9797859	down	JCMS vs. **K279a**
Y67D8C.3	Y67D8C.3a	0.041092202	1.9769925	up	JCMS vs. **K279a**
T01G6.10	T01G6.10	0.0475993	1.9708395	down	JCMS vs. **K279a**
nca-1	C11D2.6c	0.049158946	1.9706376	down	JCMS vs. **K279a**
srd-15	C04E6.10	0.03448531	1.970504	down	JCMS vs. **K279a**
spp-4	T08A9.8	0.03570299	1.9567186	down	JCMS vs. **K279a**
C27A7.8	C27A7.8b	0.048808206	1.9563383	down	JCMS vs. **K279a**
trk-1	D1073.1a	0.03352219	1.9550177	down	JCMS vs. **K279a**
F59F3.6	F59F3.6	0.04869883	1.9527658	down	JCMS vs. **K279a**
ZK484.6	ZK484.6	0.032857217	1.9519114	up	JCMS vs. **K279a**

C09D4.1	C09D4.1b	0.03112937	1.951794	down	JCMS vs. **K279a**
F15E11.4	F15E11.4	0.045410227	1.9517282	down	JCMS vs. **K279a**
tba-7	T28D6.2	0.034811806	1.9516444	up	JCMS vs. **K279a**
C41G7.7	C41G7.7	0.018985962	1.9487039	down	JCMS vs. **K279a**
lgc-47	F47A4.1b	0.041303933	1.9486008	down	JCMS vs. **K279a**
ugt-1	AC3.7	0.03570299	1.9485377	down	JCMS vs. **K279a**
Y75B8A.28	Y75B8A.28	0.041625626	1.9451518	up	JCMS vs. **K279a**
glr-5	ZC196.7	0.022911746	1.9406823	down	JCMS vs. **K279a**
F25D1.5	F25D1.5	0.016857263	1.9337225	down	JCMS vs. **K279a**
R05G9.2	R05G9.2a	0.034480248	1.9336687	down	JCMS vs. **K279a**
fbxa-79	Y82E9BL.13	0.03490468	1.9314344	up	JCMS vs. **K279a**
C01B12.5	C01B12.5	0.046601456	1.9314051	down	JCMS vs. **K279a**
Y7A5A.1	Y7A5A.1	0.03490468	1.930615	down	JCMS vs. **K279a**
twk-14	K01D12.4	0.04869883	1.9296019	down	JCMS vs. **K279a**
F23H11.7	F23H11.7	0.039219737	1.9276334	down	JCMS vs. **K279a**
M01F1.8	M01F1.8b	0.044151295	1.9257995	up	JCMS vs. **K279a**
fbxa-53	F07G6.7	0.03464283	1.9233339	down	JCMS vs. **K279a**
C10C5.2	C10C5.2	0.04994786	1.917649	up	JCMS vs. **K279a**
prx-3	C15H9.8a	0.033573005	1.9159106	down	JCMS vs. **K279a**
F19C6.4	F19C6.4a	0.03570299	1.9151382	down	JCMS vs. **K279a**
nhr-123	M02H5.7	0.030908348	1.9132345	up	JCMS vs. **K279a**
clec-206	F59A7.1	0.046352427	1.9109688	down	JCMS vs. **K279a**
F19C6.4	F19C6.4b	0.03307332	1.9094397	down	JCMS vs. **K279a**
egl-13	T22B7.1d	0.040522408	1.9074717	down	JCMS vs. **K279a**
F53B2.8	F53B2.8	0.031215737	1.904862	up	JCMS vs. **K279a**
cyp-13A10	ZK1320.4	0.048808206	1.9036196	down	JCMS vs. **K279a**
cyp-34A2	T10H4.11	0.038228717	1.9032409	down	JCMS vs. **K279a**
trk-1	D1073.1b	0.030908348	1.9029629	down	JCMS vs. **K279a**
C35E7.2	C35E7.2a	0.040522408	1.9017708	down	JCMS vs. **K279a**
R05G9.2	R05G9.2b	0.036853842	1.9014893	down	JCMS vs. **K279a**
T25B6.6	T25B6.6	0.03570299	1.9012824	down	JCMS vs. **K279a**
C03A7.13	C03A7.13	0.03570299	1.899925	up	JCMS vs. **K279a**
prx-3	C15H9.8b	0.040651113	1.8987058	down	JCMS vs. **K279a**
tag-178	B0495.10a	0.03307332	1.8976481	down	JCMS vs. **K279a**
crn-2	CD4.2	0.024452075	1.8921117	up	JCMS vs. **K279a**
R03H10.7	R03H10.7	0.04345726	1.8876929	up	JCMS vs. **K279a**
lgc-47	F47A4.1a	0.042742778	1.8854693	down	JCMS vs. **K279a**
nhr-284	T20C7.2	0.04869883	1.884926	down	JCMS vs. **K279a**
T21D12.9	T21D12.9a	0.048145175	1.8848114	down	JCMS vs. **K279a**
F16F9.3	F16F9.3b	0.032612246	1.883988	down	JCMS vs. **K279a**
R05A10.3	R05A10.3	0.030055868	1.8816506	down	JCMS vs. **K279a**
F12E12.11	F12E12.11	0.033139024	1.8757962	down	JCMS vs. **K279a**
lys-10	F17E9.11	0.018985962	1.8751354	down	JCMS vs. **K279a**

egl-13	T22B7.1b	0.03490468	1.8731508	down	JCMS vs. **K279a**
T02B11.4	T02B11.4	0.040522408	1.8683612	down	JCMS vs. **K279a**
asic-2	T28F4.2	0.040911943	1.8663714	down	JCMS vs. **K279a**
mgl-1	ZC506.4a	0.040522408	1.8657176	down	JCMS vs. **K279a**
C04G6.5	C04G6.5	0.034811806	1.8650703	up	JCMS vs. **K279a**
ggr-1	C09G5.1	0.04934841	1.8632022	down	JCMS vs. **K279a**
F09F7.4	F09F7.4a	0.039219737	1.857894	down	JCMS vs. **K279a**
ZK1321.2	ZK1321.2b	0.021732002	1.8570946	down	JCMS vs. **K279a**
set-28	Y73B3B.2	0.028550781	1.8521025	up	JCMS vs. **K279a**
F44E7.7	F44E7.7	0.034480248	1.8495271	down	JCMS vs. **K279a**
ZC239.21	ZC239.21	0.03483439	1.8418937	down	JCMS vs. **K279a**
hint-3	C26F1.7	0.03490468	1.8392067	down	JCMS vs. **K279a**
bbs-2	F20D12.3	0.040522408	1.8350352	down	JCMS vs. **K279a**
tag-178	B0495.10c	0.038274776	1.8348339	down	JCMS vs. **K279a**
sue-1	F07A5.5	0.040522408	1.832486	down	JCMS vs. **K279a**
R102.4	R102.4b	0.041206796	1.8299972	down	JCMS vs. **K279a**
F55A12.6	F55A12.6	0.03570299	1.8230835	up	JCMS vs. **K279a**
K04D7.6	K04D7.6	0.04934841	1.8198856	down	JCMS vs. **K279a**
ugt-2	AC3.8	0.041837987	1.8110349	down	JCMS vs. **K279a**
tag-178	B0495.10b	0.03570299	1.8089328	down	JCMS vs. **K279a**
Y17D7B.3	Y17D7B.3	0.03131432	1.806058	up	JCMS vs. **K279a**
pme-5	ZK1005.1b	0.030055868	1.8024597	up	JCMS vs. **K279a**
kqt-1	C25B8.1a	0.040522408	1.8001318	down	JCMS vs. **K279a**
nhr-156	C17E7.1	0.044269264	1.7950081	down	JCMS vs. **K279a**
F13B12.3	F13B12.3	0.044408925	1.7939677	down	JCMS vs. **K279a**
F40G126.	F40G12.6	0.04869883	1.7925559	down	JCMS vs. **K279a**
ugt-62	M88.1	0.033528186	1.7923853	down	JCMS vs. **K279a**
F53B7.4	F53B7.4	0.045368545	1.7922591	down	JCMS vs. **K279a**
Y57G11C.44	Y57G11C.44	0.037168607	1.789421	down	JCMS vs. **K279a**
ZK1321.2	ZK1321.2d	0.023416784	1.7859691	down	JCMS vs. **K279a**
F43E2.5	F43E2.5	0.044833507	1.7854958	down	JCMS vs. **K279a**
nhr-188	F47C10.7	0.040893234	1.7806431	down	JCMS vs. **K279a**
nlp-16	T13A10.5	0.04086817	1.7797147	down	JCMS vs. **K279a**
ZK1321.2	ZK1321.2e	0.024452075	1.7744285	down	JCMS vs. **K279a**
flp-11	K02G10.4a	0.028550781	1.7734762	down	JCMS vs. **K279a**
dod-23	F49E12.2	0.030055868	1.7721064	up	JCMS vs. **K279a**
K07H8.11	K07H8.11	0.04869883	1.7709447	down	JCMS vs. **K279a**
ZK1321.2	ZK1321.2c	0.028550781	1.7697113	down	JCMS vs. **K279a**
Y7A5A.11	Y7A5A.11	0.04994786	1.7693335	up	JCMS vs. **K279a**
Y54G2A.11	Y54G2A.11a	0.03742433	1.768225	down	JCMS vs. **K279a**
C10G11.6	C10G11.6	0.047759306	1.7667325	up	JCMS vs. **K279a**
C18G1.6	C18G1.6	0.030908348	1.7640367	down	JCMS vs. **K279a**
ugt-55	T04H1.7	0.048808206	1.7638787	down	JCMS vs. **K279a**

