Modeling Host-Microbiome Interactions in *Caenorhabditis elegans*

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Abstract: The microbiome influences host processes including nutritional availability, development, immunity, and behavioral responses. *Caenorhabditis elegans* is a powerful model to study molecular mechanisms of host–microbial interactions. Recent efforts have been made to profile the natural microbiome of *C. elegans*, laying a foundation for mechanistic studies of host–microbiome interactions in this genetically tractable model system. Studies using single-species microbes, multi-microbial systems, and humanized worm–microbiome interaction studies reveal metabolic and microbial–microbial interactions relevant in higher organisms. This article discusses recent developments in modeling the effects of host–microbiome interactions in *C. elegans*.
Key words: Caenorhabditis elegans, Giardia, host, microbiome interactions, humanized worms, microbiome.

Recent advances in sequencing technologies have enabled an unprecedented view of the composition and diversity of the human microbiome (Consortium of the Human Microbiome Project, 2012). Our body organs including those previously thought to be sterile (e.g., breast, lung) are colonized by microbes (Morris et al., 2013; Urbaniak et al., 2014). Research findings implicate the microbiome in most if not all aspects of host biological processes ranging from metabolism, development, and immunity to behavioral responses (Belkaid and Hand, 2014; Nieuwdorp et al., 2014; Kostic et al., 2015; Rogers et al., 2016). Significant strides have been made in understanding the composition and diversity of the microbiome, but much remains to be done to characterize functions of selected members of the microbiome. The dynamic interactions between host, microbiome, and environment, and the implications that these interactions have on host health and disease are just beginning to be unraveled. Functional characterization of the microbiome and its causal relationship with malfunctions in mammalian model systems is faced with cost, logistics, and inherent biological complexity challenges. *Caenorhabditis elegans* is one of the simplest multicellular model organisms that has been developed to investigate different biological processes including development, neurobiology, host–pathogen interactions, and aging, to name a few (Sifri et al., 2005; Harrington et al., 2010; Marsh and May, 2012; Cohen and Troemel, 2015; Tissenbaum, 2015). RNAi, mutant libraries, transparent imaging, transcriptomics, and proteomics are well established for mechanistic and systems biology studies in *C. elegans*. The ease of maintenance of germ-free worms, high-throughput applicability, and their relatively short lifespan makes *C. elegans* a practical choice to study interactions between host, microbiome, and the environment (Kaletta and Hengartner, 2006; Cabreiro and Gems, 2013; Clark and Hodgkin, 2014; Yilmaz and Walhout, 2014). The simplicity of laboratory growth

and maintenance of germ-free worms for use in host–microbe interaction studies is summarized in Fig. 1.

In this article, we review recent developments in microbiome research in the *C. elegans* model system, including the use of humanized worms for modeling host–microbiome interactions and provide future directions for the study of mechanisms of interactions between host, microbiome, and the environment. We emphasize interactions between microbes and the host that may also benefit *C. elegans* as reviews on host–pathogen interactions in *C. elegans* have been recently published (Irazoqui et al., 2010; Pukkila-Worley and Ausubel, 2012; Balla and Troemel, 2013; Clark and Hodgkin, 2014; Ermolaeva and Schumacher, 2014; Cohen and Troemel, 2015; Sorathia and Rajadhyaksha, 2016). Clark and Hodgkin (2014) also summarize insights learned from modeling the effects of human commensal and probiotic bacteria in *C. elegans*. We also refer readers to a recent review by Zhang et al. (2017) that summarizes findings on the natural microbiome of *C. elegans* in a new meta-analysis.

