# Title: Understanding host-microbiome and pathobiome interactions using *C. elegans*

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The two-phase human microbiome project (HMP) successfully identified associations between microbial species and healthy or diseased individuals. However, a major challenge they identified was the absence of model systems for studying host-microbiome interactions, which would increase our capacity to uncover molecular interactions, understand organ-specificity, and discover new microbiome-altering health interventions.

*Caenorhabditis* elegans has been a pioneering model organism for over 70 years but was largely studied in the absence of a microbiome. Recently, ecological sampling of wild nematodes has uncovered a large amount of natural genetic diversity as well as a slew of associated microbiota. The field has now explored the interactions of *C. elegans* with its associated gut microbiome and found a defined, non-random microbial community, highlighting its suitability for dissecting host-microbiome interactions. This core microbiome is being used to study the impact of host genetics, age, and stressors on microbiome composition. Furthermore, single microbiome species are being used to dissect molecular interactions occurring between microbes and the animal gut. Being amenable to health altering genetic and non-genetic interventions, *C. elegans* has emerged as a promising system to generate and test new hypotheses regarding host-microbiome interactions, with the potential to uncover novel paradigms relevant to other systems.

## Introduction

Microbes are evolutionarily the oldest and the most abundant life form, and believed to colonize all other metazoans and plants, likely in symbiotic, pathogenic, or commensal-like associations. The term microbiome is used for all the harboring microbes, including bacteria, fungi and viruses, in any given biotic or abiotic habitat. Microbiomes are known to play fundamental roles in many biological processes, from being required for development, immunity, metabolism, organ function, and behavior, to causing disease, morbidity, and death.

Until very recently, the microbiota was commonly referred to as “the forgotten organ”. However, even though the study of animal microbiomes is perceived as a young discipline, we are already using probiotics and fecal microbiota transplant (FMT) procedures to manage health complications and diseases in the humans. The two-phase human microbiome project (HMP) conducted between 2007-2016, took a comprehensive, multi-disciplinary approach to characterize the human microbiome from different organs of healthy and diseased North Americans (1, 2). These studies have characterized the composition and abundance of microbes from different organs of healthy individuals (3, 4). Additionally, they have discovered changes in microbial composition in organs that are associated with specific diseases, including gut microbiome changes associated with inflammatory bowel disease (IBD), nasal and gut changes associated with pre-diabetes, and vaginal microbiome changes associated with preterm births (5). In addition to creating a wealth of knowledge and community resources, this decade long research initiative also identified potential gaps and limitations (2).

The HMP opened up a multitude of research avenues to understand the interactions between an animal host and its microbiota, including the development of new tools and techniques in genetically amenable model systems such as *C. elegans* and *D. melanogaster.* These and other model organisms are now being used to address the role of host genetics in shaping their microbiomes. This was an understudied aspect of the HMP, as the human subjects belonged to different ethnicities with limited genetic diversity, and it is generally difficult to investigate a causal role of host genetics in humans. Over the last 70 years, *C. elegans* has been extensively used to decode genes and pathways that regulate complex organismal phenotypes, including development, neurobiology, health, aging, and pathogenesis. *C. elegans* has several traits that makes it a suitable model to study host-microbiome interactions, including a short life span, genetic tractability, and

well-defined tissue systems (6). Additionally, *C. elegans* is transparent throughout its life, allowing for easy visualization of microbial colonization using simple microscopes, with sufficient spatial resolution (7, 8). They can also quickly and easily be made germ-free by using a rather simple bleaching method. These properties make *C. elegans* suitable for studying its interaction with a single microbial species or more diverse microbial communities (9, 10). However, until recently, the vast majority of studies in *C. elegans* were conducted in the absence of a microbiome. Generally, *C. elegans* is conventionally grown on the *Escherichia coli* strain OP50 as a sole food source, which does not colonize the gut of healthy, young animals.

