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***C. elegans* and its bacterial diet as a model for systems-level understanding of host-microbiota interactions**

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Abstract

Resident microbes of the human body, particularly the gut microbiota, provide essential functions for the host, and, therefore, have important roles in human health as well as mitigating disease. It is difficult to study the mechanisms by which the microbiota affect human health, especially at a systems-level, due to heterogeneity of human genomes, the complexity and heterogeneity of the gut microbiota, the challenge of growing these bacteria in the laboratory, and the lack of bacterial genetics in most microbial species. In the last few years, the interspecies model of the nematode *Caenorhabditis elegans* and its bacterial diet has proven powerful for studying host-microbiota interactions, as both the animal and its bacterial diet can be subjected to large-scale and high-throughput genetic screening. The high level of homology between many *C. elegans* and human genes, as well as extensive similarities between human and *C. elegans* metabolism, indicates that the findings obtained from this interspecies model may be broadly relevant to understanding how the human microbiota affects physiology and disease. In this review, we summarize recent systems studies on how bacteria interact with *C. elegans* and affect life history traits.

Introduction

Humans are inhabited by microorganisms that altogether form our microbiota. Most of these microbes are harmless bacteria that, in fact, provide great benefits to human physiology and health. For instance, the human gut microbiota aids in the digestion of complex vegetables by breaking down fibers and producing short chain fatty acids propionate and butyrate [1]. The microbiota also provides essential micronutrients such as vitamins [2]. In addition, the microbiota plays a plethora of complex roles in human development, metabolism, immunity and lifespan [3–5]. The importance of the microbiota is underscored by the discoveries that its disruption is correlated with a broad range of diseases, including obesity, insulin resistance that is a precursor to type 2 diabetes, metabolic syndrome, autoimmune disorders, autism, neurodevelopmental diseases, inflammatory diseases, cancer and aging [6–9]. Therefore, a thorough understanding of the molecular mechanisms that govern different

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types of human-microbiota interactions will likely provide important steps toward utilizing or perturbing the microbiota to support health and treat disease.

Studying the mechanisms by which bacteria affect human physiology at a systems-level is challenging because the human genome, diet and microbiota are highly heterogeneous, and many known human microbiota species are difficult to propagate and manipulate in the laboratory. Thus, large-scale genetic screens with the human microbiota are not yet feasible, even using mammalian model organisms.

The nematode *Caenorhabditis elegans* and its bacterial diet can be used to rapidly gain broad systems-level and deep mechanistic insights into bacterial effects on host physiology. We refer to this as an “interspecies model” because both the animal and the bacteria it eats are genetically tractable. Here, we summarize recent findings obtained with this interspecies model in both the laboratory and in the wild that demonstrate its utility in examining host-microbiota interactions.

The nematode *C. elegans*

C. elegans is a free-living nematode with a short developmental cycle of about three days and a lifespan of approximately two weeks. It is easy and cost-efficient to culture *C. elegans* in the laboratory as it can be maintained at a range of temperatures from 15°C to 25°C and needs no special animal facility for growth. *C. elegans* has a simple body plan of ~1,000 somatic cells, the identity of which is invariant and known [10]. The transparent body of the animal enables the phenotypic characterization of tissues and cells. In addition, the use of fluorescent protein reporters allows researchers to monitor gene expression in living animals [11–15]. Much of basic *C. elegans* biology, including metabolism, is highly conserved in human, and an estimated 50% of *C. elegans* genes have clear orthologs in humans [16–18]. Finally, *C. elegans* can be subjected to forward genetics in which a phenotype is linked to a gene after mutagenizing the genome and whole genome sequencing can be used to identify the causal mutation, and to reverse genetic screens that allow the assignment of phenotypes to individual genes, for instance by RNA interference (RNAi). Genetic resources for *C. elegans* include publicly available gene deletion, point mutant, and CRISPR mutant strains that can be obtained through the *Caenorhabditis* genetics center (CGC) (Figure 1). Different RNAi libraries are also available, including two genome-scale libraries [19,20], a metabolic gene library [21], and a transcription factor library [22].

C. elegans is a bacterivore that can grow and reproduce on a variety of bacterial diets, including bacteria found in the human microbiota such as *Escherichia coli*. In fact, the diet most widely used for *C. elegans* in the laboratory is an *E. coli* strain called OP50. For reverse genetic screens, RNAi libraries have only been generated in another *E. coli* strain, HT115, thus generally limiting the use of RNAi to study bacterial effects on host phenotypes [19,20]. Recently, however, an *E. coli* OP50 RNAi-compatible strain has been generated expanding the possibilities of performing RNAi in *C. elegans* with different *E. coli* strains [23*]. Finally, other bacteria can be mixed in low dilutions with *E. coli* HT115, but still confer a particular phenotype [24*]. In such cases the *E. coli* HT115 RNAi resources can be used by simply adding a small amount of the relevant other bacteria [25*].