K01C8.1	K01C8.1	0.030055868	1.7619791	down	JCMS vs. **K279a**
M01F1.8	M01F1.8a	0.042742778	1.757884	up	JCMS vs. **K279a**
ZK1321.2	ZK1321.2a	0.028550781	1.7507949	down	JCMS vs. **K279a**
F53B6.2	F53B6.2b	0.0374213	1.7477375	down	JCMS vs. **K279a**
exp-2	F12F3.1b	0.039219737	1.7447168	down	JCMS vs. **K279a**
bath-19	F59H6.1	0.041117292	1.7321781	up	JCMS vs. **K279a**
ins-35	K02E2.4	0.030570457	1.7315233	down	JCMS vs. **K279a**
dlc-3	Y10G11A.2b	0.048500955	1.7276474	down	JCMS vs. **K279a**
R09E12.9	R09E12.9	0.048500955	1.725788	down	JCMS vs. **K279a**
srw-24	C41G6.7	0.03808078	1.7249954	down	JCMS vs. **K279a**
M163.8	M163.8	0.04877365	1.7188601	down	JCMS vs. **K279a**
exp-2	F12F3.1d	0.040911943	1.717512	down	JCMS vs. **K279a**
Y45G12C.1	Y45G12C.1	0.04877365	1.7145842	up	JCMS vs. **K279a**
C41G7.8	C41G7.8	0.03742433	1.7127185	up	JCMS vs. **K279a**
nhr-60	F57A10.5	0.04815337	1.7092502	up	JCMS vs. **K279a**
str-156	Y9C9A.11	0.04815337	1.7061661	down	JCMS vs. **K279a**
DH11.2	DH11.2	0.03131432	1.7013183	down	JCMS vs. **K279a**
acr-7	T09A5.3	0.028550781	1.6990278	down	JCMS vs. **K279a**
srj-1	ZK829.8	0.026941897	1.6898063	down	JCMS vs. **K279a**
F22E5.9	F22E5.9	0.034811806	1.6897995	up	JCMS vs. **K279a**
ugt-22	C08F11.8	0.040522408	1.6847504	down	JCMS vs. **K279a**
fbxa-15	Y82E9BL.11	0.040522408	1.6841569	up	JCMS vs. **K279a**
fipr-1	F23H12.8	0.034480248	1.6805714	down	JCMS vs. **K279a**
flp-11	K02G10.4b	0.04888181	1.6800139	down	JCMS vs. **K279a**
clec-4	Y38E10A.5	0.034480248	1.677538	up	JCMS vs. **K279a**
cyp-33E2	F42A9.5	0.04090871	1.6771789	down	JCMS vs. **K279a**
fbxa-94	F28F8.8	0.044833507	1.6767371	up	JCMS vs. **K279a**
C25H3.12	C25H3.12	0.041092202	1.6726538	up	JCMS vs. **K279a**
C04G2.11	C04G2.11	0.04877365	1.6724299	down	JCMS vs. **K279a**
Y66D12A.6	Y66D12A.6	0.04086817	1.6714369	up	JCMS vs. **K279a**
kqt-1	C25B8.1b	0.04872105	1.668859	down	JCMS vs. **K279a**
F16H6.10	F16H6.10	0.049546637	1.6676933	up	JCMS vs. **K279a**
F55A4.7	F55A4.7	0.038411703	1.6611776	down	JCMS vs. **K279a**
stl-1	F30A10.5	0.030055868	1.6605046	down	JCMS vs. **K279a**
twk-43	F32H5.7	0.044564333	1.6596388	down	JCMS vs. **K279a**
avr-14	B0207.12b	0.049129996	1.6571361	down	JCMS vs. **K279a**
T19D12.4	T19D12.4a	0.028550781	1.6570386	up	JCMS vs. **K279a**
pqn-66	T16A1.7	0.048808206	1.6545621	up	JCMS vs. **K279a**
D2023.4	D2023.4	0.04934841	1.6531273	down	JCMS vs. **K279a**
srr-1	W07G4.6	0.046548683	1.6515795	down	JCMS vs. **K279a**
T19D12.4	T19D12.4b	0.028550781	1.6493986	up	JCMS vs. **K279a**
srh-204	E03D2.3	0.043472152	1.6434877	down	JCMS vs. **K279a**
T20D4.17	T20D4.17	0.048808206	1.6431254	up	JCMS vs. **K279a**

Y41C4A.6	Y41C4A.6	0.030908348	1.639913	down	JCMS vs. **K279a**
C43F9.5	C43F9.5	0.048500955	1.6361848	down	JCMS vs. **K279a**
C06A8.8	C06A8.8a	0.046601456	1.6324382	down	JCMS vs. **K279a**
C27H5.2	C27H5.2d	0.04888181	1.6285645	down	JCMS vs. **K279a**
C16D6.2	C16D6.2	0.048885334	1.6130383	down	JCMS vs. **K279a**
Y37D8A.18	Y37D8A.18	0.03570299	1.6124098	up	JCMS vs. **K279a**
F46C3.2	F46C3.2	0.046601456	1.6074984	down	JCMS vs. **K279a**
B0391.10	B0391.10	0.040651113	1.606022	up	JCMS vs. **K279a**
clec-10	C03H5.1	0.046601456	1.6056792	down	JCMS vs. **K279a**
cdh-10	C45G7.5	0.044269264	1.6031455	down	JCMS vs. **K279a**
F42G8.10	F42G8.10a	0.04815337	1.5972579	down	JCMS vs. **K279a**
cpr-4	F44C4.3	0.038228717	1.597035	down	JCMS vs. **K279a**
K08D8.4	K08D8.4c	0.046601456	1.5943484	up	JCMS vs. **K279a**
M02H5.8	M02H5.8	0.039219737	1.5893376	up	JCMS vs. **K279a**
T01D3.6	T01D3.6a	0.04090871	1.5858722	up	JCMS vs. **K279a**
H25P06.4	H25P06.4	0.0419092	1.5850699	up	JCMS vs. **K279a**
T18D3.7	T18D3.7	0.04934841	1.5755402	down	JCMS vs. **K279a**
R08D7.1	R08D7.1	0.04090871	1.5746074	up	JCMS vs. **K279a**
F35A5.2	F35A5.2	0.044269264	1.57101	down	JCMS vs. **K279a**
C17C3.1	C17C3.1b	0.040911943	1.5676676	down	JCMS vs. **K279a**
mpk-2	C04G6.1a	0.04877365	1.566992	down	JCMS vs. **K279a**
mpk-2	C04G6.1c	0.046601456	1.5583347	down	JCMS vs. **K279a**
C26B2.1	C26B2.1	0.040911943	1.5533066	up	JCMS vs. **K279a**
C18G1.7	C18G1.7	0.04090871	1.5437826	down	JCMS vs. **K279a**
T26E3.8	T26E3.8	0.040651113	1.5364281	down	JCMS vs. **K279a**
grp-1	K06H7.4	0.04090871	1.535427	up	JCMS vs. **K279a**
R102.4	R102.4a	0.043652575	1.5330052	down	JCMS vs. **K279a**
F28A12.4	F28A12.4	0.043064047	1.5161399	down	JCMS vs. **K279a**
spp-2	T08A9.12	0.047187086	1.5130311	down	JCMS vs. **K279a**
F09F7.4	F09F7.4b	0.046176136	1.5082997	down	JCMS vs. **K279a**
F42G8.10	F42G8.10b	0.044151295	1.5016463	down	JCMS vs. **K279a**

Differential expression was determined via all pairwise comparisons of *C. elegans* gene expression on *S. maltophilia* JCMS, *S. maltophilia* K279a and *E. coli* OP50. The bolded strain was the baseline treatment in the indicated comparison. Statistical significance was determined using a moderated T test and a Benjamini-Hochberg multiple testing correction with a 1.5 fold change cut off. Only the corrected p values are listed for each transcript and comparison. A transcript was considered significantly differentially expressed if the corrected p value was less than 0.05. There were 438 significantly differentially expressed transcripts representing 395 unique genes. All differentially expressed transcripts are shown here listed by fold change from largest to smallest. The gene isoform is denoted in the sequence name column if applicable.