The Natural Microbiome of *C. elegans*

Composition and diversity

In nature, *C. elegans* can be found in soil and microorganism-rich rotting fruit and plant matter (Petersen et al., 2014). Very few earlier studies explored the association between *C. elegans* and microbes in nature (Grewal, 1991; Grewal and Wright, 1992; Venette and Ferris, 1998; Avery and Shtonda, 2003). Driven by the recent surge of interest in the microbiome and advances in sequencing technologies, more comprehensive attempts have been made to profile the natural microbiome of *C. elegans* using both culture and 16S rDNA sequencing (Montalvo-Katz et al., 2013; Berg et al., 2016a; Dirksen et al., 2016; Niu et al., 2016; Samuel et al., 2016). Two different approaches were used to characterize the worm microbiome. In the first approach, germ-free worms were exposed to laboratory-simulated natural environments (soil, rotten plant matter, or soil supplemented with rotten plant matter) to allow for the assembly of microbiome in the intestine of the worm. In the second approach, worm strains were
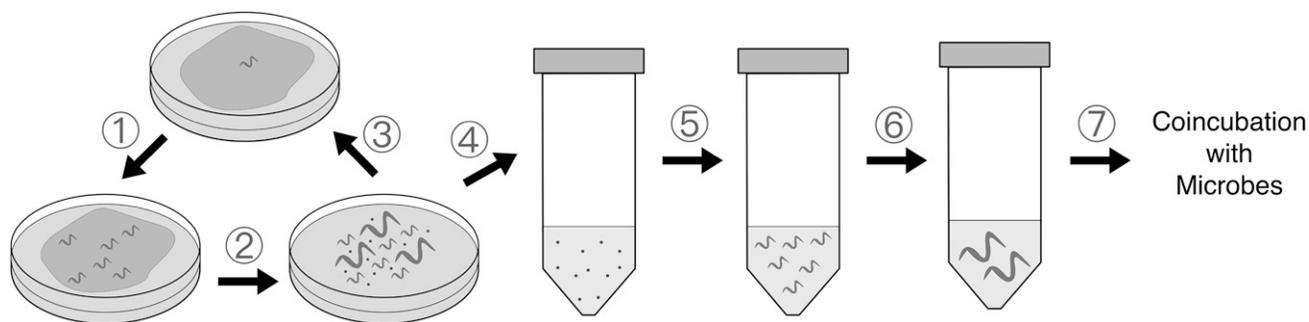
Fig. 1. Laboratory growth and maintenance of *Caenorhabditis elegans* for use in host–microbe interaction studies. Gravid adult worms are transferred to a nematode growth medium agar plate previously seeded with OP50 *Escherichia coli*, a standard food source for *C. elegans*. (1) The worms will reproduce and populate the plate. (2) Gravid adult worms from the plate can then be transferred to a new plate (3) continuing the cycle. When there are enough eggs and gravid adults on the plate, they can be collected, transferred to a tube, and treated with bleach, which dissolves the worms and kills bacteria, leaving only the bleach-resistant eggs. (4) The eggs are washed and allowed to hatch and arrest at the L1 developmental stage in S-basal buffer, creating an age-synchronized germ-free population. (5) L1 worms are then resuspended in S-basal complete media, fed OP50 *E. coli*, and allowed to develop over 2 to 3 d to L4/adult worms. (6) These worms are ready for host–microbe interaction studies (7) as in Gerbaba et al. (2015).

collected from the wild, and their native microbiome characterized. Montalvo-Katz et al. (2013) used culture-dependent approaches to identify the worm microbiome by growing wild-type larvae of *C. elegans* in soil amended with compost or rotting fruits. Seventeen bacterial species were isolated and identified, mainly belonging to *Bacillus* and *Pseudomonas* genera. Culture-dependent approaches select for culturable microbes and hence limit the depth of diversity and composition characterization of the microbiome. Dirksen et al. (2016) used 16S rDNA deep sequencing to characterize the natural microbiomes of *C. elegans, Caenorhabditis remanei*, and *Caenorhabditis briggsae* isolated from natural environments, including plant stems, fruit, and compost. The study revealed the complexity of native gut microbial taxa of nematodes obtained from natural environments. Although Proteobacteria is the most dominant bacteria taxa belonging to Bacteroidetes, Actinobacteria and Firmicutes are also represented in this microbiome (Dirksen et al., 2016). Berg et al. (2016a) characterized the microbiome of *C. elegans* N2 strain assembled from soil supplemented with plant matter (bananas, potatoes, oranges, or strawberries). A recent report offers an exhaustive survey of bacterial communities inhabiting *C. elegans* natural environments, rotten fruits (apples, orange, cactus fruit, and black bryony) and a vector (snail), using culture-dependent and culture-independent approaches (Samuel et al., 2016). The *C. elegans* environment harbors dominant bacterial taxa belonging to Proteobacteria, Firmicutes, Bacteriodetes, and Actinobacteria. More than 500 bacterial isolates have been obtained from these environments and exhaustively analyzed to determine the effects of single, paired, and multispecies bacterial community on worm growth (Samuel et al., 2016). Researchers commonly use *C. elegans* in monoxenic association with single-species bacteria. The studies discussed previously revealed that the *C. elegans* microbiome is

not as simple as originally thought. Indeed, diverse groups of bacteria are found associated with *C. elegans* in its natural environment.