Bacterial mutant collections

Mutant libraries have been generated for several bacterial species that can be fed to *C. elegans*. A mutant library can be made either by recombination, which generates a precise gene deletion, or by using randomized transposon-based mutagenesis in which a transposon was inserted in an unknown location in the genome (Figure 1).

The most widely used bacterial mutant library is known as the *E. coli* Keio deletion collection [26]. This collection contains 3,985 strains each of which harbors a deletion in a single non-essential gene and this collection covers 93% of all annotated genes in this strain. Transposon-based mutagenesis has been used to create mutant strain collections for *Pseudomonas aeruginosa* PAO1 [27], *Comamonas aquatica* DA1877 [28**] and *E. coli* OP50 [29*]. The *P. aeruginosa* PAO1 library contains more than 30,000 clones and was converted into an organized collection of non-redundant mutants that covers 88% of 5,570 predicted genes [30]. *Comamonas* DA1877 genome sequencing led to a predicted ~4,000 genes, and the deletion library contains 5,760 mutants. Several genes are represented by independent mutant clones and therefore, this library does not represent a genome-scale, complete deletion collection [28**]. For the laboratory *C. elegans* food source, *E. coli* OP50, a library comprising about 2,000 mutants was also generated by transposon mutagenesis [29*].

There are several advantages and disadvantages with regards to constructing and using precise deletion compendia versus random transposon-based mutant collections. The main advantage of using directed deletion libraries is that they are comprehensive, provide complete loss-of-function alleles, and provide a direct link between mutant and the identity of the gene knockout due to the known location of each mutant in the compendium. Disadvantages of constructing such a library include the need for costly primer pairs for each gene and the labor-intensive process of strain generation and verification. Advantages of random libraries include the low cost and relative ease of generation. However, with such libraries, the identity of the genes mutated need to be determined by PCR amplification and sequencing. Further, random mutagenesis may result in partial loss of function alleles, the phenotypes of which may be more difficult to interpret. Finally, depending on the size of the library, only a subset of genes is likely covered.

The natural microbiome of *C. elegans*

Bacteria are the major dietary source of nutrition for *C. elegans*. In the wild, live bacteria can also be found in the animal's gut [31,32]. Advances in high-throughput sequencing of 16S ribosomal DNA to define bacterial species, together with worldwide sample collection, has facilitated the characterization of the composition of microbes digested by *C. elegans* in the wild. Several recent studies have contributed to our understanding of the natural diet and microbiota of *C. elegans*. These studies performed systematic sequencing of the gut bacteria from animals grown in natural-like environments made of soil and rotting fruits [33], and nematodes isolated from natural habitats [34**]. Although there are variations in the composition of the microbiota, and not all of the dominant microbes detected in these studies are the same, broad trends exist, particularly at the phylum level (Figure 2). A high

abundance of *Enterobacteriaceae*, *Pseudomonadaceae*, *Xanthomonadaceae* and *Sphingobacteriaceae* was found in different studies using different methods, which indicates that *C. elegans* carries a microbiome that can be comprised of different species.

Bacterial effects on *C. elegans* life history traits, pathogen resistance and behavior

Bacteria can interact with *C. elegans* in different ways. First, bacteria serve as a natural food source. Bacterial diets supply the animal with macronutrients such as carbohydrates, fats and proteins to support the animal's growth and reproduction. In addition, bacteria provide essential micronutrients such as vitamins and cofactors. Finally, bacteria can be pathogenic to *C. elegans*.

In the laboratory, *C. elegans* fed different bacterial diets can exhibit remarkable differences in life history traits such as developmental rate, fecundity and life span. For instance, when fed *Comamonas aquatica* DA1877, *C. elegans* developed faster, exhibited reduced fecundity and a shorter lifespan compared to animals fed the *E. coli* OP50 diet [24*]. Interestingly, mixing only a very small amount of *Comamonas aquatica* DA1877 into *E. coli* OP50 was sufficient to exert developmental acceleration and reduce fecundity, demonstrating that *Comamonas aquatica* DA1877 produces a dilutable metabolite that modulates the animal's life history traits.