The recent extensive worldwide sampling of wild *Caenorhabditis* nematode populations has led to the isolation and identification of a vast array of microbes naturally associated with these animals {reviewed in (11)}. In their habitat of rotten fruits and plant matter, *C. elegans* and related nematodes are now known to interact with a variety of obligate and non-obligate pathogens, such as bacteria, viruses, fungi, and parasites (11-17). More recently in 2016, three parallel studies provided the first description of the *C. elegans* microbiome and found that it assembles a gut microbiome in a deterministic, non-random manner, which is distinct from the microbiome of its habitat {(18-20), and reviewed in (21)}. These discoveries were combined to define a core intestinal microbiome of *C. elegans*. Initially a set of 12 bacteria from 9 different families (called CeMbio) were chosen based on taxonomic diversity, capacity for easy in vitro culture, and high prevalence in microbes associated with wild-isolated *C. elegans* (9). Later a set of 51 bacteria from 10 different families were added to the core CeMbio (called BIGbiome) for more comprehensive studies (10). In addition to CeMbio and BIGbiome, several individual bacterial species found in wild nematodes also allow for direct mechanistic studies of host-bacterial interactions (7, 10, 22, 23). The genetic amenability of *C. elegans* and their bacterial microbiome, coupled with the ease of implementing complex experimental designs and large-scale genetic screens, makes it an excellent model for dissecting the genetic basis of host-microbiome interactions. In addition, *C. elegans* is ideal for studying the impact of microbiome on host fitness, tissue-specific health outcomes, metabolism, and behaviors, using large genetically homogenous populations.

The objective of this review is to provide a brief analysis of different factors that sculpt the host microbiome, focused on studies conducted in the *C. elegans* model. In addition, the review underlines the role of host and microbial genes in shaping the microbiome, as well as the amenability of the *C. elegans* holobiome to unravel yet unknown genetic determinants that drive host-microbiome associations. In the end, this review identifies some of the understudied aspects of host-microbiome research that can be tackled in *C. elegans*.

## 1. Understanding factors regulating host-microbiome interactions

Host-microbe interactions are expected to be shaped by a complex interplay between several abiotic and biotic factors. The impact of a microbe on host fitness and health is dynamic and context-dependent, and defines whether the relationship is pathogenic, mutualistic, or commensal-like. Here, we cover general factors that have been shown to regulate host-microbiome interactions in *C. elegans*. First, we discuss how host-microbe associations can influence each other's behavior. Next, we describe how microbiota are critical for optimal development of host immunity and pathogen resistance. Additionally, several microbiota-derived vitamins and metabolites, otherwise unobtainable for the host, are essential for host function and fitness. Finally, niche-specific selection pressures are exerted by abiotic factors such as pH and oxygen conditions, and adhesion factors and receptors confer specificity and stability to various host-microbiota interactions. Besides this, the composition and abundance of a microbial community is shaped by inter-species microbial competition and cooperation.

### *1.1 Microbe-seeking and microbe-driven behaviors: stay, avert, or hitchhike*

How microbial associations and microbe-derived metabolites modify animal behaviors is an emerging area of study in host-microbiome interactions {reviewed in (24)}. Can the gut-microbiota influence and manipulate what we eat and how we behave? The answer is, yes! Gut microbiota derived metabolites and

neurohormones can manipulate our eating behaviors by impacting satiety (25) and can even modify our sense of taste and food cravings {reviewed in (24)}. In addition, gut microbiota-derived metabolites such as SCFAs (short-chain fatty-acids) can directly impact gut hormone metabolism and indirectly modulate brain functions via the vagal nerve in the gut-brain axis, to manipulate animal behaviors {reviewed in (24, 26)}.

In the wild, *C. elegans* is exposed to a variety of microbes, from pathogens to commensals. Given that *C. elegans* are bacterivores, many microbes also serve as food sources. It is likely that *C. elegans* uses their innate sense of smell and touch to quickly profile their habitat to make immediate decisions, including foraging, avoidance, eating, or egg-laying (27). In addition to these innate behaviors, worms are also capable of long-term learned behaviors elicited by changes in their internal-state {reviewed in (28)}. While ingestion of nutritive bacteria leads to learned attraction for exploitation of a bacterial food source, ingestion of pathogens and toxins leads to learned aversion for avoiding stress and damage (29-31), **(Figure- Panel A)**.

Microbial-derived odors are expected to impact seeking and colonization of a food source by *C. elegans*. Interestingly, several bacteria from the natural habitat of *C. elegans* were found to release odors that include previously known attractants and repellents, studied for decades in *C. elegans* neurobiology and behavior (32-34). For example, *Lactobacillus paracasei* bacteria were isolated from rotten citrus fruits that also harbored wild *C. elegans*, and this bacteria was found to naturally produce the attractant diacetyl (35). Diacetyl is a volatile byproduct of citric acid metabolism in *Lactobacillus paracasei*, that themselves do not colonize *C. elegans,* but possibly attracts them to rotting citrus fruits that are rich in other microbes (10).