Microbiome assembly: effects of host genotype and development stage

The association between *C. elegans* and its microbes is much more than a dietary relationship. The *C. elegans* microbiome is distinct from the microbiome in its environment and from the microbiome of *C. remanei* (Dirksen et al., 2016). The microbiomes of *C. elegans* and *C. briggsae* protect each species against infections, but the microbiome of one species fails to protect the other species (Berg et al., 2016b). The reason for the lack of cross protection is not clear, and phylogenetic distinctions between the microbes of the two species using 16s rDNA analysis are not available. Although 16s rDNA and phylogenetics-based microbiome characterization allow in-depth characterization of the complexity and diversity of microbial ecosystems, these methodologies are not always able to assign all operational taxonomic units to distinct species level, a hurdle in understanding functional contributions at species and strain levels. Functional characterization of microbial isolates in *C. elegans* should help reveal differential contributions of closely related species/strains as well as functional redundancy between distinct bacterial taxa. The composition and diversity of the microbiome in the environment correlates with developmental stages of the worms (Dirksen et al., 2016). Alpha-Proteobacteria–rich environments support proliferation, whereas higher levels of gamma-Proteobacteria and Bacteoidetes promote nonproliferating dauer-stage development (Samuel et al., 2016). The use of defined synthetic microbiota ecosystems, guided by the natural history of *C. elegans*, should help optimize the development of a relevant model system for host–microbiome interaction studies (Samuel et al., 2016).

The genotype and development of worms appear to have been shaped at least in part by host–microbiome interactions. However, further studies into the interactions between *C. elegans* and its natural microbiome are required to provide conclusive evidence on the microbiome impact on the host genotype.

Few attempts have been made to explore microbiome of other nematodes. For a recent review on the microbiota of parasitic nematodes and the reciprocal interactions between host microbiota and parasitic nematodes, we refer the readers to Midha et al. (2017). The environment in the host organism appears to influence the composition of the microbiome of parasitic nematodes. Bacteria identified from *Ascaris suum* appear to have been derived from the host intestine (Hsu et al., 1986). Adult *Heligmosomoides polygyrus bakeri* microbiota shows similarity to that of its murine host ileum (Walk et al., 2010). It is unknown if host-associated parasitic nematodes have less or more diverse microbiota compared with free-living forms. Further studies on the microbiome of parasitic nematodes using culture-independent approaches should shed light on the impact of selective pressures from their mammalian host. Understanding the interactions between parasitic nematodes and their native microbiome may open up new avenues for therapeutics. A good example for this is the interaction between filarial worms and their endosymbiont gram-negative intracellular bacterium, *Wolbachia*. *Wolbachia* is necessary for normal larval growth and development, embryogenesis, and survival of adult filarial worms (Taylor et al., 2005), and attempts are being made to target *Wolbachia* for the treatment of filarial infection (Taylor et al., 2014).

Simplified host–microbiome interaction models: Escherichia coli OP50, a uracil auxotroph of an *E. coli* B strain, serves as a standard food source for *C. elegans* in the laboratory (Stiernagle, 2006). It also has non-dietary benefits and age-dependent pathogenic effects. *Escherichia coli* accumulates in the intestine of older worms, which appears associated with decreased lifespan, and antibiotics or UV-treated bacteria extend worm lifespan (Gems and Riddle, 2000; Garigan et al., 2002; Herndon et al., 2002; McGee et al., 2011). Association of *C. elegans* with a single bacterial species such as *E. coli* serves as a simplified model to study the role of host–microbiome interactions at the molecular level, which allows to characterize effects of microbial factors and host pathways on various biological processes as discussed in the later paragraphs (summarized in Fig. 2).