Table A.8 Gene ontology and terms for all *C. elegans* significantly differentially expressed genes on *E. coli* OP50, *S. maltophilia* JCMS or K279a.

Gene public name	Sequence name	GO Term(s)
ugt-1	AC3.7	BP: positive regulation of growth rate, determination of adult life span, carbohydrate metabolic process and lipid glycosylation MF: transferase activity, transferring hexosyl groups and carbohydrate binding CC: integral to membrane
ugt-2	AC3.8	BP: carbohydrate metabolic process and lipid glycosylation MF: transferase activity, transferring hexosyl groups, carbohydrate binding CC: integral to membrane
B0024.4	B0024.4	BP: positive regulation of growth rate
che-12	B0024.8	BP: chemotaxis, sensory cilium assembly, hyperosmotic response, dauer entry MF: binding
B0244.5	B0244.5	CC: integral to membrane
B0391.10	B0391.10	Unknown
sre-1	B0495.1	BP: sensory perception of chemical stimulus MF: transmembrane receptor activity CC: integral to membrane
C01B12.5	C01B12.5	CC: integral to membrane
C01G10.15	C01G10.15	CC: integral to membrane
C02B4.4	C02B4.4	CC: integral to membrane
C02C2.4	C02C2.4	BP: transmembrane transport CC: integral to membrane
ugt-51	C03A7.11	BP: carbohydrate metabolic process and lipid glycosylation MF: transferase activity, transferring hexosyl groups, carbohydrate binding CC: integral to membrane
C03A7.13	C03A7.13	BP: metabolic process MF: transferase activity, transferring hexosyl groups CC: integral to membrane
tag-293	C03G6.13	CC: integral to membrane
clec-10	C03H5.1	MF: binding
C03H5.7	C03H5.7	CC: integral to membrane
srd-15	C04E6.10	CC: integral to membrane
C04G2.11	C04G2.11	CC: membrane
C04G6.5	C04G6.5	CC: integral to membrane
C05E7.3	C05E7.3	CC: integral to membrane

glr-1	C06E1.4	BP: response to mechanical stimulus, ionotropic glutamate receptor signaling pathway, ion transport, feeding behavior, lipid storage and sensory perception of bitter taste MF: alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionate selective glutamate receptor activity and ion channel activity CC: integral to membrane, postsynaptic membrane, cell soma, neuron, ionotropic glutamate receptor complex, dendrite and outer membrane-bounded periplasmic space
irg-1	C07G3.2	BP: defense response to Gram-negative bacterium and innate immune
fbxa-161	C08E3.4	Unknown
fbxa-162	C08E3.5	Unknown
fbxa-163	C08E3.6	BP: positive regulation of growth rate
C08F11.1	C08F11.1	CC: integral to membrane
fbxa-98	C08F11.5	Unknown
ugt-22	C08F11.8	BP: carbohydrate metabolic process and lipid glycosylation MF: transferase activity, transferring hexosyl groups, carbohydrate binding CC: integral to membrane
ggr-1	C09G5.1	BP: taxis and ion transport MF: extracellular ligand-gated ion channel activity CC: membrane, integral to membrane and postsynaptic membrane
C10C5.2	C10C5.2	Unknown
C10G11.6	C10G11.6	Unknown
C12D5.3	C12D5.3	BP: protein folding MF: ATP binding and unfolded protein binding
C14C6.2	C14C6.2	Unknown
C16D6.2	C16D6.2	BP: G-protein coupled receptor protein signaling pathway MF: neuropeptide Y receptor activity CC: integral to membrane
nhr-156	C17E7.1	BP: regulation of transcription MF: transcription factor activity, sequence-specific DNA binding and zinc ion binding CC: nucleus
C18B2.2	C18B2.2	BP: carbohydrate biosynthetic process MF: sulfotransferase activity CC: integral to membrane
C18G1.6	C18G1.6	CC: integral to membrane
C18G1.7	C18G1.7	Unknown
C18H7.11	C18H7.11	CC: integral to membrane

C18H7.4	C18H7.4	MF: ATP binding, protein binding, protein kinase activity, protein serine/threonine kinase activity and protein tyrosine kinase activity BP: protein amino acid phosphorylation
C25D7.5	C25D7.5	CC: integral to membrane
C25F9.11	C25F9.11	Unknown
C25F9.6	C25F9.6	Unknown
srw-86	C25F9.7	Unknown
C25G6.1	C25G6.1	Unknown
C25G6.4	C25G6.4	Unknown
C25H3.12	C25H3.12	Unknown
dnc-4	C26B2.1	BP: embryonic development ending in birth or egg hatching and pronuclear migration
hint-3	C26F1.7	MF: catalytic activity CC: integral to membrane
che-11	C27A7.4	BP: dauer entry, determination of adult life span, response to heat, hyperosmotic response, intraflagellar transport and response to oxidative stress MF: protein binding CC: intraflagellar transport particle A
nex-4	C37H5.1	MF: calcium-dependent phospholipid binding
C41C4.3	C41C4.3	Unknown
srw-24	C41G6.7	CC: integral to membrane
C41G7.7	C41G7.7	Unknown
C41G7.8	C41G7.8	CC: integral to membrane
C43F9.5	C43F9.5	CC: integral to membrane
ugt-15	C44H9.1	BP: carbohydrate metabolic process and lipid glycosylation MF: transferase activity, transferring hexosyl groups, carbohydrate binding CC: integral to membrane
ilys-3	C45G7.3	MF: lysozyme activity
cdh-10	C45G7.5	BP: cell adhesion and homophilic cell adhesion MF: calcium ion binding CC: membrane, plasma membrane and integral to membrane
acs-17	C46F4.2	BP: determination of adult life span, metabolic process MF: catalytic activity CC: integral to membrane (IEA)
C48B4.1	C48B4.1	BP: metabolic process and oxidation reduction MF: acyl-CoA dehydrogenase activity and oxidoreductase activity, acting on the CH-CH group of donors and FAD binding

rom-2	C48B4.2	MF: serine-type endopeptidase activity and calcium ion binding CC: integral to membrane
nhr-167	C49F5.4	BP: regulation of transcription MF: transcription factor activity, steroid hormone receptor activity, sequence-specific DNA binding and zinc ion binding CC: nucleus
C49G7.12	C49G7.12	Unknown
srsx-29	C51E3.4	BP: G-protein coupled receptor protein signaling pathway CC: integral to membrane
C53A3.1	C53A3.1	Unknown
C54D10.12	C54D10.12	Unknown
ftn-1	C54F6.14	BP: cellular iron ion homeostasis, embryonic development ending in birth or egg hatching, determination of adult life span and iron ion transport MF: binding and ferric iron binding
crn-2	CD4.2	BP: DNA fragmentation involved in apoptosis, DNA fragmentation involved in apoptosis, apoptotic cell clearance MF:deoxyribonuclease activity, endodeoxyribonuclease activity, producing 5'-phosphomonoesters
D2023.4	D2023.4	Unknown
DH11.2	DH11.2	Unknown
E02C12.6	E02C12.6	Unknown
srh-204	E03D2.3	CC: integral to membrane
str-162	E03H12.1	CC: integral to membrane
E04A4.6	E04A4.6	CC: integral to membrane
pho-1	EGAP2.3	BP: nematode larval development, growth, embryonic development ending in birth or egg hatching, reproduction and receptor-mediated endocytosis MF: acid phosphatase activity CC: integral to membrane
F02C12.2	F02C12.2	BP: metabolic process and oxidation reduction MF: oxidoreductase activity, 2,3-dihydro-2,3-dihydroxybenzoate dehydrogenase activity, enterobactin biosynthetic process, catalytic activity and binding
sue-1	F07A5.5	Unknown
str-116	F07B10.2	CC: integral to membrane
clec-45	F07C4.2	MF: binding CC: integral to membrane
F07C4.6	F07C4.6	Unknown
fbxa-53	F07G6.7	Unknown