In yet another interesting example, *Providencia alcalifaciens* isolate JUb39, a known *C. elegans* commensal (10), uses sensory attraction and override mechanisms to manipulate *C. elegans* feeding behaviors. The JUb39 bacteria releases another well-studied attractant, isoamyl alcohol (34), possibly making them a preferred food choice over the standard *E. coli* diet (36). Then, on colonizing the *C. elegans* gut, JUb39 produces the bioactive neurotransmitter tyramine, subverting the need for *C. elegans'* tyramine while utilizing their octopamine signaling to essentially manipulate *C. elegans* into selecting a food beneficial for both of them (36). In an example of beneficial selection by the host, *C. elegans'* preferably colonizes the beneficial strain of the commensal-like *Pantoea* sp. that hinders the pathogenic colonization of *Pseudomonas aeruginosa* over an environmental strain that lacks this benefit (22). This implies to a possible habitat driven co-evolution between this beneficial *Pantoea* sp. and *C. elegans.*

How does the microbiome shape *C. elegans* behavior during food scarcity? Food deprivation is known to elicit behavioral changes from enhanced risk-taking during food search (37) to alternate developmental decisions, such as dauer formation. During unfavorable conditions including food scarcity, instead of further development into reproductive adults, *C. elegans* larva develop into a metabolically quiescent state called the dauer that is capable of surviving for prolonged periods (38). Consistently, dauers are very prevalent in the natural habitat of *C. elegans*, and are likely the first to enter and the last to exit a new food source in a given habitat (11, 14). Dauers exhibit a phoretic behavior called nictation, that enable their dispersal to geographically isolated habitats, by hitchhiking on snails and isopods (11, 14). However, it remains unknown if microbial odors impact the dauer entry and exit decisions, or if dauer-associated microbes define the microbial communities in a new habitat. This is a fascinating area for future research in *C. elegans*, as it is likely that many host-microbiome co-evolutionary processes have occurred with the dauer state, given that this stage likely acts as a transport mechanism for some bacteria between discontinuous food sources (39) and possibly long-term survival across geological time scales (40).

### *1.2. Host and microbe-derived immunity together sculpt the microbiome: from commensal-like to a pathobiont*

The microbiome is known to play a major role in the development and maintenance of host immunity. In parallel, host immunity has been identified as a common and critical factor in shaping the microbiome. The composition of the first human microbiota is critical for postnatal development of gut immunity. For example, compared to cesarean delivery, vaginal births are considered ideal as it results in greater gut-microbiota diversity, required for development and priming of neonatal immune system {reviewed in (41)}, and subsequently impacting childhood pathologies and immune-mediated diseases {reviewed in (42)}. Likewise, in the absence of gut microbiota, germ-free mice have morphological and functional defects in the development of adaptive immunity and regulation of immune cells {reviewed in (43)}.

The natural *C. elegans* microbiota are also known to regulate immunity (7, 44). For example, several *C. elegans'* gut commensals have been shown to prime host immunity, either directly or indirectly to counteract pathogens (20, 45) **(Figure-Panel B).** Of the several studied *Pseudomonas* spp. commensals, the *Pseudomonas lurida* strain MYb11 and *Pseudomonas fluorescens* strain MYb115, are known to modulate host immunity using distinct immune-protective mechanisms. Both MYb11 and MYb115 are commensals that efficiently colonize the *C. elegans* gut and provide more nutrition than the standard *E. coli* diet, allowing for *C. elegans* to have a higher population growth, faster development, and early reproduction with an overall increase in fertility (9).

Conversely, the state of host immunity can impact the composition of the microbiota. For example, a decline in *C. elegans'* immunity is accompanied by an increase in commensals with pathogenic potential (pathobionts) and otherwise non-colonizing *E. coli* OP50 in the gut. More specifically, the known *C. elegans* commensal bacteria, *Enterobacter* sp. CEent1, is known to protect from Gram-positive *E. faecalis* infection (45). However, CEent1 itself becomes a pathogen in immune-compromised *C. elegans* strains mutated in the TGFβ/BMP pathway, evidenced by a selective *Enterobacter* spp. bloom that shortens host lifespan with enhanced susceptibility towards *E. faecalis* **(Figure-Panel B)**. In addition to having a conserved role in regulating gut immunity (46, 47), the TGF-β/BMP signaling pathway regulates the intestinal gut microbiota via neurons and the epidermis, implying that inter-tissue communication plays a role in microbiome colonization (7).