Development and aging

Bacterial signals affect worm development and aging. *Escherichia coli* folate synthesis reduces *C. elegans* lifespan (Virk et al., 2016). *Comamonas aquatica* produces
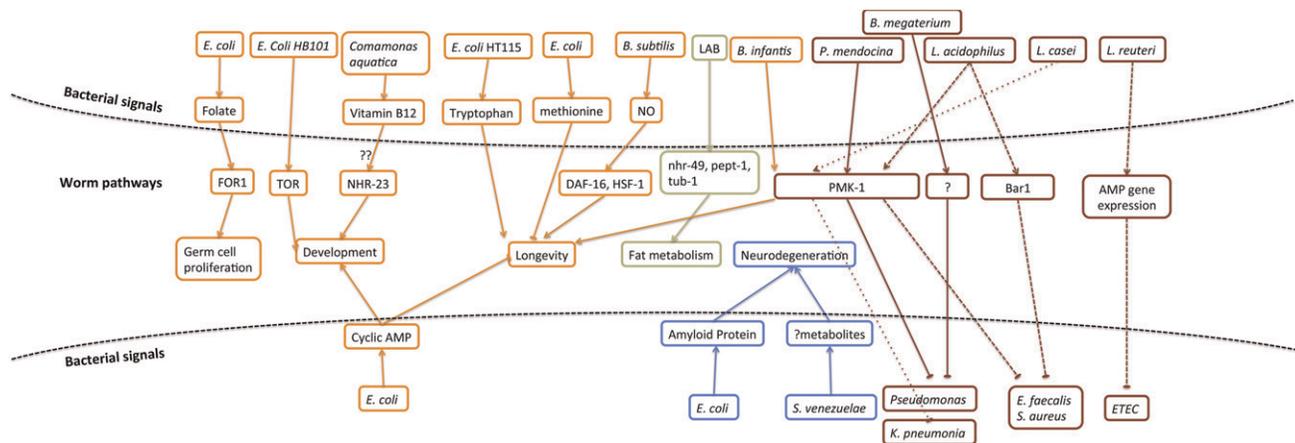
Fig. 2. Host–microbe interactions in *Caenorhabditis elegans*. This figure summarizes host–microbe interactions in *C. elegans* discussed in the present review. Bacteria metabolic signals, such as folate, vitamin B12, tryptophan, methionine, nitric oxide (NO), cyclic antimicrobial peptide (AMP), and amyloid protein, influence various biological processes including germ cell proliferation, development, longevity, and neurodegeneration in *C. elegans*. Expression of folate receptors (FOR1) in *C. elegans* is essential to the modulatory effects of bacterial folate on germ cell proliferation. The nuclear hormone receptor (NHR-23) is likely involved in regulating the molting cycle during larval development (MacNeil et al., 2013), but the link between the effects of *Caenorhabditis aquaticus* vitamin B12 on the acceleration of development and NHRs is not clear (Watson et al., 2014). The effects of *Escherichia coli* HB101 on worm development (speeding growth) are target of rapamycin (TOR) dependent (MacNeil et al., 2013). *Escherichia coli* HT115 tryptophan mediates detoxification responses in nhr-114 mutant worms. Microbes are important sources of dietary methionine in *C. elegans*, and interference with microbial methionine synthesis induces methionine restriction, which in turn extends lifespan (Cabreiro et al., 2013). *Bacillus subtilis* NO extends worm lifespan, which is dependent on DAF-16 and HSF-1 transcription factors (Gusarov et al., 2013; Donato et al., 2017). Several studies using *C. elegans* have illustrated how the host microbiome may protect against pathogens (shown in dark red). *Pseudomonas mendocina*, *Lactobacillus acidophilus*, and *Lactobacillus casei* protect against pathogenic infection in a PMK-1 (p38 MAPK) or β-catenin signaling (BAR1) dependent manner. *Lactobacillus reuteri* protects against enterotoxigenic *E. coli* (ETEC) possibly through induction of expression of AMP genes. *Bacillus megaterium*–mediated protection against Pseudomonas infection is linked to impaired egg laying, the potential host response pathways involved remain obscure. Various lactic acid bacteria (LAB) alter expression of obesity phenotype genes (in green). Bacterial metabolite effects in eliciting neurodegeneration (light blue) have also been studied in *C. elegans*, but the specific bacterial metabolites implicated in these effects require further identification. The effects of *C. elegans*-microbial interactions on longevity, development, and germ cell proliferation are shown in orange.