numr-1	F08F8.5	CC: nucleus
F08G2.5	F08G2.5	Unknown
F09G8.5	F09G8.5	CC: integral to membrane
F10A3.1	F10A3.1	CC: integral to membrane
fbxa-88	F10A3.2	Unknown
str-204	F10D2.1	CC: integral to membrane
F10E7.2	F10E7.2	BP: embryonic development ending in birth or egg hatching CC: integral to membrane
clec-7	F10G2.3	MF: binding
srh-279	F11A5.2	CC: integral to membrane
F11C7.7	F11C7.7	Unknown
lgc-38	F11H8.2	BP: ion transport MF: GABA-A receptor activity and extracellular ligand-gated ion channel activity CC: membrane, integral to membrane and postsynaptic membrane
F12E12.11	F12E12.11	BP: metabolic process, oxidation reduction and enterobactin biosynthetic process MF: oxidoreductase activity, 2,3-dihydro-2,3-dihydroxybenzoate dehydrogenase activity, catalytic activity and binding
F13B12.3	F13B12.3	CC: integral to membrane
cyp-13A12	F14F7.3	BP: oxidation reduction MF: monooxygenase activity, iron ion binding, electron carrier activity and heme binding CC: integral to membrane
F14F9.4	F14F9.4	Unknown
F14H8.4	F14H8.4	Unknown
F15B9.6	F15B9.6	CC: integral to membrane
F15E11.4	F15E11.4	CC: membrane
F16H6.10	F16H6.10	Unknown
lys-10	F17E9.11	BP: carbohydrate metabolic process MF: catalytic activity and cation binding
F19B10.4	F19B10.4	Unknown
F19B2.5	F19B2.5	Unknown
F19C7.5	F19C7.5	CC: integral to membrane
F19C7.6	F19C7.6	CC: integral to membrane
F19G12.4	F19G12.4	Unknown
F20B6.6	F20B6.6	BP: protein amino acid dephosphorylation MF: protein tyrosine phosphatase activity

bbs-2	F20D12.3	Unknown
F20G2.5	F20G2.5	BP: defense response
F22E5.6	F22E5.6	BP: potassium ion transport MF: protein binding, voltage-gated potassium channel activity CC: voltage-gated potassium channel complex and membrane
F22E5.9	F22E5.9	CC: integral to membrane
F23H11.7	F23H11.7	Unknown
fipr-1	F23H12.8	BP: nematode larval development, growth, positive regulation of growth rate and reproduction CC: integral to membrane
F25D1.5	F25D1.5	BP: enterobactin biosynthetic process, metabolic process and oxidation reduction MF: oxidoreductase activity, 2,3-dihydro-2,3-dihydroxybenzoate dehydrogenase activity, catalytic activity and binding
F27C8.2	F27C8.2	Unknown
F28A12.3	F28A12.3	MF: transmembrane receptor protein serine/threonine kinase activity, transforming growth factor beta receptor activity CC: membrane and integral to membrane
F28A12.4	F28A12.4	BP: proteolysis MF: aspartic-type endopeptidase activity
fbxa-94	F28F8.8	Unknown
stl-1	F30A10.5	BP: nematode larval development, growth, embryonic development ending in birth or egg hatching and reproduction CC: membrane
twk-43	F32H5.7	BP: potassium ion transport MF: potassium channel activity CC: membrane and integral to membrane
F33H12.7	F33H12.7	Unknown
F35A5.2	F35A5.2	CC: integral to membrane
F35E12.4	F35E12.4	CC: integral to membrane
F35E8.1	F35E8.1	CC: integral to membrane
F36D1.6	F36D1.6	Unknown
F40G12.6	F40G12.6	Unknown
cyp-33E2	F42A9.5	BP: determination of adult life span and oxidation reduction MF: monooxygenase activity, iron ion binding, electron carrier activity and heme binding CC: integral to membrane
F43C1.7	F43C1.7	BP: positive regulation of growth rate
F43C11.8	F43C11.8	MF: protein binding and zinc ion binding CC: intracellular
F43C9.1	F43C9.1	Unknown

msra-1	F43E2.5	BP: protein metabolic process and oxidation reduction MF: oxidoreductase activity, acting on sulfur group of donors, disulfide as acceptor
F44A6.5	F44A6.5	Unknown
cpr-4	F44C4.3	BP: proteolysis MF: cysteine-type endopeptidase activity and cysteine-type peptidase activity
F44D12.9	F44D12.9	BP: transport MF: nucleoside transmembrane transporter activity CC: membrane and integral to membrane
F44E7.7	F44E7.7	BP: transmembrane transport CC: integral to membrane
F46B6.2	F46B6.2	CC: integral to membrane
F46C3.2	F46C3.2	CC: integral to membrane
F46C5.1	F46C5.1	CC: integral to membrane
nas-25	F46C5.3	BP: proteolysis MF: metalloendopeptidase activity and zinc ion binding CC: integral to membrane
nhr-188	F47C10.7	BP: regulation of transcription MF: transcription factor activity, steroid hormone receptor activity, sequence-specific DNA binding and zinc ion binding CC: nucleus
lgc-11	F48E3.7	BP: ion transport MF: extracellular ligand-gated ion channel activity CC: membrane, integral to membrane and postsynaptic membrane
F49C12.14	F49C12.14	Unknown
dod-23	F49E12.2	BP: determination of adult life span
F49F1.6	F49F1.6	BP: positive regulation of growth rate
F49H6.13	F49H6.13	CC: integral to membrane
F49H6.3	F49H6.3	CC: integral to membrane
F53A9.2	F53A9.2	Unknown
F53B2.8	F53B2.8	Unknown
F53B7.2	F53B7.2	BP: G-protein coupled receptor protein signaling pathway MF: zinc ion binding CC: integral to membrane and intracellular
F53B7.4	F53B7.4	CC: integral to membrane
F54D12.4	F54D12.4	BP: nematode larval development, growth, locomotion and body morphogenesis
F55A12.6	F55A12.6	CC: integral to membrane
F55A4.7	F55A4.7	Unknown
F55B11.4	F55B11.4	BP: lipid storage MF: zinc ion binding CC: intracellular

dod-22	F55G11.5	BP: determination of adult life span
F55G11.8	F55G11.8	Unknown
F55G7.1	F55G7.1	Unknown
F56B3.9	F56B3.9	Unknown
clec-67	F56D6.2	MF: binding
nhr-60	F57A10.5	BP: hatching, embryonic development ending in birth or egg hatching and regulation of transcription MF: transcription factor activity, steroid hormone receptor activity, sequence-specific DNA binding and zinc ion binding CC: nuclear envelope and nucleus
nhr-193	F57G8.6	BP: regulation of transcription MF: transcription factor activity, steroid hormone receptor activity, sequence-specific DNA binding and zinc ion binding CC: nucleus
lys-5	F58B3.2	BP: peptidoglycan catabolic process, cell wall macromolecule catabolic process and carbohydrate metabolic process MF: lysozyme activity and cation binding
lys-6	F58B3.3	BP: peptidoglycan catabolic process, cell wall macromolecule catabolic process and carbohydrate metabolic process MF: lysozyme activity and cation binding
dsl-5	F58B3.8	BP: regulation of meiosis, regulation of cell proliferation, positive regulation of growth rate and cell communication CC: membrane
F58B4.5	F58B4.5	Unknown
F58G6.8	F58G6.8	BP: sodium ion transport MF: sodium channel activity CC: membrane and integral to membrane
clec-206	F59A7.1	MF: binding
F59D6.3	F59D6.3	BP: proteolysis MF: aspartic-type endopeptidase activity CC: integral to membrane
F59F3.6	F59F3.6	BP: potassium ion transport MF: protein binding and voltage-gated potassium channel activity CC: voltage-gated potassium channel complex, membrane
bath-19	F59H6.1	MF: protein binding
H12D21.4	H12D21.4	BP: sulfate transport MF: thiosulfate sulfurtransferase activity CC: integral to membrane
H25P06.4	H25P06.4	BP: proteolysis MF: calcium-dependent cysteine-type endopeptidase activity and protein dimerization activity CC: intracellular