C. elegans have been fed individual bacteria isolated from wild habitats. Of all bacteria tested, ~80% supported robust nematode growth, while ~20% impaired growth and induced a stress reporter [35]. Strikingly, mixing the harmful bacteria at low ratios with the standard laboratory diet of *E. coli* OP50 was detrimental to *C. elegans* growth, indicating that the harmful bacteria are not merely nutritionally deficient, but rather that they may produce toxins that suppresses *C. elegans* growth.

Certain bacteria can also protect *C. elegans* from microbial pathogenicity. For instance, *C. elegans* fed *B. subtilis* GS67 (GS67) showed strong resistance to the pathogenic effects of *B. thuringiensis* DB27 (DB27) [36]. GS67 can inhibit growth of DB27 directly and also reduce the colonization of DB27 in *C. elegans* intestine. A GS67 mutant defective in fengycin production lost inhibition against DB27 and was unable to protect *C. elegans* from DB27, indicating that fengycin may mediate inhibition of pathogens and *C. elegans* protection. Further, animals grown on *B. megaterium* and *P. mendocina* both show protection to pathogenic *P. aeruginosa* compared to animals grown on *E. coli* [37]. *B. megaterium* and *P. mendocina* increase *C. elegans* resistance to infection through distinct mechanisms. The ability of *B. megaterium* to enhance infection resistance was linked to its effects on reproduction, while *P. mendocina* functions by activating the p38-dependent MAP kinase pathway to prime the *C. elegans* innate immune system [37].

The *C. elegans* natural microbiome can also protect the nematode from fungal pathogens [34*]. For instance, *Pseudomonas* MYb11 isolated from wild *C. elegans* strains impaired fungal growth in comparison to *E. coli*. These studies have provided important first insights into how bacteria affect *C. elegans* life history traits and pathogen resistance.

Bacterial metabolites that modulate *C. elegans* life history traits

Bacterially produced metabolites that have been reported to modulate *C. elegans* life history traits include nitric oxide, folate, and vitamin B12. Nitric oxide is a diffusible signaling molecule that is produced by some bacteria but not by the nematode. Feeding *C. elegans* nitric oxide-deficient *B. subtilis* shortens the animal's lifespan, while exogenous supplementation of nitric oxide results in an increase in lifespan. Nitric oxide-mediated lifespan extension may function through DAF-16 and HSF-1, which induce aging-related genes and an anti-stress response [38].

Folate, or vitamin B9, plays an important role in nucleotide biosynthesis, the salvage of methionine from homocysteine, and the generation of methyl donors used in various metabolic reactions [39]. A serendipitously isolated *E. coli* HT115 mutant that is deficient in the 3-dehydroquinate dehydratase-encoding gene *aroD* was found to extend *C. elegans* lifespan [40**]. The *aroD*-encoded enzyme is involved in the synthesis of aromatic compounds, including folates. Adding the folate precursor para-aminobenzoic acid (PABA) to *aroD* mutants reversed the lifespan increase in *C. elegans*, suggesting that decreased *E. coli* folate from this mutant bacterial diet is the major cause of the increased *C. elegans* lifespan [40**]. Interestingly, drugs inhibiting *E. coli* folate synthesis also increased *C. elegans* lifespan [40**]. A follow-up study identified two more genes involved in *E. coli* folate synthesis as effecting *C. elegans* longevity [41**]. These findings indicated that folate is required by *E. coli*, rather than by *C. elegans*, to modulate the animal's lifespan.

Bacterial genetics revealed that the micronutrient provided by the *Comamonas aquatica* DA1877 diet described above is vitamin B12 [28**], which is exclusively synthesized by a minority of bacterial species [42]. Vitamin B12 is an essential micronutrient for both humans and *C. elegans*, but is dispensable for other model organisms such as yeasts, flies and plants. Vitamin B12 is a critical cofactor for two metabolic enzymes: methylmalonyl-CoA mutase, which is involved in the breakdown of propionate, and methionine synthase, which converts homocysteine into methionine in the methionine/S-adenosylmethionine cycle [43]. In *C. elegans*, genetically perturbing the canonical propionate breakdown pathway mimics vitamin B12 depletion [25*]. However, it is clear from more than four decades of *C. elegans* research that nematodes can be maintained on low vitamin B12 diets such as *E. coli* OP50. Recently, it has been discovered that *C. elegans* can grow on low vitamin B12 conditions because, under such conditions, it transcriptionally activates a propionate shunt, thereby preventing the toxic buildup of this metabolite [21].