vitamin B12 that affects *C. elegans* development and fertility and also breaks down propionic acid to prevent its toxic buildup (Watson et al., 2014). *Escherichia coli* HT115 tryptophan metabolism prevents sterility in nuclear receptor nuclear hormone receptor (NHR-114) mutant worms (Gracida and Eckmann, 2013b). NHR-144 deficient worms grown on a B strain bacteria such as OP50 and BL21 *E. coli* are sterile unless supplemented with tryptophan (Gracida and Eckmann, 2013a). Bacterial signals affect the role of the mechanistic target of rapamycin complex 2 (mTORC2) and serum- and glucocorticoid-regulated kinase 1 (SGK-1) pathway in aging. mTORC2 directs SGK-1 to inhibit SKN-1 and release of this inhibition depends on bacterial cues, which allows SKN-1 to increase stress resistance and lifespan (Mizunuma et al., 2014). *Bacillus subtilis* produces nitric oxide, which extends *C. elegans* lifespan through expression of heat shock and stress response genes controlled by DAF-16 and HSF-1 transcription factors (Gusarov et al., 2013; Donato et al., 2017).

Neurodegenrative disorders

The simplicity of the *C. elegans* model system allows the study of molecular pathways that are not easily tractable in higher organisms such as that of Parkinson's disease (Harrington et al., 2010). To study microbe-mediated neurodegeneration, human alpha-synuclein (AS) expressed in wild-type worms was used to analyze effects of *E. coli* amyloid protein curli on AS aggregation (Chen et al., 2016). Curli-producing bacteria fed AS-expressing *C. elegans* had enhanced AS aggregation, suggesting that bacterial amyloid functions may trigger AS aggregation. Studies also reported on the effects of *Streptomyces venezuelae* metabolites in combination with Parkinson's disease susceptibility gene mutations in eliciting neurodegeneration (Ray et al., 2014; Watkins et al., 2016). *Streptomyces venezuelae* metabolites cause excessive production of reactive oxygen species, upregulate mitochondrial unfolded protein response pathway, and impair ATP production, which may in part be responsible for neurotoxicity and cell death (Ray et al., 2014).

Genetics of host–microbiome interactions

Caenorhabditis elegans is suitable to study effects of host–microbiome interactions in a semi-highthroughput manner. A combination of *C. elegans* and bacterial genetic screening reveal novel host–microbe interaction mechanisms. Coolon et al. (2009) identified candidate genes differentially regulated in response to different bacteria isolates. Using mutations that inactivate 21 of the identified host genes, they showed that most bacteria contribute to lifespan and fitness (Coolon et al., 2009). Khanna et al. (2016) identified 56 *E. coli* deletion mutants that enhance dauer formation. cyaA mutant bacteria defective in cyclic antimicrobial peptide (AMP) production extends lifespan and enhances dauer formation in an insulin-like receptor (*daf-2*) mutant worms through the modulation of transforming growth factor-beta (*daf-7*) signaling (Khanna et al., 2016).

Germ cell proliferation

Bacterial signals appear to modulate germ cell proliferation in *C. elegans*. Bacteria-derived folates promote worm germ cell proliferation independently of their roles as vitamins, and folate receptor-1 (FOLR-1) is required for the stimulation of germ cell proliferation (Chaudhari et al., 2016). FOLRs are overexpressed in many human cancers and associated with neoplastic progression and poor prognosis (Kelemen, 2006), and studies implicate the microbiome in the development of cancer (Schwabe and Jobin, 2013). It will be interesting to see the potential use of *C. elegans* as a model to investigate the role of the microbiome in cancer pathogenesis.

Drug resistance

Caenorhabditis elegans is a suitable model to study pathogenic nematode drug resistance and it has been used for screening antihelminthics (Geary and Thompson, 2001; Burns et al., 2015). The role of the host gut microbiome in the development of drug resistance in pathogenic nematodes remains unclear. Thirty gut Enterobacteriaceae that catabolize benzimidazole-class antihelminthics have been isolated and tested for their effects on *C. elegans* survival in the presence of benzimidazoles (Whittaker et al., 2016). The Enterobacteriaceae microbiota protects *C. elegans* from benzimidazoles and adult ascarids from the effects of albendazole (Whittaker et al., 2016). Research on clinical interactions between gastrointestinal helminths and resident microbiota during drug treatment is important in attempts to understand interactions between human parasite nematodes and the microbiome. By taking into account the importance of the microbiome, drug screening and resistance studies in *C. elegans* may help devise novel approaches to curb resistance to antimicrobials and antihelminthics.