H34P18.1	H34P18.1	CC: integral to membrane
K01C8.1	K01C8.1	BP: metabolic process MF: catalytic activity, pyridoxal phosphate binding, amino acid binding and threonine ammonia-lyase activity
twk-14	K01D12.4	BP: potassium ion transport MF: potassium channel activity CC: membrane and integral to membrane
ins-35	K02E2.4	CC: integral to membrane
lipl-4	K04A8.5	BP: positive regulation of growth rate and lipid metabolic process
K04D7.6	K04D7.6	CC: integral to membrane
grp-1	K06H7.4	BP: regulation of ARF protein signal transduction MF: ARF guanyl-nucleotide exchange factor activity CC: intracellular
aqp-9	K07A1.16	BP: transport MF: transporter activity CC: membrane and integral to membrane
cyp-35A5	K07C6.5	BP: oxidation reduction, response to xenobiotic stimulus, response to xenobiotic stimulus, lipid storage MF: monooxygenase activity, iron ion binding, electron carrier activity and heme binding
K07H8.11	K07H8.11	Unknown
K08D10.10	K08D10.10	CC: integral to membrane
scrm-5	K08D10.8	Unknown
cyp-14A2	K09A11.3	BP: oxidation reduction MF: monooxygenase activity, iron ion binding, electron carrier activity and heme binding CC: integral to membrane
K10C2.2	K10C2.2	CC: integral to membrane
K10D11.2	K10D11.2	Unknown
odc-1	K11C4.4	BP: polyamine biosynthetic process MF: catalytic activity
cwp-4	K11D12.1	BP: determination of adult life span
K11H12.10	K11H12.10	CC: integral to membrane
K11H12.3	K11H12.3	BP: reproduction CC: integral to membrane
K11H12.4	K11H12.4	Unknown
cal-3	M02B7.6	MF: calcium ion binding
M02F4.1	M02F4.1	Unknown
nhr-123	M02H5.7	BP: regulation of transcription MF: transcription factor activity, steroid hormone receptor activity, sequence-specific DNA binding and zinc ion binding CC: nucleus
M02H5.8	M02H5.8	Unknown

xbx-3	M04D8.6	CC: integral to membrane
M117.4	M117.4	Unknown
M163.8	M163.8	Unknown
M176.11	M176.11	CC: integral to membrane
ugt-62	M88.1	BP: carbohydrate metabolic process and lipid glycosylation MF: transferase activity, transferring hexosyl groups, carbohydrate binding CC: integral to membrane
R02D5.6	R02D5.6	CC: integral to membrane
R03H10.7	R03H10.7	Unknown
cyp-14A4	R04D3.1	BP: oxidation reduction MF: monooxygenase activity, iron ion binding, electron carrier activity and heme binding CC: integral to membrane
R05A10.3	R05A10.3	Unknown
R06F6.7	R06F6.7	CC: integral to membrane
R08D7.1	R08D7.1	BP: embryonic development ending in birth or egg hatching, meiotic chromosome segregation, hermaphrodite genitalia development, locomotion, morphogenesis of an epithelium, receptor-mediated endocytosis, nematode larval development and growth
R08E5.1	R08E5.1	BP: lipid biosynthetic process and tRNA modification MF: tRNA (guanine-N7-)-methyltransferase activity and methyltransferase activity
R09A1.3	R09A1.3	Unknown
R09E12.9	R09E12.9	Unknown
R09H10.7	R09H10.7	CC: integral to membrane
srw-145	R10D12.17	CC: integral to membrane
R10E11.5	R10E11.5	Unknown
R11E3.2	R11E3.2	BP: transmembrane transport MF: transporter activity CC: membrane and integral to membrane
T01G6.10	T01G6.10	BP: cellular metabolic process, oxidation reduction and enterobactin biosynthetic process MF: catalytic activity, coenzyme binding, oxidoreductase activity, alcohol dehydrogenase (NAD) activity, 2,3-dihydro-2,3-dihydroxybenzoate dehydrogenase activity and binding
T02B11.4	T02B11.4	Unknown
T02B5.1	T02B5.1	BP: metabolic process MF: hydrolase activity CC: integral to membrane
T02B5.3	T02B5.3	BP: metabolic process MF: hydrolase activity CC: integral to membrane

gcy-22	T03D8.5	BP: cyclic nucleotide biosynthetic process, cGMP biosynthetic process, intracellular and protein amino acid phosphorylation MF: ATP binding, phosphorus-oxygen lyase activity, peptide receptor activity, protein kinase activity protein, serine/threonine kinase activity and guanylate cyclase activity CC: membrane and integral to membrane
ugt-55	T04H1.7	BP: carbohydrate metabolic process and lipid glycosylation MF: transferase activity, transferring hexosyl groups, carbohydrate binding CC: integral to membrane
clec-140	T05A7.2	MF: binding CC: integral to membrane
T05A7.3	T05A7.3	Unknown
T05E12.3	T05E12.3	BP: potassium ion transport MF: protein binding and voltage-gated potassium channel activity CC: voltage-gated potassium channel complex and membrane
T05F1.11	T05F1.11	Unknown
T05F1.9	T05F1.9	Unknown
spp-2	T08A9.12	Unknown
spp-4	T08A9.8	CC: integral to membrane
T08B2.3	T08B2.3	CC: integral to membrane
T08G11.3	T08G11.3	Unknown
T08G5.1	T08G5.1	Unknown
T08G5.7	T08G5.7	CC: intracellular MF: zinc ion binding
acr-7	T09A5.3	BP: ion transport and transport MF: extracellular ligand-gated ion channel activity CC: membrane, integral to membrane and postsynaptic membrane
T09B9.3	T09B9.3	BP: glycerol metabolic process and lipid metabolic process MF: glycerophosphodiester phosphodiesterase activity and phosphoric diester hydrolase activity CC: integral to membrane
cyp-13A4	T10B9.1	BP: reproduction, locomotion, positive regulation of growth rate, positive regulation of multicellular organism growth, determination of adult life span and oxidation reduction MF: monooxygenase activity, iron ion binding, electron carrier activity and heme binding CC: integral to membrane
cyp-13A7	T10B9.10	BP: oxidation reduction MF: monooxygenase activity, iron ion binding, electron carrier activity and heme binding CC: integral to membrane

cyp-13A5	T10B9.2	BP: positive regulation of growth rate, oxidation reduction, locomotion and positive regulation of multicellular organism growth MF: monooxygenase activity, iron ion binding, electron carrier activity and heme binding CC: integral to membrane
cyp-13A6	T10B9.3	BP: positive regulation of growth rate and oxidation reduction MF: monooxygenase activity, iron ion binding, electron carrier activity, heme binding CC: integral to membrane
cyp-34A2	T10H4.11	BP: oxidation reduction MF: monooxygenase activity, iron ion binding, electron carrier activity and heme binding
T12D8.5	T12D8.5	BP: positive regulation of growth rate
nlp-16	T13A10.5	Unknown
pqn-66	T16A1.7	CC: integral to membrane
T16G1.4	T16G1.4	Unknown
T18D3.7	T18D3.7	BP: regulation of transcription, translation and tRNA aminoacylation for protein translation MF: transcription factor activity, nucleotide binding, aminoacyl-tRNA ligase activity and ATP binding CC: cytoplasm
T19D12.5	T19D12.5	BP: protein amino acid phosphorylation MF: ATP binding and protein serine/threonine kinase activity
T19D7.5	T19D7.5	CC: integral to membrane
nhr-284	T20C7.2	BP: regulation of transcription MF: transcription factor activity and steroid hormone receptor activity CC: nucleus
T20D4.17	T20D4.17	Unknown
lbp-8	T22G5.6	BP: lipid transport MF: lipid binding, transporter activity and binding
spp-12	T22G5.7	BP: determination of adult life span
T24C4.3	T24C4.3	Unknown
T24E12.5	T24E12.5	CC: integral to membrane
T25B6.6	T25B6.6	BP: carbohydrate metabolic process MF: hydrolase activity, hydrolyzing O-glycosyl compounds
T26E3.8	T26E3.8	Unknown
srsx-36	T26E4.15	BP: G-protein coupled receptor protein signaling pathway CC: integral to membrane
tba-7	T28D6.2	BP: determination of adult life span, lipid storage, microtubule-based process and protein polymerization MF: GTP binding, structural molecule activity and GTPase activity CC: microtubule, protein complex

asic-2	T28F4.2	BP: sodium ion transport (IEA) MF: sodium channel activity CC: membrane and integral to membrane
clec-218	W02D7.2	MF: binding
W03F9.4	W03F9.4	MF: acyltransferase activity CC: integral to membrane
W04C9.6	W04C9.6	BP: transmembrane transport CC: integral to membrane
srr-1	W07G4.6	CC: integral to membrane
W09G12.9	W09G12.9	CC: integral to membrane
Y113G7B.14	Y113G7B.14	MF: DNA binding, ATP binding, nucleic acid binding and ATP-dependent helicase activity
clec-194	Y116A8A.8	MF: binding
Y119C1B.3	Y119C1B.3	CC: integral to membrane
cyp-33D3	Y17D7A.4	BP: oxidation reduction MF: monooxygenase activity, iron ion binding, electron carrier activity and heme binding CC: integral to membrane
Y17D7B.2	Y17D7B.2	Unknown
Y17D7B.3	Y17D7B.3	Unknown
Y19D10B.3	Y19D10B.3	CC: integral to membrane
srd-64	Y22D7AR.8	CC: integral to membrane
Y37A1B.5	Y37A1B.5	MF: selenium binding
Y37D8A.18	Y37D8A.18	BP: nematode larval development, growth, embryonic development ending in birth or egg hatching, positive regulation of growth rate, reproduction and translation MF: structural constituent of ribosome CC: intracellular and ribosome
Y37H2B.1	Y37H2B.1	Unknown
clec-4	Y38E10A.5	MF: binding
Y38H6C.19	Y38H6C.19	BP: vitelline membrane formation MF: structural constituent of vitelline membrane
Y38H6C.21	Y38H6C.21	CC: integral to membrane
ugt-31	Y39G10AR.6	BP: carbohydrate metabolic process and lipid glycosylation MF: transferase activity, transferring hexosyl groups, carbohydrate binding CC: integral to membrane
Y40B1A.2	Y40B1A.2	MF: protein binding
Y41C4A.6	Y41C4A.6	Unknown
Y41D4B.18	Y41D4B.18	Unknown