Finally, immunity can be conferred by inter-species interactions between microbes that shape the microbiome through cooperation or competition. This can be conceptualized as the collective immunity of the host and its microbiome, which for *C. elegans* includes multiple innate immunity pathways combined with acquired immunity from the associated microbes that together dictate the carrying capacity and composition of its microbiome. For example, the *Pseudomonas lurida* strain, MYb11, provides broad-range protection against the fungal pathogen *Drechmeria coniospora* (19), gram-negative *P. aeruginosa*, and gram-positive *B. thuringiensis* bacteria (48). Some of this broad range protection may be due to the bacteria modulating host immunity, as MYb11 can directly activate some *C. elegans* innate immune and defense response pathways (49), but this has yet to be experimentally tested. Instead, MYb11 was found to directly antagonize *B. thuringiensis* growth by generating two cyclic lipopeptide surfactants, namely, massetolide and viscosin, that have a bacteriostatic effect on the pathogen. As such, worms colonized by MYb11 exhibited significant improvement in intestinal barrier dysfunction induced by *B. thuringiensis* (48) **(Figure-Panel B)**. While MYb11 benefits its host in multiple ways, colonization of *C. elegans* does result in a small reduction in host lifespan, suggesting that there are some trade-offs to the host when colonized by this protective bacteria.

On the contrary, *Pseudomonas fluorescens* strain, MYb115, protects *C. elegans* without reducing the pathogen burden of *B. thuringiensis*. Therefore, it is likely that MYb115 helps *C. elegans* cope with infection, possibly by enhancing damage repair in gut epithelium (48). This idea is supported by the fact that MYb115 decreases the epithelial barrier dysfunction induced by *B. thuringiensis* infection (48), likely by altering *C. elegans*' lipid metabolism, and cytoskeleton reorganization through intermediate filaments

(IFs) (49), which are known to protect against microbial insults (50). However, the impact of *C. elegans'* microbiome on cytoskeleton reorganization and epithelial barrier function requires further investigation.

It has become evident from these studies in *C. elegans*, that both host immunity and microbe-derived immunity dictate microbiome composition, with a decline in host immunity playing a role in the shift of commensal-like microbes to pathobionts **(Figure-Panel B)**. However, it is also possible that some microbes can actively switch from commensal-like to pathogenic traits by inducing gene expression changes (51), an aspect that needs investigation in *C. elegans*.

***1.3. Diet and metabolism: a nourishing factor in host-microbiome interactions***

Host-microbiota assemblies are expected to be governed by the availability and distribution of nutrients in a given habitat. For microbiome bacteria, it is expected that the bulk of nutrition comes from the host. However, microbes reciprocally allow their host to utilize resources that they otherwise can't digest or metabolize on their own, including essential vitamins. Consistent with this ideas, the lack of a metabolically active gut microbiome causes caloric restriction in germ-free mice (52) and axenically-grown *C. elegans* (53), showing a host's dependence on their microbiome for optimal nutrition. Similarly, our diet can dramatically impact our microbiome, as a Mediterranean-style diet rich in fruits and fibers, and low in fat, can have a positive impact on the gut microbial diversity and metabolism (54-56). By contrast, a Western-style diet low in fiber and high in fat, can result in reduced levels of beneficial bacteria with low diversity (57), resulting in obesity and altered behaviors (58).

*C. elegans* are bacterivores and thus have a more complicated relationship with their microbiota, which can also serve as a nutrition source. As such, it is not so straightforward to separate the non-dietary commensal- or pathogen-like impact of microbes from their potential dietary (or nutritive) roles. We have only begun to understand the impact of microbiome derived metabolites on *C. elegans* life history traits. For example, of the several known essential nutrients commonly required by both humans and *C. elegans* {reviewed in (59)}, vitamin B12 is an example of a microbiota-derived essential cofactor whose deficiency leads to slowed growth **(Figure-Panel C)**. B12 deficiency in *C. elegans* results in stunted development, loss of fertility, and reduced lifespan (60), as the cofactor is required in Methionine/SAM cycle for converting homocysteine to methionine (61). As another example, *C. elegans* are cholesterol auxotrophs and lack the first three enzymes in canonical cholesterol biosynthesis pathway. However, it was recently found that these animals are capable of converting dietary sterols obtained from fungi and plants into cholesterol (62), making it possible that microbiome bacteria or fungi can provide sterols for cholesterol production in *C. elegans,* an area that remains to be investigated **(Figure-Panel C)**.