Survival assays and functional screening

The single-species microbe–host interaction model system is instrumental in understanding the function of specific members of the microbiome. Beneficial and detrimental microbes can be screened in a relatively short time using worm survival as a readout. Samuel et al. (2016) studied the response of *C. elegans* to 565 bacterial species isolated from *C. elegans* natural environment (Samuel et al., 2016). These studies showed that 78% of the bacterial isolates had beneficial effects on survival, whereas the remaining 22% of the bacterial strain had detrimental effects.

Fat metabolism

Microbiome dominated with Firmicutes rather than Bacteriodetes have been associated with obesity in humans

(Turnbaugh et al., 2006; Turnbaugh and Gordon, 2009; Turnbaugh et al., 2009a). A consortium of *Lactobacillus delbrueckii*, *Lactobacillus fermentum*, and *Leuconostoc lactis* affect energy metabolism in *C. elegans*. The lactic acid bacteria (LAB) microbial consortia decrease worm lifespan when compared with animals fed with conventional *E. coli*, cause accumulation of lipid droplets, and alter expression of obesity phenotype genes, nhr-49, pept-1, and tub-1 (Zanni et al., 2015).

Microbiome and host–pathogen interactions

Host–pathogen interaction studies in *C. elegans* have allowed the study of various pathways and mechanisms conserved in higher organisms. These studies explore mechanisms of host–pathogen interactions to uncover host and pathogen factors important in susceptibility and resistance to infections. The effect of pathogens on worm survival is used as a proxy for pathogenic effects, as determined by feeding worms *E. coli* OP50 alone, or in the presence of a pathogen of interest. The role of microbes in protection against infection is determined by exposing worms to members of the microbiome and assessing the response to infecting pathogens. Members of the *C. elegans* natural microbiome, *Bacillus megaterium* and *Pseudomonas mendocina*, enhance worm resistance to subsequent infection with *Psudomonas aeruginosa* (Montalvo-Katz et al., 2013). The protective microbe *P. mendocina* primes the immune response to subsequent infection with the phylogenetically closely related species *P. aeruginosa*. In hamsters and humans, nontoxigenic *Clostridium difficile* protect against highly virulent *C. difficile* infections (Seal et al., 1987; Shim et al., 1998; Villano et al., 2012; Nagaro et al., 2013; Gerding et al., 2015). The mechanisms are not clear although colonization resistance through niche exclusion is hypothesized to be at play (Natarajan et al., 2013; Gerding et al., 2015). *Caenorhabditis elegans* may serve as a novel tool to study and distinguish between niche exclusion and immune priming as mechanisms of microbiota-mediated protection against pathogens. Another *C. elegans* native microbiome member, *Pseudomanas* sp., affords protection against the *C. elegans* fungal pathogen, *Drechmeria coniospora* (Dirksen et al., 2016). *Enterobacter cloacae*, an isolate from *C. elegans*, protects its host against infection with *Enterococcus faecalis*, but fails to protect a nonhost species, *C. briggsae* (Berg et al., 2016b). Similar host-specific protective effects were observed for *E. cloacae* isolates from *C. briggsae* (Berg et al., 2016b).

Caenorhabditis elegans, in its natural environment, is exposed to diverse beneficial and detrimental microbes. Beneficial bacteria (*Gluconobacter* sp., *Enterobacter* sp., and *Providencia* sp.) isolated from natural habitats of *C. elegans* have protective effects against detrimental bacteria (*Serratia* sp., *Pseudomonas* sp., and *Chryseobacterium* sp.) isolated from the same environment (Samuel et al., 2016).

Caenorhabditis elegans presents an interesting model system to experimentally dissect the role of the microbiome in host evolution and adaptation to changing environments including infections. The short lifespan of *C. elegans* allows for studies on aging and co-evolution. Ford et al. (2016) studied co-evolutionary dynamics between a defensive microbe and a pathogen driven by fluctuating selection (Ford et al., 2016). Co-passaging protective *E. faecalis* bacteria and a *Staphyloccoccus aureus* pathogen results in reciprocal adaptation, revealing patterns of pathogen local adaptation and genetic diversification (Ford et al., 2016). Research into competition and resilience between founder and introduced bacteria using *E. coli* and *S. typhymurium* shows the persistent gut colonization by *Salmonella* efficiently outcompetes *E. coli* strains; by contrast, repeated in vivo passage enhances the ability of *E. coli* to compete (Portal-Celhay and Blaser, 2012). *Caenorhabditis elegans* also allowed to investigate synergistic commensalism of opportunistic pathogens, using *Candida albicans* and gram-positive *E. faecalis*, both normal residents of the human gut microbiome. Co-infection of *C. elegans* with these microorganisms is less pathogenic than with either species alone because *E. faecalis* derived products inhibit hyphal morphogenesis in *C. albicans* (Garsin and Lorenz, 2013).