Y43D4A.2	Y43D4A.2	BP: metabolic process MF: transferase activity, transferring hexosyl groups
Y43F8B.15	Y43F8B.15	Unknown
Y45F10D.2	Y45F10D.2	Unknown
Y45G12C.1	Y45G12C.1	CC: integral to membrane
clec-174	Y46C8AL.2	MF: binding
clec-70	Y46C8AL.3	MF: binding
clec-76	Y46C8AR.1	MF: binding
bcmo-1	Y46G5A.24	BP: positive regulation of growth rate
fbxa-218	Y49E10.17	Unknown
Y50D7A.5	Y50D7A.5	CC: integral to membrane
Y51B9A.6	Y51B9A.6	BP: transmembrane transport CC: integral to membrane
Y53G8AR.1	Y53G8AR.1	Unknown
Y57G11C.44	Y57G11C.44	BP: sodium ion transport MF: sodium channel activity CC: membrane and integral to membrane
Y58A7A.3	Y58A7A.3	Unknown
Y58A7A.4	Y58A7A.4	Unknown
Y58A7A.5	Y58A7A.5	CC: integral to membrane
Y60A9.1	Y60A9.1	Unknown
Y66D12A.6	Y66D12A.6	Unknown
Y69A2AR.12	Y69A2AR.12	Unknown
Y69A2AR.13	Y69A2AR.13	Unknown
Y69A2AR.25	Y69A2AR.25	Unknown
daao-1	Y69A2AR.5	BP: oxidation reduction and metabolic process MF: binding, catalytic activity, oxidoreductase activity and D-amino-acid oxidase activity
Y71A12B.10	Y71A12B.10	Unknown
set-28	Y73B3B.2	Unknown
Y73F8A.35	Y73F8A.35	CC: integral to membrane
Y75B7B.2	Y75B7B.2	Unknown
Y75B8A.28	Y75B8A.28	Unknown
Y75B8A.39	Y75B8A.39	Unknown
Y7A5A.1	Y7A5A.1	MF: oxidoreductase activity and FAD binding CC: integral

		to membrane
Y7A5A.11	Y7A5A.11	Unknown
fbxa-15	Y82E9BL.11	Unknown
fbxa-79	Y82E9BL.13	Unknown
Y82E9BL.18	Y82E9BL.18	Unknown
Y82E9BR.13	Y82E9BR.13	BP: positive regulation of growth rate
str-156	Y9C9A.11	CC: integral to membrane
glr-5	ZC196.7	BP: ion transport, MF: ionotropic glutamate receptor activity, extracellular-glutamate-gated ion channel activity CC: membrane, outer membrane-bounded periplasmic space and integral to membrane
sdz-35	ZC239.12	BP: potassium ion transport MF: protein binding and voltage-gated potassium channel activity CC: voltage-gated potassium channel complex and membrane
ZC239.21	ZC239.21	Unknown
ZC404.11	ZC404.11	BP: G-protein coupled receptor protein signaling pathway CC: integral to membrane
gcy-14	ZC412.2	BP: cyclic nucleotide biosynthetic process, intracellular and protein amino acid phosphorylation MF: ATP binding, phosphorus-oxygen lyase activity, protein kinase activity and protein serine/threonine kinase activity CC: integral to membrane
fbxa-30	ZC47.4	Unknown
ZK105.5	ZK105.5	Unknown
ZK1055.7	ZK1055.7	Unknown
ZK1240.6	ZK1240.6	MF: protein and zinc ion binding CC: intracellular
ZK1240.9	ZK1240.9	MF: protein and zinc ion binding CC: intracellular
twk-9	ZK1251.8	BP: potassium ion transport MF: potassium channel activity CC: membrane and integral to membrane
cyp-13A10	ZK1320.4	BP: oxidation reduction MF: monooxygenase activity, iron ion binding, electron carrier activity, heme binding CC:integral to membrane
ZK177.3	ZK177.3	Unknown
ZK177.9	ZK177.9	Unknown

asm-2	ZK455.4	BP: sphingomyelin catabolic process and lipid metabolic process MF: sphingomyelin phosphodiesterase activity and hydrolase activity CC: extracellular region and intracellular
ZK484.6	ZK484.6	BP: metabolic process MF: catalytic activity
pqn-97	ZK488.10	CC: integral to membrane
pqn-98	ZK488.7	CC: integral to membrane
srj-1	ZK829.8	CC: integral to membrane

All terms called for each gene are listed or consolidated into a summative GO term for each GO ontology category: Biological Process (BP), Molecular Function (MF) and cellular component (CC). All differentially expressed genes were annotated using the WormMart tool biomart version 0.7 dataset WS220-bugFix in WormBase Version: WS247. Genes that were unannotated in WormMart were individually checked manually in WormBase Version: WS247. Genes are listed alphabetically by sequence name.

Table A.9 Gene ontology enrichment of all *C. elegans* significantly differentially expressed genes on *E. coli* OP50, *S. maltophilia* JCMS or K279a.

Cluster 1 EASE 4.29			
GO Category	**Term**	**Genes**	**Benjamini**
MF	electron carrier activity	16	3.30E-04
MF	heme binding	14	2.40E-04
MF	iron	15	8.20E-03
MF	iron ion binding	23	2.20E-02
MF	monooxygenase activity	14	9.60E-06
BP	oxidation reduction	21	4.40E-03
MF	tetrapyrrole binding	14	2.30E-04
MF	transition metal ion binding	39	4.00E-01
Cluster 2 EASE 4.21			
GO Category	**Term**	**Genes**	**Benjamini**
CC	cell	199	1.90E-01
CC	cell part	199	2.00E-01
CC	integral to membrane	166	6.60E-05
CC	intrinsic to membrane	166	5.20E-05
CC	membrane	172	8.10E-05
CC	membrane part	167	1.30E-04
Cluster 3 EASE 3.14			
GO Category	**Term**	**Genes**	**Benjamini**
MF	alkali metal ion binding	6	7.70E-02
MF	cation channel activity	14	1.40E-03
CC	cation channel complex	7	1.40E-02
MF	cation transmembrane transporter activity	15	7.70E-02
BP	cation transport	17	2.40E-02
CC	cell junction	7	2.00E-01
MF	channel activity	23	8.50E-06
BP	establishment of localization	35	7.70E-01
MF	extracellular ligand-gated ion channel activity	8	5.30E-02
MF	gated channel activity	16	2.70E-04
CC	integral to plasma membrane	7	5.60E-02
CC	intrinsic to plasma membrane	7	5.30E-02
MF	ion channel activity	23	1.60E-05
CC	ion channel complex	8	7.80E-03
MF	ion transmembrane transporter activity	25	1.40E-03
BP	ion transport	28	1.30E-04
MF	ligand-gated channel activity	8	6.50E-02
MF	ligand-gated ion channel activity	8	6.50E-02
BP	localization	36	9.10E-01