Bacterial metabolites can also influence the *C. elegans* response to pathogenic microbes. For instance, two secondary metabolites produced by *P. aeruginosa*, phenazine-1-carboxamide and pyochelin, can activate a G protein-signaling pathway in *C. elegans* chemosensory neuron, and induce expression of DAF-7/TGF- β , which activates the canonical TGF- β signaling pathway in *C. elegans* interneurons, thereby promoting the animal to seek environments with higher oxygen levels and avoid pathogens [44].

Bacterial respiration affects *C. elegans* life history traits

More than 10 years ago, it was found that *C. elegans* fed *E. coli* GD1, which has reduced levels of coenzyme Q, have a prolonged life span [45]. Coenzyme Q is an essential component of the respiratory chain in both prokaryotes and eukaryotes [46]. Remarkably, however, *C. elegans* life span extension produced by feeding GD1 was found not to be due to a lack of coenzyme Q, since adding exogenous coenzyme Q to the animal did not reverse the phenotype [47]. Thus, this is a second example, with folate described above, where the gene found initially can be misleading with regard to phenotypic modulation of *C. elegans* life history traits. Lacking coenzyme Q causes compromised respiration in the GD1 strain, suggesting that reduced bacterial respiration may affect *C. elegans*. Indeed, a deletion in another *E. coli* respiratory chain gene, *atpA*, also confers a robust life span extension in *C. elegans* [48]. Respiration-deficient *E. coli* proliferates slowly compared to wild-type *E. coli*, and it is known that bacterial proliferation inside the animal influences *C. elegans* life span [49]. Thus, *C. elegans* life span may be extended by certain mutants because they do not grow as well as wild type bacteria. *E. coli cyoA* mutants, which are also deficient in respiration, cause developmental delay when fed to *C. elegans*. This delay is thought to be caused by excessive reactive oxygen species produced by *cyoA* that induce a mitochondrial stress response in *C. elegans* and thereby affects development [29*]. These studies indicate that different components of the bacterial respiratory chain affect *C. elegans* development and life span.

Conclusions and future perspectives

The interspecies systems biology model of *C. elegans* and its bacterial diet has proven to be powerful for the elucidation of the interactions between microbes and animals, and the mechanisms involved. Moving forward, it will be important to decipher precisely how bacterial metabolic networks interact with *C. elegans* metabolic and gene regulatory networks. Such studies will be greatly facilitated by the recently reconstructed *C. elegans* metabolic network, called iCEL1273, that can be combined with mathematical flux balance analysis to computationally model *C. elegans* metabolism under different nutritional or environmental conditions [50**]. In the future, combining *C. elegans* network with bacterial metabolic networks, such as *E. coli* [51], *B. subtilis* [52], *P. aeruginosa* PAO1 [53], *Lactococcus lactis* [54] and human gut microbiota [55], will provide a stepping stone toward the deep mechanistic understanding of how bacteria affect *C. elegans* life history traits.

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Highlights

- *C. elegans* and its bacterial diet is an excellent model system for mammalian host-microbiota interactions.
- *C. elegans* carries a distinct and species-rich microbiome.
- Metabolites produced by bacteria modulate *C. elegans* life history traits.
- Bacterial respiration affects *C. elegans* development and life span.

Figure 1.

C. elegans and its bacterial diet are both tractable model organisms for studying host-microbiota interactions at a systems-level. *C. elegans* phenotypes (green) can be screened by RNAi-mediated knock down libraries, or by examining collections of deletion, point, or CRISPR mutants. Bacterial mutant libraries (blue) for certain strains are available from targeted deletion or transposon insertion collections.

Figure 2.

The structure of the native microbiome in *C. elegans*. The most abundant phyla and families are indicated. Phyla are represented by color and families are represented by name. This figure was produced with data from [34].

Table 1

The effects of various bacterial diets on *C. elegans* and the known metabolites involved in these effects. Bacterial diet effect is relative to *E. coli* OP50.

Bacterial strain	Metabolite Involved	Effect	Reference
<i>C. aquatica</i> DA1877	Vitamin B12	Accelerated growth	21, 24, 25, 28
<i>B. subtilis</i> nos	Nitric oxide	Decreased lifespan	38
<i>E. coli</i> HT115 <i>aroD</i>	Folate	Increased lifespan	40, 41
<i>E. coli</i> GD1	Co-enzyme Q	Increased lifespan	45, 47, 48
<i>E. coli</i> OP50 <i>cyoA</i>		Decelerated growth	29
<i>B. subtilis</i> GS67	Fengycin	Anti-pathogen	36
<i>P. mendocina</i>		Anti-pathogen	37
<i>B. megaterium</i>		Anti-pathogen	37
<i>Pseudomonas</i> MYb11		Anti-fungal	34