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

Jingyan Zhang, Amy D Holdorf and Albertha JM Walhout

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 microbiota 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 aides 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 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 ~1000 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 humans, 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

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.
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.

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 Ref. [34**].
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 C. 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 C. aquatica DA1877 into E. coli OP50 was sufficient to exert developmental acceleration and reduce fecundity, demonstrating that C. 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 (Table 1). 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

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**Table 1**

<table>
<thead>
<tr>
<th>Bacterial strain</th>
<th>Metabolite involved</th>
<th>Effect</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. subtilis Δnos</td>
<td>Nitric oxide</td>
<td>Decreased lifespan</td>
<td>[38]</td>
</tr>
<tr>
<td>E. coli HT115 ΔaroD</td>
<td>Folate</td>
<td>Increased lifespan</td>
<td>[40**,41**]</td>
</tr>
<tr>
<td>E. coli GD1</td>
<td>Co enzyme Q</td>
<td>Increased lifespan</td>
<td>[46,47,48]</td>
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<tr>
<td>E. coli OP50 cyaA</td>
<td>Fengycin</td>
<td>Decelerated growth</td>
<td>[39]</td>
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<tr>
<td>B. subtilis GS67</td>
<td></td>
<td>Anti-pathogen</td>
<td>[36]</td>
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<td>P. mendocina</td>
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<td>Anti-pathogen</td>
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<tr>
<td>B. megaterium</td>
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<td>Anti-pathogen</td>
<td>[37]</td>
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<tr>
<td>Pseudomonas MYb11</td>
<td></td>
<td>Anti-fungal</td>
<td>[34**]</td>
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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 *C. 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 [28**]. 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* choemo sensory neurons, 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 caused by 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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**References and recommended reading**

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
- of outstanding interest


The authors genetically engineer the E. coli OP50 strain to make it possible to perform RNAi experiments in C. elegans fed this strain.


With [41••], these papers describes how the inhibition of E. coli folate synthesis increases C. elegans lifespan. Folate synthesis is required by E. coli, but not by C. elegans, to modulate the animal’s lifespan.


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The first genome-scale reconstruction of the C. elegans metabolic network starting with a systematic annotation of metabolic genes enables predictions of gene essentiality and the unraveling of the mechanisms of different nutrient contributions influencing the animal’s physiology.