CC	macromolecular complex	13	1.00E+00
MF	metal ion transmembrane transporter activity	15	1.50E-03
BP	metal ion transport	17	1.60E-03
BP	monovalent inorganic cation transport	15	7.40E-03
MF	neurotransmitter binding	7	1.50E-01
MF	neurotransmitter receptor activity	7	1.50E-01
MF	passive transmembrane transporter activity	23	8.50E-06
CC	plasma membrane	17	1.20E-02
CC	plasma membrane part	15	1.00E-02
CC	postsynaptic membrane	8	5.60E-02
MF	potassium channel activity	11	1.30E-03
CC	potassium channel complex	7	1.20E-02
MF	potassium ion binding	4	1.90E-01
BP	potassium ion transport	11	8.00E-03
CC	protein complex	11	9.80E-01
MF	substrate specific channel activity	23	1.60E-05
MF	substrate-specific transmembrane transporter activity	26	1.30E-03
MF	substrate-specific transporter activity	26	6.30E-03
CC	synapse	8	1.00E-01
CC	synapse part	8	7.60E-02
MF	transmembrane transporter activity	27	2.10E-03
BP	transport	34	7.00E-01
MF	transporter activity	30	1.60E-02
MF	voltage-gated cation channel activity	7	2.50E-02
MF	voltage-gated channel activity	7	4.30E-02
MF	voltage-gated ion channel activity	7	4.30E-02
MF	voltage-gated potassium channel activity	7	1.90E-02
CC	voltage-gated potassium channel complex	7	1.20E-02

Cluster 4 EASE 1.66

GO Category	Term	Genes	Benjamini
MF	carbohydrate binding	10	6.30E-02
BP	carbohydrate metabolic process	10	9.60E-01
BP	cellular lipid metabolic process	9	7.10E-01
BP	lipid glycosylation	5	3.70E-01
BP	lipid metabolic process	11	9.00E-01
BP	lipid modification	6	2.60E-01
MF	transferase activity	23	9.90E-01
MF	transferase activity, transferring glycosyl groups	12	4.40E-02

Cluster 5 EASE 1.65

GO Category	Term	Genes	Benjamini
MF	cation binding	54	4.50E-02
MF	ion binding	55	3.20E-02

MF	metal ion binding	51	7.90E-02
MF	transition metal ion binding	39	4.00E-01
MF	zinc ion binding	24	9.80E-01

GO term enrichment was determined using the functional annotation tool in DAVID Bioinformatics Resources 6.7. DAVID categorizes genes in functional annotation clusters with an enrichment score (EASE). Each GO term has an associated global p value correction. Only clusters with significant (Benjamini FDR < 0.05) GO terms were included in this table. GO terms are listed by EASE and cluster. Each GO term has an associated GO ontology category: Molecular function (MF), Biological process (BP) and Cellular component (CC).

Table A.10 Gene ontology enrichment of the network of *C. elegans* differentially expressed genes on *E. coli* OP50, *S. maltophilia* JCMS or K279a.

Cluster 1 EASE 1.76			
Category	**Term**	**Genes**	**Benjamini**
CC	cell	59	5.00E-01
CC	cell part	59	6.00E-01
CC	integral to membrane	49	7.00E-02
CC	membrane	52	1.10E-01
CC	membrane part	50	9.30E-02

Cluster 2 EASE 1.21			
Category	**Term**	**Genes**	**Benjamini**
MF	alkali metal ion binding	3	7.30E-01
MF	cation channel activity	4	8.20E-01
MF	cation transmembrane transporter activity	4	9.80E-01
BP	cation transport	6	8.00E-01
CC	cell junction	3	6.80E-01
MF	channel activity	7	6.60E-01
BP	establishment of localization	14	6.20E-01
MF	extracellular ligand-gated ion channel activity	3	8.80E-01
MF	gated channel activity	5	7.20E-01
MF	ion channel activity	7	8.30E-01
MF	ion transmembrane transporter activity	8	6.90E-01
BP	ion transport	11	5.80E-02
MF	ligand-gated channel activity	3	8.70E-01
BP	localization	14	6.90E-01
MF	metal ion transmembrane transporter activity	4	8.70E-01
BP	metal ion transport	6	6.10E-01
BP	monovalent inorganic cation transport	5	9.70E-01
MF	passive transmembrane transporter activity	7	5.60E-01
CC	plasma membrane	6	2.70E-01
CC	plasma membrane part	6	1.40E-01
CC	postsynaptic membrane	3	5.60E-01
MF	potassium channel activity	3	8.80E-01
BP	potassium ion transport	3	9.90E-01
MF	receptor activity	8	1.00E+00
MF	substrate specific channel activity	7	8.30E-01
MF	substrate-specific transmembrane transporter activity	9	6.00E-01
MF	substrate-specific transporter activity	9	6.50E-01
CC	synapse	3	7.70E-01

CC	synapse part	3	6.30E-01
MF	transmembrane transporter activity	10	4.40E-01
BP	transport	14	8.60E-01
MF	transporter activity	11	2.00E-01

GO term enrichment was determined on the gene network using the functional annotation tool in DAVID Bioinformatics Resources 6.7. DAVID categorizes genes in functional annotation clusters with an enrichment score (E). Each GO term has an associated global p value correction. Only clusters with significant (Benjamini FDR < 0.1) GO terms were included in this table. Each GO term has an associated GO ontology category: Molecular function (MF), Biological process (BP) and Cellular component (CC).

Table A.11 Survival of wild-type nematodes versus mutants that were not array candidates on *S. maltophilia* JCMS or K279a.

	***S. maltophilia* JCMS**					***S. maltophilia* K279a**				
Genotype	**M**	**SE**	**N**	**Hazard Ratio**	**p value**	**M**	**SE**	**N**	**Hazard Ratio**	**p value**
wildtype (WT)	4.0	0.27	30	N/A	N/A	8.2	0.68	28	N/A	N/A
gcy-14(pe1102)	5.1	0.18	30	0.63	0.0851	8.2	0.33	29	1.6	0.0894
kcnl-2(ok2818)	4.3	0.20	29	0.97	0.913	10.0	0.59	25	0.56	0.0516
numr-1(ok2239)	4.0	0.15	30	1.2	0.483	9.3	0.49	29	0.77	0.327
srw-145(ok495)	4.2	0.18	30	1.1	0.825	7.03	0.36	29	2.5	0.0027
acr-7(tm863)	3.8	0.13	26	1.4	0.206	8.5	0.47	29	1.2	0.486
lgc-11(tm627)	3.1	0.25	28	1.8	0.0319	10.1	0.38	29	0.66	0.128
tctn-1(ok3021)	2.8	0.25	30	4.0	1.9E-06	4.0	0.36	17	6.1	2.8E-06

p values are given for the survival predictor of treatment (mutant nematode genotype) for Cox proportional hazard models in R. Hazard ratios represent the hazard of the treatment divided by the control (wild-type) of the same bacteria. A hazard is the probability that a nematode at a given time dies. p values less than 0.05 were considered significant. Number of nematodes tested = Number. Mean = mean survival units (days). Mutants of *lgc-11* and *tctn-1* were short lived on *S. maltophilia* JCMS. *srw-145* and *tctn-1* mutants were susceptible while, *kcnl-2* mutants were marginally significantly long lived on *S. maltophilia* K279a. Only three of seven mutants had significant phenotypes on JCMS or K279a.

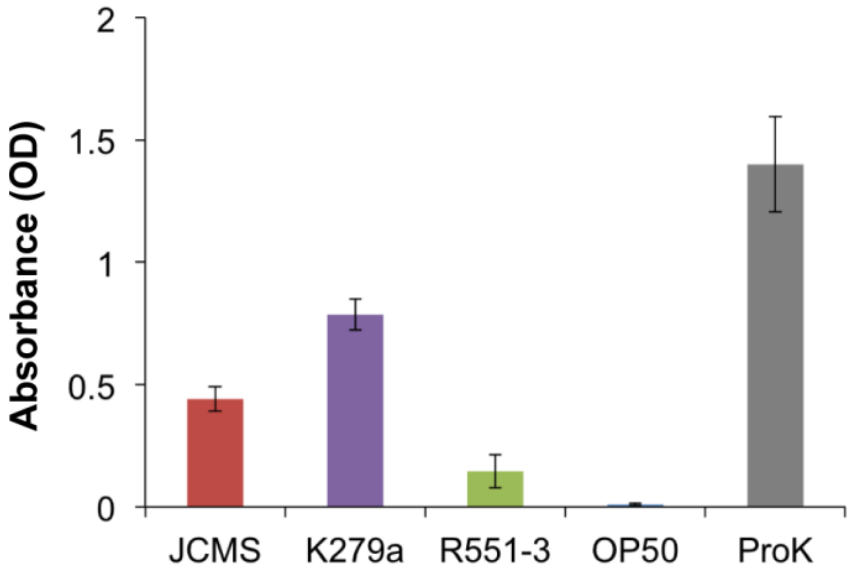

Figure A.6 Protease activity of *E. coli* and *S. maltophilia* strains.