Along these lines, a recent study has shown how some *C. elegans* microbiome bacteria can utilize diverse metabolic competencies to colonize and flourish in the gut. Two *C. elegans* commensals, *Pseudomonas lurida*, MYb11, and *Ochrobactrum* sp., MYb71, were shown to use unique and shared biochemical pathways to colonize the *C. elegans* gut, which provide the host with essential metabolites. The study identified two key metabolic competencies exhibited by these bacteria to facilitate gut colonization, including the ability to ferment pyruvate to acetoin and the ability to degrade hydroxyproline (63). MYb11 was also found to increase vitellogenin protein (yolk protein) production in young *C. elegans* adults believed to hasten their reproductive timing (49). Therefore, for successful colonization, microbes likely integrate their metabolism with host's metabolism to provide an optimal growth condition for both.

Finally, an interesting aspect of host-microbiome associations is the ability of a host to provide niche-specific food and foraging avenues for their microbiota. This is an area that has yet to be studied with the *C. elegans* microbiome, but holds potential. In mammals, the mucus layer in the gut is known to provide nutrition to its microbes, in addition to its role as a protective physical barrier for the epithelia. Mucus is made of heavily glycosylated mucin proteins which can become a foraging avenue for glycolytic microbes that metabolize the complex glycosylated moieties to release sugars for their

consumption {reviewed in (55)}. The production, diversification and maintenance of a mucus layer is akin to a metabolic collaboration between the host and their microbiota. Consistent with this, germ-free mice have an underdeveloped mucus layer (64) and glycan metabolism is known to shape the human gut microbiota (65). Several prominent human gut commensals include mucin-degrading bacteria, such as *Akkermansia muciniphila,* that can utilize mucin as their sole source of carbon and nitrogen {reviewed in (66)}. Similarly, *Bacteroides thetaiotaomicron* exhibit adaptive mucus foraging to utilize glycan sugars in the absence of dietary polysaccharides (64), thereby contributing to the metabolic homeostasis in the gut. However, in *C. elegans*, we do not yet know whether its microbiota can induce glycan and mucin production, nor whether there are mucolytic and glycolytic foragers in its microbiota **(Figure-Panel C)**.

***1.4. Mucins, epithelial barrier and intestinal X factors: shaping the confines of the gut niche***

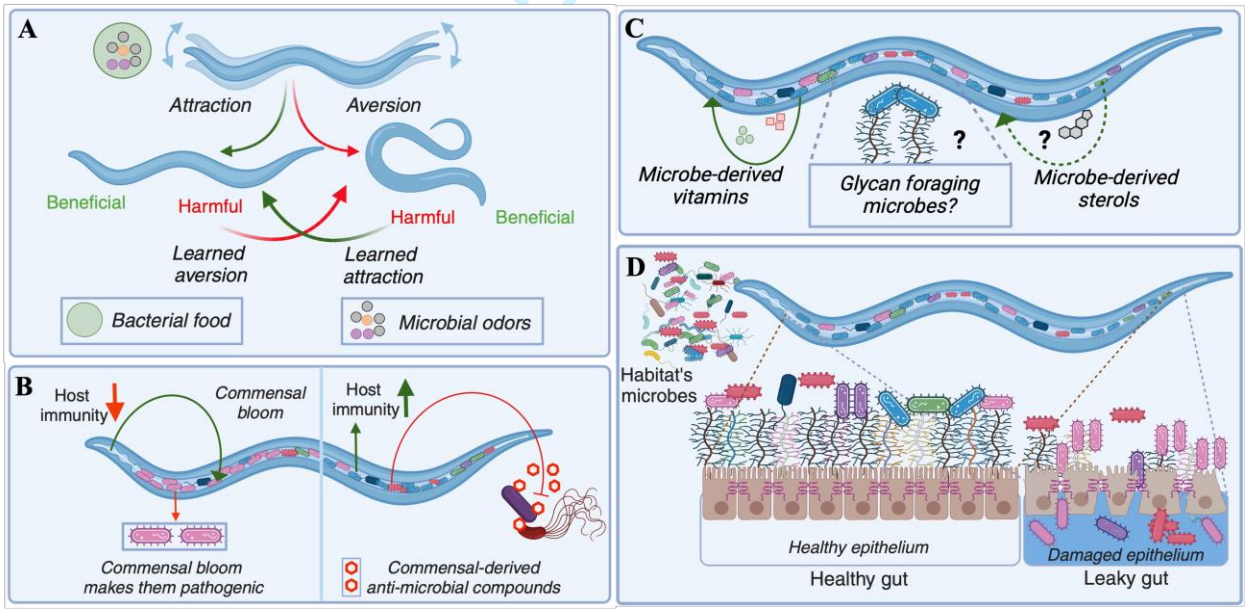
Anatomical and tissue-specific factors are expected to drive the community composition of a microbiome, and here we focus specifically on the animal gut. In mammals, the gut is compartmentalized along the length, into the stomach, small intestine (duodenum and ileum), and the large intestine, each having unique structure and function. The different microbial composition in these tissues is predominantly dictated by their niche-specific environments, such as pH, oxygen, nitrate, and bile acid metabolites {reviewed in (67)}. Microbial interactions in the gut mainly occur with the secreted mucus layer or the glycocalyx, a layer of heavily glycosylated proteins (including membrane-bound mucins) at the apical surface of the epithelia. These coats create a protective barrier between the underlying gut epithelium and microbes. Besides being the first-line of defense, the mucus layer can provide a selective substrates for bacterial adhesion, with host mucins bound by bacterial fimbriae or pili (68). Research suggests that there is a potential cross-communication between the host gut and the microbiota. In fact, altered mucous glycosylation can impact the microbiota composition and intestinal architecture (69). In turn, microbes are known to induce host secretion of mucus. This is supported by the fact that germ-free mice have an underdeveloped mucus layer (70), and the ability of the mucolytic human gut commensal, *Bacteroides thetaiotaomicron*, to induce mucin secretion by regulating the differentiation of goblet cells (71).