Infection with certain pathogens is known to lead to microbiome dysbiosis (Beatty et al., 2017), which has been implicated in a variety of post-infectious inflammatory disorders in humans. Parasitic (e.g., *Giardia duodenalis*) and bacterial (e.g., *Campylobacter jejuni*) infections have been found to cause post-infectious irritable bowel syndrome and flares in patients with inflammatory bowel (Buret, 2016). The mechanisms remain obscure. Studies of changes in intestinal microbiome composition and function on exposure to enteropathogens such as *G. duodenalis* will improve our understanding of the mechanisms of microbiome-mediated post-infectious disorders. The effects of *G. duodenalis* on host–microbiome interactions in *C. elegans* were recently investigated (Gerbaba et al., 2015). *Giardia* secretes factors that induce functional changes in non-pathogenic *E. coli* bacteria by altering expression of a broad range of *E. coli* metabolic genes, possibly converting them into pathobionts. Indeed, exposure to *Giardia* alters expression of *E. coli* genes involved in cysteine metabolism, and concurrent exposure to both microorganisms, but not to each organism alone, causes lethal toxicity in *C. elegans* (Gerbaba et al., 2015). Niu et al. (2016) studied effects of a soil-dwelling opportunistic pathogen *Bacillus nematocida* B16 on the relative abundance of *C. elegans* microbiome that were assembled from soil and rotten fruits. Although the interpretation of parts of the data was limited in view of the lack of some controls (non-pathogenic *Bacillus*), it is worthwhile to mention here that the authors suggested that *C. elegans* infection with *B. nematocida* B16 increases the relative

abundance of Firmicutes and decreases the representation of Proteobacteria, Actinobacteria, Cyanobacteria, and Acidobacteria (Niu et al., 2016).

The mechanisms underlying the protective roles of members of the microbiome warrant further investigation. Protective mechanisms of bacteria have also been studied using the *C. elegans* model. *Lactobacillus casei* protects *C. elegans* from the effects of *Klebsiella pneumonia*; *L. casei* triggers a toll-like receptor mediated receptor for activated C kinase 1 dependent p38 MAPK pathway to help *C. elegans* resist *K. pneumonia* infection (Kamaladevi and Balamurugan, 2016). Heat-killed *Lactobacillus* sp. enhances the survival of *C. elegans* exposed to *Salmonella* sp. and *Yersinia* sp. Exposure to heat-killed LAB increases the expression of genes (acdh-1 and cnc-2) related to the defense response and the innate immune response (Lee et al., 2015). *Bacillus subtilis*, often found in the natural habitat of *C. elegans*, persists in the *C. elegans* intestine and protects against infection with *Bacillus thuringiensis* by producing fengycin, a lipopeptide acting as an antibiotic (Iatsenko et al., 2014). The *C. elegans* model system has proven useful for screening protective bacteria (Iatsenko et al., 2014; Park et al., 2014). Iatsenko et al. (2014) identified *B. subtilis* GS67 strain with the strongest effect against *B. thuringiensis* among eight other natural *B. subtils* isolates. Park et al. (2014) identified four *Lactobacillus plantarum* strains originally isolated from infant feces that persist in the nematode gut, significantly prolong longevity and improve worm survival upon exposure to pathogenic *S. aureus* (Park et al., 2014). Another screening study allowed the identification of LAB bacteria that protect against enterotoxigenic *E. coli* (Zhou et al., 2014a). One of the isolates, *Lactobacillus reuteri*, promotes host defensive AMP genes *clec-60* and *clec-85* (Zhou et al., 2014b). In addition to the aforementioned observations, ever-increasing evidence supports the hypothesis that microbial–microbial interactions determine the outcome of infection in the host. Co-infecting microorganisms modulate gene expression in pathogens and commensals during polymicrobial infections (Reti et al., 2015; Ibberson et al., 2017). Identification of microbial genes that may be essential for disease production is important for developing new therapies. The findings discussed here illustrate the importance of studying pathogens in the context of complex polymicrobial communities and further underscore modeling such microbial–microbial interactions and their effect in *C. elegans* provides a powerful testing ground for such studies.