This experiment was competed to assess if protease activity was different between *S. maltophilia* JCMS, K279a, R551-3 and *E. coli* OP50. Liquid cultures of JCMS (red), K279a (purple), R551-3 (green) and OP50 (blue) were filtered at log/stationary phase to remove bacterial cells. Insoluble Azocoll substrate (Azo+ dye + gelatin, Calbiochem) was washed with assay buffer (50mM Tris hydrochloride and 1 mM calcium chloride, pH 7.5) to remove unbound dye and 5mL of washed Azocoll (5mg/mL) was mixed with culture supernatant. Cultures were measured at OD_{600} and equalized by OD prior to the assay. Samples were incubated at 35˚C with end over end rotation. Absorbance was measured at 5 hours using a 1420 Multilabel Counter (Victor 3 model) at OD_{490}. 1µg of protease K (ProK, gray) was used as a positive control and all samples were standardized using a blank (Azocoll + buffer). Three technical replicates were performed during each assay and three biological replicates were completed for each strain. Statistical analysis was completed with GraphPad Software, Inc. All bacterial strain comparisons were significantly different (two tailed, unpaired Student's t test, $p < 0.05$) except for OP50 versus R551-3. Error bars indicate standard error of the mean.

Table A.12 Survival of *daf-2* mutants fed *S. maltophilia*, *P. aeruginosa* and *E. coli* on fast killing, enriched and nematode growth medium.

S. maltophilia JCMS

Control	M	SE	N	Treatment	M	SE	N	Hazard ratio	p value
NGM	5.0	0.17	49	PGS	5.9	0.48	16	0.51	0.0349
NGM	5.0	0.17	49	BHI	7.7	0.49	28	0.22	2.12e-06

S. maltophilia R551-3

Control	Mean	SE	N	Treatment	Mean	SE	N	Hazard ratio	p value
NGM	13.2	0.58	46	PGS	5.68	0.65	22	6.88	4.11e-10
NGM	13.2	0.58	46	BHI	7.7	0.62	26	5.01	5.85e-08

S. maltophilia K279a

Control	Mean	SE	N	Treatment	Mean	SE	N	Hazard ratio	p value
NGM	16.1	1.02	48	PGS	7.4	0.76	37	5.39	8.77E-09
NGM	16.1	1.02	48	BHI	7.5	0.5	54	5.969	5.5E-10

P. aeruginosa PA14

Control	Mean	SE	N	Treatment	Mean	SE	N	Hazard ratio	p value
NGM	5.5	0.2	35	PGS	2.9	0.22	39	4.69	2.02E-08

E. coli OP50

Control	Mean	SE	N	Treatment	Mean	SE	N	Hazard ratio	p value
NGM	19.4	1.3	47	PGS	6.81	0.559		7.894	8.81e-09
NGM	19.4	1.3	47	BHI	9.44	0.824	27	5.704	2.33e-07

Control: *daf-2(e1368)* nematodes fed wild-type *S. maltophilia* JCMS, R551-3, K279a, *P. aeruginosa* PA14 or *E. coli* OP50 on NGM. Treatment: *daf-2* nematodes fed wild-type *S. maltophilia* JCMS, R551-3, K279a, *P. aeruginosa* PA14 or *E. coli* OP50 on PGS or BHI. p values are given for the survival predictor of medium treatment for Cox proportional hazard models in R. Hazard ratios represent the hazard of the treatment medium divided by the control medium of the same row. A hazard is the probability that a nematode at a given time dies.

Number of nematodes tested = N. Mean = mean survival units (days). p values less than 0.05 were considered significant. Mutants of *daf-2* were significantly shorter lived when fed *S. maltophilia* R551-3, K279a, *P. aeruginosa* PA14 or *E. coli* OP50 on both BHI and PGS medium. Survival on PGS was more hazardous when nematodes were fed *S. maltophilia* R551-3 or *E. coli* OP50 while, survival on BHI was more hazardous when nematodes were fed *S. maltophilia* K279a.

Table A.13 Survival of wild-type versus *daf-2* mutant nematodes fed *S. maltophilia, P. aeruginosa* and *E. coli* on fast killing, enriched and nematode growth medium.

***S. maltophilia* JCMS**

Control	M	SE	N	Treatment	M	SE	N	Hazard ratio	p value
WT NGM	4.71	0.077	145	*daf-2(e1368)* NGM	4.9	0.16	47	0.802	0.194
WT PGS	5.2	0.29	27	*daf-2(e1368)* PGS	5.7	0.37	14	0.627	0.19
WT BHI	5.9	0.304	28	*daf-2(e1368)* BHI	7.9	0.52	28	0.347	0.0012

***S. maltophilia* R551-3**

Control	M	SE	N	Treatment	M	SE	N	Hazard ratio	p value
WT NGM	6.7	0.27	47	*daf-2(e1368)* NGM	13	0.54	46	0.0944	4.1E-13
WT PGS	5.4	0.44	28	*daf-2(e1368)* PGS	5.6	0.61	22	0.877	0.659
WT BHI	4.3	0.25	29	*daf-2(e1368)* BHI	7.2	0.49	26	0.215	1.24E-05

***S. maltophilia* K279a**

Control	M	SE	N	Treatment	M	SE	N	Hazard ratio	p value
WT NGM	8.9	0.47	46	*daf-2(e1368)* NGM	16	1.02	48	0.195	1.4E-08
WT PGS	5.6	0.26	55	*daf-2(e1368)* PGS	7.4	0.76	37	0.561	0.0168
WT BHI	5.8	0.304	54	*daf-2(e1368)* BHI	7.4	0.46	54	0.408	9.2E-05

***S. maltophilia* JV3**

Control	M	SE	N	Treatment	M	SE	N	Hazard ratio	p value
WT	2.02	0.017	83	*daf-2(e1368)* NGM	2.4	0.056	80	0.366	1.4E-08
WT	2.02	0.017	83	*daf-2(e1370)* NGM	3.1	0.17	76	0.274	2.6E-10

***S. maltophilia* FW**

Control	M	SE	N	Treatment	M	SE	N	Hazard ratio	p value

WT NGM	7.2	0.22	56	*daf-2(e1368)* NGM	7.7	0.31	46	0.614	0.0204
WT NGM	7.2	0.22	56	*daf-2(e1370)* NGM	8.7	1.07	37	0.494	0.00986

***P. aeruginosa* PA14**

Control	M	SE	N	Treatment	M	SE	N	Hazard ratio	p value
WT NGM	3.7	0.13	73	*daf-2(e1368)* NGM	5.5	0.2	35	0.246	2.1E-08
WT PGS	2.9	0.27	43	*daf-2(e1368)* PGS	2.8	0.21	39	0.887	0.604

***E. coli* OP50**

Control	M	SE	N	Treatment	M	SE	N	Hazard ratio	p value
WT NGM	8.62	0.19	240	*daf-2(e1368)* NGM	19	1.22	47	0.0903	5.55E-16
WT PGS	5.1	0.35	27	*daf-2(e1368)* PGS	6.6	0.48	27	0.488	0.0143
WT BHI	6.6	0.18	25	*daf-2(e1368)* BHI	8.7	0.63	27	0.362	0.00358

Control: wild-type nematodes fed wild-type *S. maltophilia* JCMS, R551-3, K279a, *P. aeruginosa* PA14 or *E. coli* OP50 on NGM, PGS or BHI. Treatment: *daf-2* nematodes fed wild-type *S. maltophilia* JCMS, R551-3, K279a, *P. aeruginosa* PA14 or *E. coli* OP50 on NGM, PGS or BHI. p values are given for the survival predictor of treatment [*daf-2(e1368) or daf-2(e1370)*] for Cox proportional hazard models in R. Hazard ratios represent the hazard of the treatment condition divided by the control condition of the same row. A hazard is the probability that a nematode at a given time dies. Number of nematodes tested = N. M = mean survival units (days). p values less than 0.05 were considered significant. Both *daf-2* loss of function alleles showed a significant increase in survival on both *S. maltophilia* JV3 and FW. Mutants of *daf-2* were significantly longer lived on *S. maltophilia* K279a and *E. coli* OP50 on all medium tested. *S. maltophilia* JCMS was equally virulent to wild-type nematodes and *daf-2* mutants on NGM and PGS medium but, *daf-2* mutants were long lived on BHI medium. *S. maltophilia* R551-3 and *P. aeruginosa* PA14 were equally virulent to wild-type nematodes and *daf-2* mutants on PGS medium. *daf-2* mutants were significantly long lived when fed *S. maltophilia* R551-3 on BHI medium and NGM medium or *P. aeruginosa* PA14 on NGM medium.