*C. elegans* has simpler and less compartmentalized alimentary canal. It begins with the pharynx, a neuro-muscular grinder that is very efficient in masticating bacteria. The grinder empties into the worm gut, an epithelial tube made of 20 polarized epithelial cells (72, 73). In order to colonize *C. elegans,* microbes must first survive mastication in the grinder. Then, they must adapt to the intestinal pH and oxygen conditions in gut, while avoiding getting flushed out by gastric motility and defecation. In the gut, some bacteria can directly adhere to the intestinal epithelial cells to create a niche, whereas others remain sessile by persisting and/or replicating in the intestinal lumen without direct adherence (74) (**Figure-Panel D**). The *C. elegans* intestinal lumen is suggested to be aerobic, as their gut microbiota includes several known obligate aerobes, but is also colonized by several fermenting bacteria (63). Additionally, the pH of the intestinal lumen of *C. elegans* is weakly acidic and discontinuous (75). The low intestinal pH of the gut is a possible selection force, also known to modulate inter-microbial interactions during community assembly (76). In turn, microbes can also condition the *C. elegans* intestinal environment to maintain the pH gradients that are critical for the function of pH-dependent intestinal transporters (53). In fact, the intestinal pathogens *P. aeruginosa* and *E. faecalis* were shown to neutralize the intestinal pH of the lumen upon infection (77) suggesting that microbiome bacteria are likely to play a role in pH alterations upon colonization.

Bacterial adhesion to host mucus glycans is a potential host selection mechanism that permits their gut retention and colonization (78). Currently, we still do not know if *C. elegans* contains a flushable mucus layer, similar to other mammals, but we have visualized a potential mucus-like layer on intestinal epithelial cells that may be a thick glycocalyx containing mucin-like genes (79). While many gut commensals in *C. elegans* have not been shown to colonize by adherence (14), a few are emerging with obvious direct interactions with gut epithelial cells (8, 74). This possible role of mucins in microbial adherence remains understudied, and is an active area of investigation. It is possible that in *C. elegans*, the heavily glycosylated glycocalyx can contribute to the adherence of gut commensals, and the gut microbiota in turn may induce

mucin-like protein secretion in the glycocalyx, somewhat similar to exchanges seen in mammals. In fact, a recent study has shown a requirement for the *C. elegans* mucin-like gene, *mul-1*, in *P. aeruginosa* pathology (80), and *mul-1* was previously shown to be upregulated during *P. aeruginosa* infection (81, 82). Super-resolution electron microscopy can be used to visualize the impact of *C. elegans* gut microbiota (CeMbio, BIGbiome, or individual members) on the mucin-glycocalyx layer, and the integrity of underlying microvilli and brush border assembly (83, 84). In addition, *in vivo* molecular imaging of *C. elegans* mucin-type *O*-glycans can be implemented to understand how microbes drive the mucobiology of the *C. elegans* gut (85).