Humanized worms and host–microbiome interactions: Humanized mice, germ free mice transplanted with human microbiota, are useful tools to study microbiome function (Martin et al., 2008; Turnbaugh et al., 2009b; Beatty et al., 2017). Humanized worms, *C. elegans* similarly associated with human microbial communities, can help model the effects of microbiome dysbiosis by systematically analyzing host, microbial, and environmental factors

important in the regulation of host–microbiome interactions that modulate health and disease.

The gut microbiota undergoes significant compositional and functional changes in intensive care patients. Changes include reduced diversity in a microbiome dominated by members of the Enterobacteriaceae and Proteobacteria. Decreased microbiome diversity has been associated with hospital-acquired infections such as *C. difficile* and sepsis (Chang et al., 2008; McDonald et al., 2016). Recent studies characterized the function of such ultra-low diversity microbial communities in patients with prolonged critical illness, where the microbiome was dominated by *Enterococcus* sp., *Staphylococcus* sp. and fungal pathogens such as *C. albicans* and *Candida glabrata* (Zaborin et al., 2014). The microbiome communities behaved as commensals or pathobionts, depending on their composition and environmental/host signals. Studies using *C. elegans* revealed the effects of host factors such as endogenous opioids and nutrient deprivation in changing the phenotype of these communities. Under conditions of nutrient deprivation, the ultra-low diversity communities behave like commensals, but opioid treatment enhances the pathogenic behavior of these communities, which is abolished in the presence of phosphate-polythylene glycol, an inhibitor of bacterial virulence expression. Endogenous opioids are released during physiologic stress and critical illness; parenteral feeding of critically ill patients results in nutrient deprivation (Zaborin et al., 2014). The study by Zaborin et al. (2014) shows the potential use of the *C. elegans* to model functional changes in the microbiome of critically ill patients by mimicking local conditions in the gut, nutrient deprivation and opioid release.

Recent studies using *C. elegans* also characterized functional differences in the microbiome of inflammatory bowel disease patients collected from active and inactive sites of the colon. Compared with the effects of microbiota from healthy donors, microbiota from inflamed sites caused significantly greater lethal toxicity, whereas microbiota from non-inflamed sites did not seem to affect worm survival (Gerbaba et al., 2015). Microbiota from inflamed sites in patients with inflammatory bowel disease is characterized by a low diversity microbiome (Sepehri et al., 2007). Furthermore, when concurrently exposed to *G. duodenalis*, microbiota from inflamed IBD sites synergized lethal toxicity in *C. elegans*, whereas microbiota from non-inflamed sites did not (Gerbaba et al., 2015). The aforementioned observations indicate that studies on the effects of human microbiome communities in humanized *C. elegans* help shed light on the functional significance of alterations in the human microbiome.

FUTURE DIRECTIONS

Interactions of single species microbiota isolates (e.g., *E. coli*) with *C. elegans* represent powerful model

systems to characterize molecular mechanisms regulating host–microbial interactions. The microbiome of *C. elegans*, although Proteobacteria species are dominant— at least in the proliferative larval stage of worms—they contain core microbiome members that are also present in higher organisms. Studies using more complex microbial consortia are now required to model the biology of microbial ecosystems in *C. elegans*. There is little specificity in the ecology of *C. elegans*, with soil, rotten fruits, and plant matter sometimes, but not always, harboring *C. elegans*. The importance of the environment in shaping the composition of *C. elegans* microbiome is an interesting area of future investigation. The simplicity of the *C. elegans* model system should allow for a systematic construction of defined microbial consortia that are suitable for modeling specific biological processes. Unraveling pathways that regulate the microbiome in *C. elegans* will help shed light on conserved evolutionary features relevant to higher organisms, including mechanisms of microbial ecosystem succession. In turn, such studies may guide the construction of synthetic microbial ecosystems to maintain the homeostasis of host and microbial ecosystem interactions. Moreover, the use of *C. elegans* humanized with disease-specific microbial communities will help devise strategies to manipulate the microbiome for therapeutics. The *C. elegans* model is also suitable to answer fundamental questions of how certain pathogens overcome the protective effects of the microbiome, and which host, microbial, and environmental factors determine susceptibility and resistance to infections.

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