There are other known factors that impact the homeostasis of the gut microbiome, its mucus layer, and the underlying epithelium. Age-related decline in the grinder efficiency and defecation frequency results in more live bacteria entering and proliferating in the gut, leading to constipation and death (86). In fact, the standard *C. elegans* diet bacteria, OP50, are efficiently destroyed in the grinder of healthy, young animals, but can colonize the intestinal lumen in aged animals (87). In addition, age-related depletion of the mucus-like layer and decline in gut-barrier function is known to enhance intestinal leakiness and gut dysbiosis, which negatively impacts overall health and lifespan (88, 89) (**Figure-Panel D**). Different members of the microbiota are known to impact the gut epithelium, with some pathogens disrupting it and a number of commensals fortifying it (90). For example, several known commensal bacteria in *C. elegans* have been shown to improve the gut barrier function following pathogenic infections that would increase gut leakiness (48, 49). However, how *C. elegans* commensals modify gut mucin layer and the underlying epithelium is not well explored. In *C. elegans*, the impact of microbes on the gut barrier function can be readily accessed by implementing Smurf assays to measure the extent of gut leakiness (89, 91).




## 2. Exploring the interaction between host and microbiota genetics

It has become evident in the past two decades that the microbiome plays a critical role in regulating a host's health (5). However, there is potentially enormous complexity in the genetic interactions occurring between a host and its microbiome. We have only begun to understand the capacity of the microbiome to expand the genomic potential of its host, and the genetic and metabolic features of the microbiome that are driven by the host (92). In the case of *C. elegans*, both the host and many key microbiome bacteria are genetically amenable, facilitating a systematic and contextual understanding of these genetic interactions.

### *2.1. Understanding the interaction between C. elegans genetics and the microbiome*

It has been less than a decade since it was first shown that *C. elegans* assembles its microbes in a deterministic process. We have only begun to understand how *C. elegans* genetics helps shape this microbiome. Recently, it was identified that DAF-2 insulin-like signaling dictates the gut microbiome composition in *C. elegans* (10). *Ochrobactrum* spp. are microbiome bacteria known to be abundant and persistent in the *C. elegans* gut, but they are scarce in the wild habitat/substrates (19). Interestingly, natural *C. elegans* variants with high levels of *Ochrobactrum* spp. were found to have high insulin/IGF signaling levels, broadly activated host immunity, and faster population growth rates. Conversely, variants with low levels of *Ochrobactrum* spp. had lower insulin signaling, higher bacterial diversity, activated stress pathways, and lower population growth rates (10). This determination of an *Ochrobactrum*-dominated gut microbiome was validated by knocking down *daf-2,* and a role of the downstream DAF-2 effectors, DAF-16/FOXO and PQM-1 were also identified (10).

These findings identified a novel role of the well-studied insulin signaling pathway in regulating microbiome composition and diversity. However, what remains to be studied is how changes in microbial compositions due to DAF-2/insulin signaling impacts the longevity and other well-studied life history traits of *daf-2* mutants, including dauer formation (93). As lower *daf-2* signaling is associated with improved stress resistance and longevity, having more diverse microbiome might correlate with better health outcomes in *C. elegans* as well. These questions can provide insight into the role that insulin/IGF signaling plays in defining the microbial composition of the gut in higher mammals.

This research has paved the way for the use of *C. elegans* genetics to systematically understand different aspects of host-microbiome interactions. *C. elegans* has been a pioneering model organism for over 70 years, studied largely in the context of its interaction OP50 *E. coli.* It has been used extensively to understand the genetics regulating many organismal phenotypes, including development, neurobiology, stress, aging, and metabolism. With the relatively recent standardization of core microbiomes, there is enormous potential in decoding how the microbiome can impact many well-studied organismal properties. This includes using *C. elegans* microbiota-gene pairs to understand gene-dependent microbiome outcomes, and the impacts on life history traits, longevity paradigms, behavior, and immunity. In addition, it will be interesting to investigate how different energy and nutrient regulating cellular processes in *C. elegans*, such as autophagy and mitochondrial energetics, influence gut microbiota assembly and composition. Furthermore, unbiased forward genetic screens can be easily implemented to dissect the underlying host genes and pathways regulating colonization and adherence (94). Also, *C. elegans* transcriptomics would capture changes in their gene expression, on being colonized by a single or more complex microbiota. Together, these studies will likely identify novel microbiome-related functions in known *C. elegans* genes and pathways, as well as novel pathways among the ~40% of *C. elegans* genes that currently lack annotation (95).

### *2.2. Understanding the interaction between microbial genetics and the host*

On the microbial side, bacterial genetics can be used to understand the genetic basis of host interactions. At present, the role of microbial genetics in shaping the *C. elegans* gut microbiome is not known. However, previous studies in *C. elegans* have shown the impact of genetic changes in dietary bacteria on several life history traits of *C. elegans*, including amelioration of Aβ accumulation and germline tumors (61, 96). Therefore, microbial genetics is expected to impact their association with *C. elegans'* gut, either by impacting their colonization and persistence in the gut, or *C. elegans* fitness and other life history traits.

In addition, by examining bacterial gene expression changes after being colonized in the *C. elegans* gut, we can identify the microbial genes and metabolites required for colonization and persistence in the *C. elegans'* gut. To this end, we will expect to identify genes coding for bacterial mucin-adhesion, pili and fimbriae, and, glycosidases in the case of glyolytic commensals. Furthermore, studying the gene expression changes in bacteria with increasing *C. elegans* age can be used to address if there is an active switch in commensal-

like traits to pathogenic traits with increasing age in *C. elegans*, besides the known age-related pharyngeal defects and immunosenescence in *C. elegans*.

## Conclusion and future prospects

Since the identification of its natural microbiota, *C. elegans* has emerged as a promising model system to study host-microbiome interactions. Microbiome assembly in *C. elegans* is primarily determined by host genetics, and shaped by other factors such as behavior, anatomy, immunity, nutrition, and inter-microbial interactions. The role of *C. elegans* genetics in dictating the microbiota composition is just becoming apparent, forging avenues to systematically study how the genetic regulation of behavior, immunity, diet, gut health, and lifespan impacts the microbiome.

The *C. elegans'* microbiota bacteria use diverse metabolic competencies to colonize the gut, likely to insure diversity in microbial composition. In agreement with this, compared to fast niche occupiers (ruderal strategy), bacteria with competitive or stress tolerating strategies become more prevalent in the *C. elegans* gut (63), which is likely to have an ethological relevance during boom-bust cycles (39). In addition, these host-microbiome associations are dynamic, and host immunity and tissue homeostasis are critical for host-commensal interaction (7). However, there are some understudied aspects of *C. elegans* microbiome, as our current understanding is limited to the gut microbiome bacteria. This is due to the lack of known microbiome commensals that colonize other tissues (like the epidermis) and the lack of knowledge of other possible microbes such as fungi and viruses that make up any tissue microbiomes. Though we can visually resolve bacteria in a tissue-specific manner, resolving them for isolation and characterization is difficult as *C. elegans* are too small for complicated dissections needed to obtain tissue-specific microbiome samples, and in particular skin microbiome would be more difficult to resolve.

However, despite these limitation, *C. elegans* as a microbiome model also has advantages that can pave the way to new biology. For example, one of the major challenge in microbiome research is the inability to culture the vast majority of microbial species outside of their host. *C. elegans* can allow for the *in vivo* study of such non-culturable microbes with relative ease, as the worms can be cultured at scale, allowing for the production of microbe preps in the host (15). In addition, *C. elegans* has a very short generation time, allowing for the uncovering of the heritable aspects of microbiome bacteria in carefully designed trans-generational studies. Furthermore, the gut microbiome can be considered as an organ in itself, which can interact and influence other organs in the body. To this end, *C. elegans* gut-microbiome studies can be used to understand inter-tissue crosstalk and the role the microbiome plays in driving animal behavior. Currently, a major medical goal of the microbiome field is to engineer bacteria with attributes to manage health and disease in humans. Using *C. elegans,* it would be possible to genetically modify commensal bacteria and study their impact on host physiology and disease to understand the probiotic attributes of the modified bacteria. As such, *C. elegans* will continue to be the model of choice to study other heterologous bacteria, including human pathogens and commensals.

## Acknowledgements

This work was supported by NIH grant R35 GM146836 to RJL.

The authors declare no conflict of interest.

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